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NONCOVALENT DERIVATIVES OF HYDROQUINONE:
BIS-(N,N-DIALKYL)BICYCLO[2.2.2]OCTANE-1,4-DICARBOXAMIDE
COMPLEXES

Bruce M. Foxman,¹ Donna J. Guarrera,² Ramdas Pai,² Carlos Tassa,³ and
John C. Warner^{3*}

¹Department of Chemistry, Brandeis University, Waltham, MA 02454, USA

²Polaroid Corporation, Media Systems Research Laboratories,
Cambridge, MA 02139, USA

³Department of Chemistry, University of Massachusetts Boston, 100 Morrissey Blvd.,
Boston, MA 02125, USA

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ABSTRACT

The solid state phase behavior of hydroquinone **1** with *bis*-(*N,N*-diethyl)bicyclo[2.2.2]octane-1,4-dicarboxamide (**4**) and with *bis*-(*N,N*-dimethyl)bicyclo[2.2.2]octane-1,4-dicarboxamide (**5**) has been investigated. A 1:1 complex, **6**, exists with hydroquinone and the diethyl derivative **4**. A 1:2 complex, **7**, and a 2:1 complex, **8**, is formed with hydroquinone and the dimethyl derivative, **5**. X-ray structure determinations of these cocrystals have been performed and the role of the alkyl side chains in the crystal packing is discussed. © 1999 Elsevier Science Ltd

KEYWORDS: organic compounds, crystal growth, crystal structure, phase transitions, thermodynamic properties

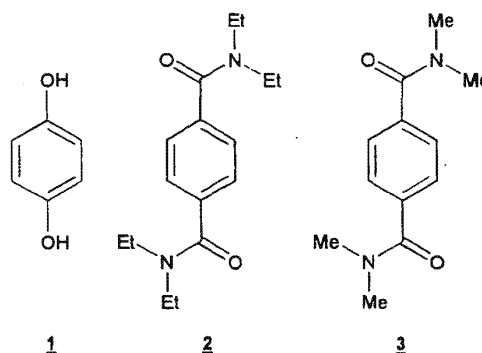
INTRODUCTION

The intentional manipulation of bulk physical properties using crystal engineering can be thought of as noncovalent derivatization [1]. Rather than replace covalently bound functional groups using conventional synthetic organic chemistry, analog libraries may be generated by

*To whom correspondence should be addressed.

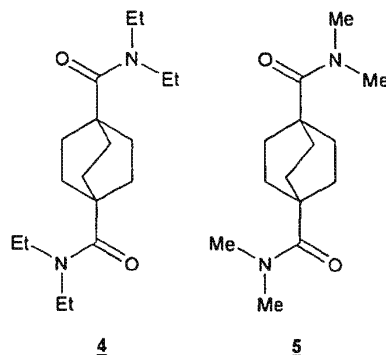
the designed incorporation of auxiliary molecules within an organic crystalline lattice. Supramolecular derivatives can be constructed by using noncovalent interactions such as hydrogen bonding, π -stacking and lipophilic-lipophilic interactions [2–6]. The new ordered structure can be viewed as a unique chemical entity distinct from either of its individual components. If the auxiliary molecules are themselves environmentally acceptable, the elimination of several solvent-requiring synthetic transformations may be bypassed by using this technique. The method of forming organic solid-state complexes can in many applications be performed under aqueous or solventless conditions [7].

We have been extrapolating the general utility of this technique across a wide spectrum of technological applications [8–10]. To do so, it is necessary for us to understand the processes available for designing, synthesizing, and characterizing these assembled systems. Using the dialkyl amide-phenol hydrogen bond as the fundamental structural feature, we have been investigating a number of binary self-assembled systems. We have recently reported the description of the phase behavior of hydroquinone **1** with *bis*-(*N,N*-dialkyl)amides; *bis*-(*N,N*-diethyl)terephthalamide **2** and *bis*-(*N,N*-dimethyl)-terephthalamide **3** [11].



We have shown that the characterization of these binary systems is possible by solid-state CP-MAS NMR and differential scanning calorimetry (DSC) [12]. These techniques allow for the determination of available stoichiometries of binary systems. This is achieved by the construction of phase diagrams.

Evaluation of the crystal structures of the stoichiometric complexes shows that the crystalline lattices are primarily defined by the hydrogen bonding networks between the phenols and the amides. Secondary effects involving interactions between the aromatic rings are difficult to separate from the hydrogen bonding. In order to view these effects separately, complexes of hydroquinone **1** with *bis*-(*N,N*-diethyl)bicyclo[2.2.2]octane-1,4-dicarboxamide **4** and with *bis*-(*N,N*-dimethyl)bicyclo[2.2.2]octane-1,4-dicarboxamide **5** have been studied. The bicyclo[2.2.2]octane system provides a linear linkage of amide groups similar to the terephthalamides, but lacks the aromaticity.



EXPERIMENTAL

Hydroquinone **1** was purchased from Aldrich Chemical (Milwaukee, WI) and used without further purification. *Bis*-(*N,N*-diethyl)bicyclo[2.2.2]octane-1,4-dicarboxamide **4** [13] and *bis*-(*N,N*-dimethyl)bicyclo[2.2.2]octane-1,4-dicarboxamide **5** were prepared from bicyclo[2.2.2]octane-1,4-dicarbonyl chloride [14] and the appropriate secondary amine.

Bis-(*N,N*-Diethyl)bicyclo[2.2.2]octane-1,4-dicarboxamide 5. Isolated as a white solid:mp. 138–139°C.

TABLE I
Crystallographic Data

Compound	6	7	8
CCDC deposit no.	CCDC-1294/74	CCDC-1294/75	CCDC-1294/76
Chemical Formula	C ₂₄ H ₃₈ N ₂ O ₄	C ₃₄ H ₅₄ N ₄ O ₆	C ₂₆ H ₃₆ N ₂ O ₆
Formula weight	418.58	614.83	472.59
Space group	Ps/n [C _{2h} ; No. 13]	Fdd2 [C _{2v} ; No. 43]	Pī [C _i ; No. 2]
T, °C	21	21	21
a, Å	19.282(8)	22.896(7)	11.002(3)
b, Å	6.417(3)	44.459(13)	12.329(4)
c, Å	19.272(8)	6.813(2)	10.750(3)
α, deg	90	90	100.59(3)
β, deg	103.04(4)	90	108.17(3)
λ, deg	90	90	108.78(3)
V, Å ³	2323(3)	6935(6)	1245(2)
Z	4	8	2
ρ _{calcd} , g cm ⁻³	1.197	1.178	1.261
λ, Å	0.71073	0.71073	0.71073
μ, mm ⁻¹	0.076	0.075	0.084
R	0.060	0.053	0.062
R _w	0.079	0.053	0.070

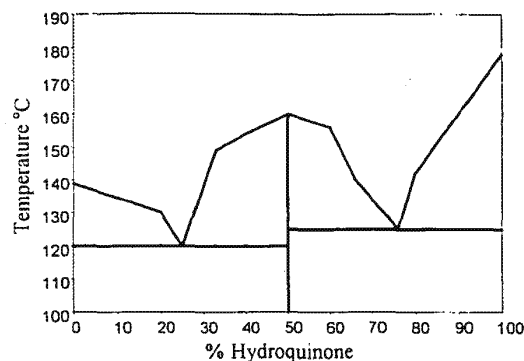


FIG. 1

Phase diagram for the hydroquinone-*bis*-(*N,N*-diethyl)bicyclo[2.2.2]octane-1,4-dicarboxamide system. the eutectic compositions were not experimentally determined.

Anal. Calcd for $C_{18}H_{32}O_2N_2$: C, 70.09; H, 10.46; N, 9.08; and C, 70.52; H, 10.17; N, 8.87.

Structure Determination of 6, 7, and 8. Routine operations were performed as described previously (Syntex P2₁ diffractometer at Brandeis University, Waltham, MA) [15–21]. The structures were refined by using anisotropic displacement parameters for N, O, and C atoms. H atoms, except those attached to O (refined by using isotropic displacement parameters), were fixed at calculated positions. Crystallographic data are presented in Table 1.

RESULTS AND DISCUSSION

Hydroquinone **1** and *bis*-(*N,N*-diethyl)bicyclo[2.2.2]octane-1,4-dicarboxamide **4** form a 1:1 solid state complex **4** as shown by the phase diagram (Fig. 1). This is similar to the 1:1 complex formed with hydroquinone **1** and *bis*-(*N,N*-diethyl)terephthalamide **2**.

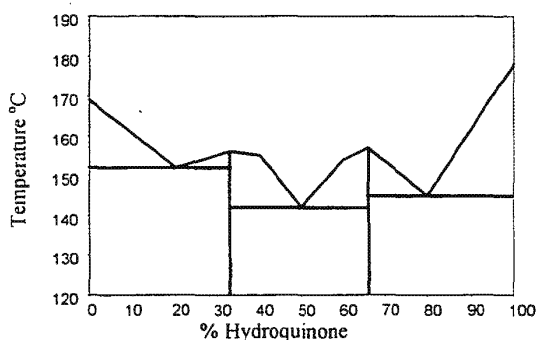


FIG. 2

Phase diagram for the hydroquinone-*bis*-(*N,N*-diethyl)bicyclo[2.2.2]octane-1,4-dicarboxamide system. the eutectic compositions were not experimentally determined.

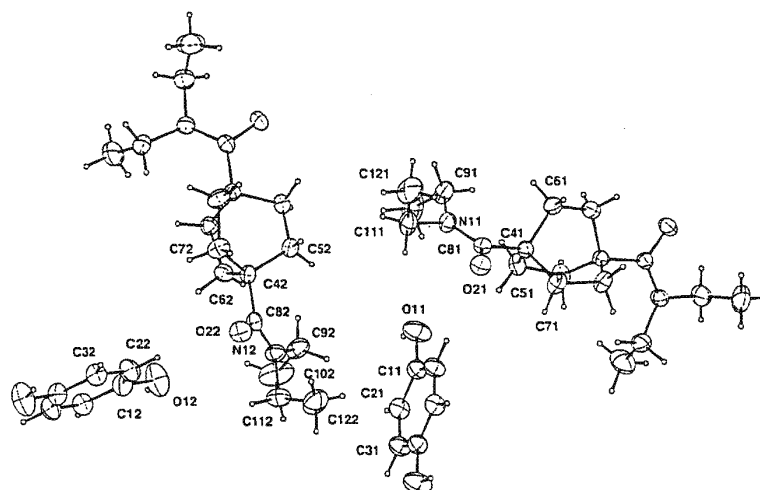


FIG. 3

Molecular structure of **6**, showing labeling scheme and 40% probability ellipsoids for atoms refined using anisotropic displacement parameters. The hydroquinone moieties crystallize on centers of symmetry at $(1/2, 0, 0)$ and $(0, 1/2, 0)$, while the terephthalamide occupies a crystallographic C_2 axis.

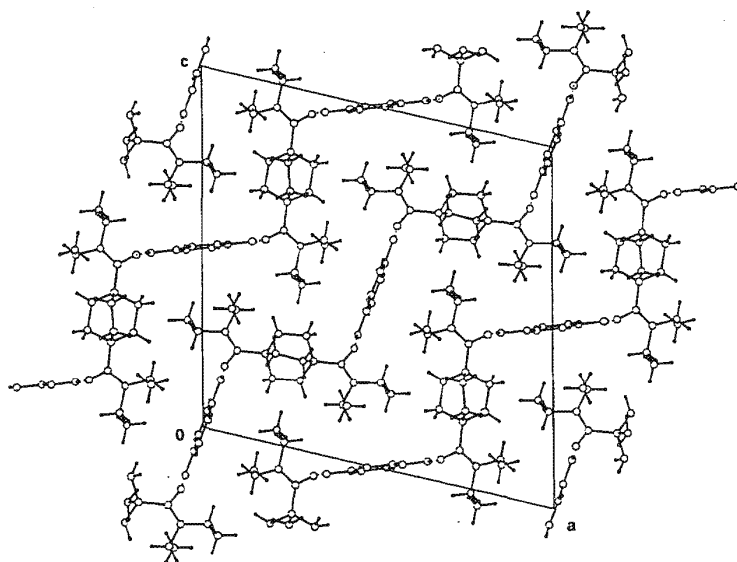


FIG. 4

Crystal packing diagram of 1:1 hydroquinone-*bis*-(*N,N*-diethyl)bicyclo[2.2.2]octane-1,4-dicarboxamide **6**.

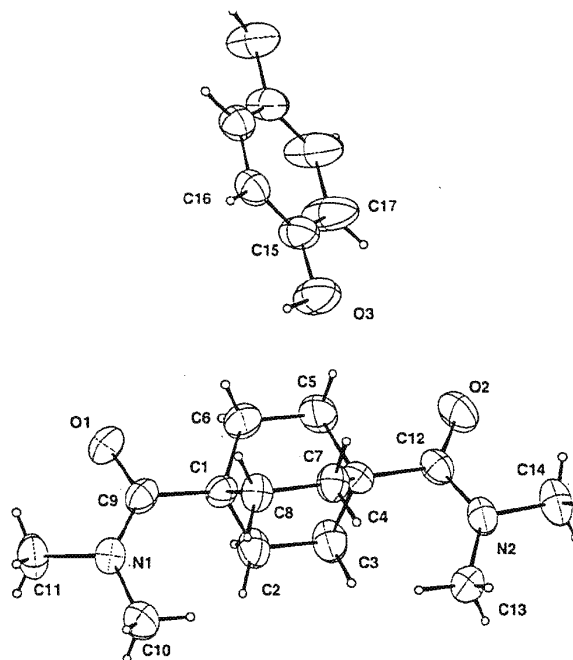


FIG. 5

Molecular structure of **7**, showing labeling scheme and 40% probability ellipsoids for atoms refined using anisotropic displacement parameters. The hydroquinone moiety occupies a crystallographic C_2 axis.

When the length of the alkyl chains in the bis-amide is reduced by one methylene unit as in *bis*-(*N,N*-dimethyl)bicyclo[2.2.2]octane-1,4-dicarboxamide **5**, different phase behavior is observed. The phase diagram (Fig. 2) that can be constructed of the binary system of hydroquinone **1** and *bis*-(*N,N*-dimethyl)bicyclo[2.2.2]octane-1,4-dicarboxamide **5** shows that instead of a 1:1 solid state complexed derivative (as is the case with the terephthalamide system), a 1:2 complex of the two components **7** is formed. There also exists a 2:1 solid state complexed derivative, **8**.

Inspection of the packing diagrams of these complex systems allows some interesting rationalization for their differing phase behavior. The molecular structure and labeling scheme of the 1:1 cocrystal **6** of hydroquinone **1** and *bis*-(*N,N*-diethylamido[2.2.2]octane-1,4-dicarboxamide **4** is shown in Figure 3. The 1:1 assembly **6** may be viewed as extended hydrogen-bonded chains with alternating hydroquinone and *bis*-amido subunits (Fig. 4 and Fig. 9a). In this structure there are two independent chains, resulting from the four independent half-molecules. The hydrogen bonds that generate the chains (along with the centers of symmetry at **1** and the C_2 axes at **4** are O11-H31...O21 [$x, 1+y, z, 2.74 \text{ \AA}, 174^\circ$] and O12-H32...O22 [$x, 1+y, z, 2.72 \text{ \AA}, 174^\circ$]. The chains are connected along the crystallographic b direction by weak C-H...O hydrogen bonds: C31-H311...O21 [$-x, 1\bar{E}y, 2\bar{E}z,$

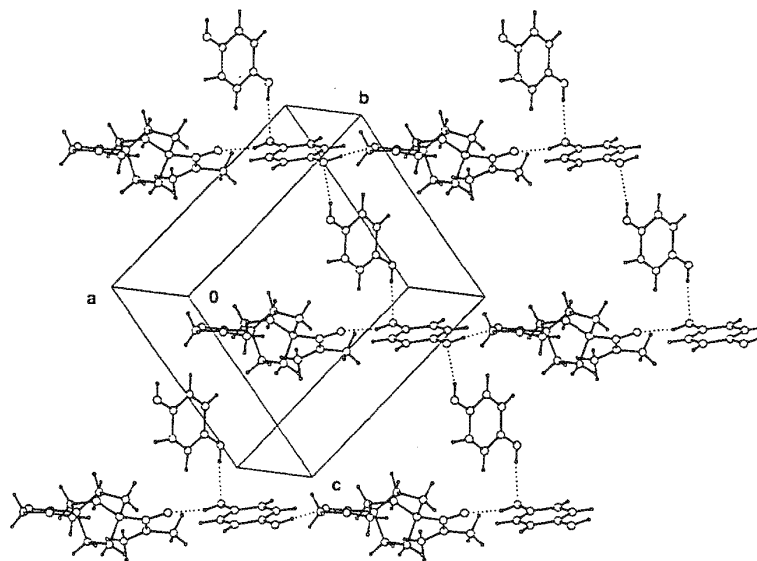


FIG. 6

Crystal packing diagram of 1:2 hydroquinone-*bis*-(*N,N*-diethyl)bicyclo[2.2.2]octane-1,4-dicarboxamide 7.

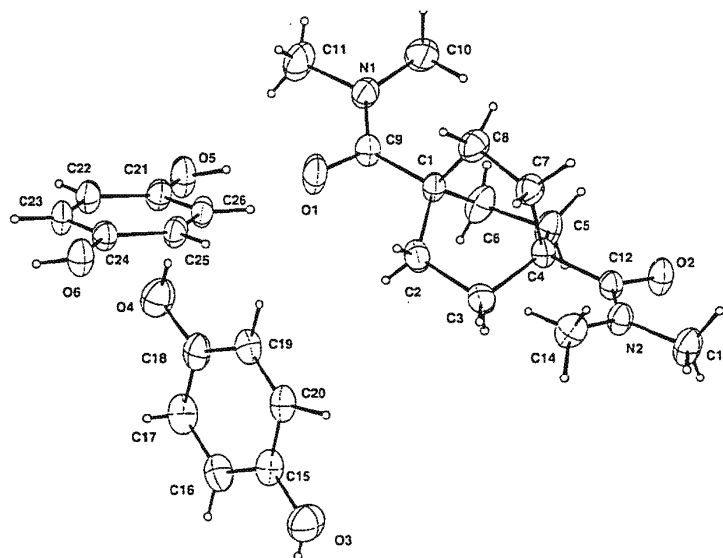


FIG. 7

Molecular structure of 8, showing labeling scheme and 40% probability ellipsoids for atoms refined using anisotropic displacement parameters.

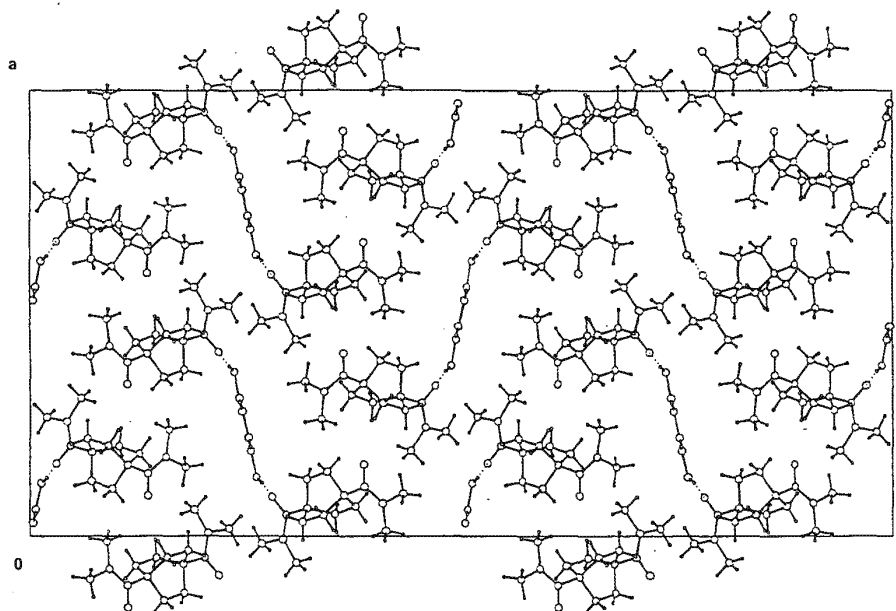


FIG. 8

Crystal packing diagram of 2:1 hydroquinone-*bis*-(*N,N*-diethyl)bicyclo[2.2.2]octane-1,4-dicarboxamide 8.

3.46 Å, 167°] and C32-H321...O22 [1-*x*, 1 $\bar{E}$ *y*, 2 $\bar{E}$ *z*, 3.38 Å, 129°]. These are not shown in the Figure.

The molecular structure and labeling scheme of the 1:2 cocrystal 7 of hydroquinone 1 and *bis*-(*N,N*-diethylamido[2.2.2]octane-1,4-dicarboxamide 4 is shown in Figure 5. The 1:2 assembly 7 has no extended hydrogen-bond network. The packing of this system features isolated hydrogen bond trimers containing two molecules of the amide and one molecule of hydroquinone (Fig. 6 and Fig. 9b). The hydrogen bond that forms the trimer (along with the *C*₂ axis at 1) is O3-H3...O2 [*x*, *y*, 1-*z*, 2.67 Å, 175°]. Weak C-H...O interactions link the *bis*-amide moieties along the crystallographic *a* direction [1/4+*x*, 1/4 $\bar{E}$ *y*, 1/4+*z*, C7-H71...O1, 3.49 Å, 130°; C8-H81...O1, 3.41 Å, 138°; C10-H101...O1, 3.48 Å, 145°]. These are not shown in the figure.

The molecular structure and labeling scheme of the 2:1 cocrystal 8 of 1 and 5 is shown in Figure 7. The 2:1 assembly 8 is in some ways similar to 6. The alternating chains of hydroquinone 1 and *bis*-amide 5 are formed by phenol-amide hydrogen bonds [*x*, 1+*y*, 1+*z*, O5-H5...O1, 2.61 Å, 163°; O6-H6...O2, 2.64 Å, 176°; Fig. 8 and Fig. 9c]. In addition, however, there are infinite chains of hydroquinone moieties that interlink the one-dimensional chains of 1 and 5 via strong O-H...O hydrogen bonds: [*x*, *y*, *z*-1, O3-H3...O6, 2.82 Å, 166°; O4-H4...O5, 2.85 Å, 172°]. There are a rich variety of C-H...O hydrogen bonds as well (seven in the range 3.29-3.50 Å). The structure appears to be dominated by the stronger O-H...O interactions, thus we will not discuss the weaker hydrogen bonds in this paper.

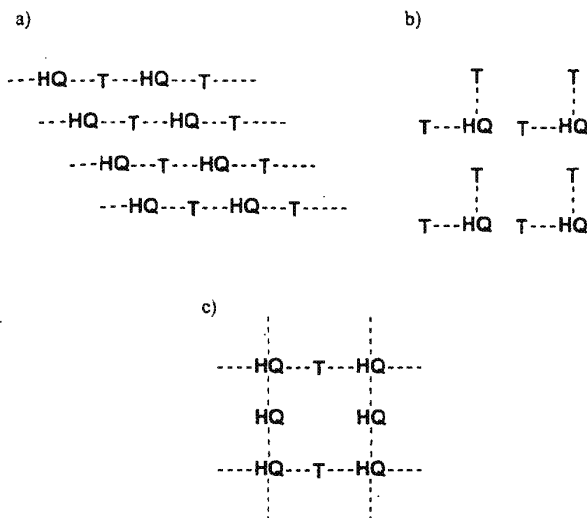


FIG. 9

Hydrogen bond assemblies: (a) extended chains, (b) isolated triads, and (c) extended networks.

The three noncovalent derivatives presented in this report represent the three possible hydrogen bonded motifs for hydrogen-bonded systems. Extended chains, isolated triads, and extended networks are each illustrated by assemblies 6, 7, and 8, respectively.

These structures are rather similar to those involving 2 and 3, [11] except that hydroquinone 1 forms a 1:1 complex with *bis*-(*N,N*-dimethyl)terephthalamide 3, defining binary tetramers rather than isolated triads as seen in the 1:2 complex of hydroquinone 1 with *bis*-(*N,N*-dimethyl)bicyclo-[2.2.2]octane-1,4-dicarboxamide 5. The difference between these systems with respect to the kinetics and thermodynamics of formation are currently under study.

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THERMAL MICROMETHODS
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by
Ludwig Kofler and Adelheid Kofler
Innsbruck, Austria

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Walter C. McCrone
McCrone Research Institute
Chicago, Illinois
U.S.A.
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Austria

THERMAL MICROMETHODS
For The Study of Organic Compounds and Their Mixtures

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I. DETERMINATION OF MICRO MELTING POINTS

A. Introduction and Apparatus

1. Scope and Advantages

The starting point and basis for the micro methods to be described is the determination of melting points under the microscope. This procedure has the advantage over other procedures that the melting process between object slide and coverslip can be observed directly with sufficient enlargement to observe the behavior of individual crystals. In addition, the entire process of heating, melting and cooling again can be minutely studied. Since many different phenomena can be observed during this heating and cooling cycle, the hot stage microscope gives not only the melting point but many other characteristic properties as well, e.g., sublimation, loss of water of hydration, polymorphic transformations etc.

The advantages of micro methods have been pointed out by many authors — first by O. Lehmann¹ many decades ago. In 1893, Goldschmidt² recommended this procedure as a simple experiment which would, with a minimum of time and material, distinguish between organic compounds. There is also no lack of publications covering apparatus and equipment for this purpose. Nevertheless, the method has been almost completely ignored by the chemist, and even today micro methods are not used in chemical work to the extent that they should be. This may be due partly to the fact that micro methods have been considered either too complicated or too inaccurate.

Only in the last few years have these methods become more common in chemical work; the statement that the melting point has been obtained by the micro method occurs more and more frequently in the literature. This statement is, of course, necessary because quite often the micro melting point differs from that determined in a capillary tube so that the two cannot always be compared. This is due partly to the differences in the two experimental methods but principally to the more accurate value obtained by the microscopical technique. Such differences are often found with hydrates, liquid crystals, polymorphic substances and materials which melt with decomposition. These factors are considered in detail in later chapters.

2. Apparatus

In most equipment the sample is heated between the slide and coverslip on the stage of the microscope with a gas flame, an alcohol lamp or electricity as the source of heat. (The last undoubtedly is the most satisfactory from a control standpoint.) The temperature may be measured with a thermometer or a thermocouple.

Many hot stages, hot stage microscopes and micro melting point bars etc., are described in the literature, i.e., Lehmann¹, Siedentopf³, Burgess⁴, Boeke⁵, Weber⁶, Jentzsch⁷, Cram⁸, Brandt⁹, Viehöver¹⁰, Clevenger¹¹, Vorländer and Haberland¹², Friedel¹³, Roberts¹⁴, Niethammer¹⁵, Klein¹⁶, Amdur and Hjort¹⁷, Kofler and Hilbck¹⁸, Weygand and Grüntzig¹⁹, Muller²⁰, Schürhoff²¹, Deininger²², L. Kofler²³, Walsh²⁴, Fuchs²⁵, Zscheile and White²⁶, Brasseko²⁷, Fonrobert²⁸, Dunbar²⁹, Halström³⁰, Shinichiro Fujise³¹, Lüdy-Tenger³² and others.

The first experiments with a hot stage microscope were carried out in 1870 by O. Lehmann¹ using a simple oil bath to heat the microscopical prep-

arations. Later he completely remodeled this equipment and constructed his "Kristallisationmikroskop". In the course of years, Lehmann with this equipment discovered and published a great many useful techniques and other findings. His work was, however, largely ignored; it is enlightening even today to read the Lehmann publications since they contain so many worthwhile ideas that have been almost completely ignored.

The micro melting point apparatus of Klein¹⁶ was somewhat more widely used. This apparatus was simple and convenient although it did not show the accuracy claimed for it (first "plus or minus 0.5°C" and later as "0.5 percent"). A check on a similar apparatus gave errors up to 7°C (Kofler and Hilbck¹⁸; Linser³³).

The apparatus of Fuchs²⁵ is designed only for reflected light studies with opaque materials and is, therefore, not useful for observations with polarized light or for determination of the refractive index of the melt.

Weygand used an apparatus developed by himself and Grüntzig¹⁹ and built by Leitz for his investigations on polymorphism. For exact temperature measurement Weygand recommends determination of the difference between the melting point of a reference substance (as a standard) and that of the test sample. He regards this procedure as the only one justified for micro melting point determinations. It is, however, difficult to find sufficient compounds of known melting point to cover the necessary temperature range. Even though many readily available chemical compounds are considered to be chemically pure, different samples show considerable variation in melting point. The use of standards in the melting point determination is more troublesome than the direct measurement of temperature at the melting point by thermometer or thermocouple.

The micro melting point apparatus described in detail below was first used with a thermocouple (Kofler and Hilbck¹⁸) although later a thermometer was substituted (Kofler²³). The apparatus consists essentially of a metal plate which can be heated electrically. An opening 1.5 millimeters in diameter in the center of the plate allows light to pass through the stage. The three-legged apparatus is fastened to the microscope stage with the help of two set screws. The preparation is shielded from air currents by a glass plate resting on a ring about six millimeters high.

When a thermocouple is used, a copper-constantan thermoelement and a millivoltmeter are employed. The junction of the thermocouple is placed in contact with the heating plate near the preparation and, therefore, always has (close to) the same temperature as the substance under examination. The "cold" junction is submerged in water, paraffin oil or sand kept in a Dewar flask. The temperature of this junction is indicated by a thermometer. The potential difference is read by a sensitive millivoltmeter which is also directly graduated in temperature units, and the exact difference in temperature between the preparation and the cold junction is, therefore, determined. A zero correction is made on the voltmeter by placing both junctions in the Dewar flask at the beginning of the experiment.

This apparatus, originally described in 1931, has proved to be quite satisfactory, so that no essential changes have been necessary. Numerous requests and inquiries have indicated a great interest in the apparatus and techniques, although the high price of the millivoltmeter often has prevented its use. To increase the use of the apparatus the thermocouple was later successfully replaced with a thermometer. This simplified hot stage, in spite of previous unsuccessful attempts, was found to be capable of high accuracy²⁹.

This apparatus has a six centimeter channel drilled below the surface of the metal heating plate to serve as a thermometer-well (Figure 1). In the past, the difficulty has always been to place the thermometer bulb in such a position that it always indicates the temperature of the preparation. Usually it is possible to achieve this only over a limited temperature range. In this range the indicated value will lie between that of the thermometer and that of the preparation, while in other temperature ranges incorrect values will be obtained. These difficulties are avoided if the thermometer is calibrated in the hot stage itself. For this purpose purified compounds of accurately known sharp melting points are used as standards. The corresponding melting points are noted on the thermometer over the necessary range and the thermometer is graduated by interpolation or, if it is already graduated, a true calibration curve is drawn. It has been possible, by this technique, to construct an accurate hot stage using a thermometer. The thermometer calibration is, of course, best only at the temperatures at which it was calibrated. Two thermometers are used, one for temperatures from about 20°C to 240°C and one for the higher temperature ranges covering from about 70°C to 350°C.

In this way a micro melting point apparatus has been constructed (Figures 1-3) having the same accuracy as the usual macro apparatus. Use of the thermocouple makes it possible to record somewhat more rapid rises in temperature and this is a distinct advantage in some cases, although here again an expensive millivoltmeter is necessary. Therefore, the thermometer hot stage is the more popular apparatus today.* The apparatus described by Halström³⁰ and Shinichiro Fujise³¹ is similar to the Kofler stage. Since this apparatus was not available during and immediately after the war, Lüdy-Tenger³² reported experiments to produce a similar electrically heated microscope hot stage. In honor of the Technikums Burdorf this stage is called the "T. B. Block"; it is described as an apparatus with a variety of useful purposes and not simply a copy of the Kofler apparatus. The essential difference between the T. B. Block and the Kofler apparatus is the presence of water cooling in the former. This complication makes the hot stage more difficult to operate but was included so that the block could be placed immediately on the microscope stage and rotated. However, in order to rotate the T. B. Block it is necessary first to remove the electrical and water connections; it is also, of course, impossible to rotate this stage at higher temperatures. Since the thermometer of the T. B. Block is not calibrated, several standard curves must be run in order to obtain corrected values.

3. Kofler Hot Stage

(a) Description and uses

The techniques described below are written with particular reference to the Kofler thermometer hot stage. They apply to and can be used, however, with other micro melting point stages, provided these permit microscopical observation of the preparation between a coverslip and slide with

*A completely standardized apparatus of this type is available either through A. H. Thomas Company, Philadelphia, Pennsylvania or Hacker, Inc., New York City. The photographs shown in Figures 1-3 were made available through the courtesy of Dr. H. K. Alber of A. H. Thomas Company.

transmitted light and with exact temperature measurement.

Adapting the Apparatus to the Microscope

The micro melting point apparatus can be used on any microscope that possesses a metal stage; needless to say, a hard rubber stage is unsuitable, since it would not be stable at high temperatures.

The hot stage is set upon the microscope so that the pins at the ends of two moving arms engage the openings provided in the microscope stage for the usual spring clips. The set screws can be loosened for free movement of the arms and the hot stage can then be centered on the microscope stage so that the central opening is co-axial with respect to the microscope objective. Once this position is found it is possible, while observing the opening through the microscope, to tighten the set screws on the movable arms. A working space free of air drafts should be chosen.

Choice of Optics

Inasmuch as the hot stage is covered with a glass plate, only objectives having a working distance greater than about seven millimeters can be used. This permits a maximum and generally adequate magnification of approximately 220X. As a rule it is recommended that observations of the micro melting point be made at 80-100X. In spite of the glass plate covering the hot stage, the objective becomes somewhat heated during use, although after many years' use under these conditions the usual objectives show no apparent damage. Special heat resistant objectives with uncemented lenses are, however, available from the microscope manufacturers.

Transmitted light is usually best for microscopical observation of the melting point, and polarized light with crossed polars is often of advantage. This is achieved using the hot stage on the stage of a polarizing microscope or by fitting a polarizer and analyzer into a simple microscope. Polaroid filters are very satisfactory for this purpose. (The polarization filter developed by Bernauer and manufactured by Zeiss is used in the Kofler equipment as sold in Europe. In the U.S. the Kofler hot stage microscope available through A. H. Thomas is equipped with Polaroid filters.) Polarization filters are very sensitive to heat, hence should not be placed near the focal spot of the illuminating beam.

Introduction of the Thermometer

Just below the surface of the stage the hot stage is provided with a thermometer well approximately six millimeters in diameter. A thermometer with the proper temperature range inserted into this channel is held tightly in position by a spring clamp built into the wall. This spring may cause some difficulty in introducing the thermometer, but judicious treatment with rotation of the thermometer will serve to insure correct placement without damage. The thermometer is introduced to the end of the well since it was calibrated in this position.

The mercury column in the thermometer should be checked to make sure that it is continuous. After the thermometer has been correctly introduced into the apparatus, the metal guard is screwed tight to protect the thermometer during the determination, and also to duplicate the conditions under which the stage was calibrated. Dependable temperature measurements

can be made only if all of these precautions are observed.

Heating

The hot stage is heated electrically using line voltage controlled by a variable slide wire resistance. The initial heating rate may be quite rapid, although in the region of the anticipated melting point the temperature rise should not exceed four degrees per minute. As a guide in future determinations, the resistance is roughly calibrated with a temperature scale. For example, to determine a melting point at about 150°C, the resistance is set for that temperature. In this way, when the current is first turned on, the temperature will rise very rapidly, but will rise less rapidly as a temperature of about 150°C is reached. This scale is calibrated in such a way that the temperature rises about four degrees a minute at the indicated temperature. Because of fluctuations in line voltage it is often not possible, however, to depend on this calibration. It is, then, necessary to check the rate of temperature rise in the desired range using a stop watch so that this rate may be maintained at very nearly four degrees per minute in the region of the melting point.

After a little experiment with the hot stage, it becomes quite easy to adjust the resistance in order to obtain the desired rate. In some cases as, for example, with compounds which decompose or sublime, it might be desirable to take a quick melting point with a high rate of heating to determine the approximate melting point. The determination could then be repeated with very rapid heating to a point close to the melting point at which time the preparation could be introduced and the heating rate changed to the prescribed four degrees per minute.

It is also true that the melting point of a hydrate or of a substance which decomposes when melted will depend on the rate of heating at the melting point. For these reasons, then, it is advisable in all cases to adhere strictly to the empirical heating procedure in order to obtain reproducible results. If, in special situations, it is necessary to vary the rate of heating at the melting point so that it is either faster or slower than four degrees per minute, this fact should be recorded with the melting point data.

If a number of melting point determinations are to be made, the cooling time can be shortened by placing a cold metal block on top of the stage. A special aluminum cooling block about 25 millimeters thick and 80 millimeters in diameter is available with a handle so that it may be moved easily to and from the hot stage. Occasionally a brass cube about 25 millimeters on edge is also used for cooling. This is placed on top of the heating stage at one side of the preparation so that the effect of the cooling process can be observed through the microscope. It may be useful to have cooling blocks of various sizes in order to be able to vary the cooling rate. Such observations are especially important in the use of the fusion method (described below).

Moving the Preparation

During the determination of melting points, mixed melting points, eutectic temperatures, indices of refraction etc., it is usually sufficient to place the preparation on the hot stage with one particular region in view and to continue the operation without moving the preparation. In other

cases, especially in observations of polymorphism or of the fusion method, it is necessary to move the preparation during the experiment. For this purpose the round glass cover plate must be lifted and the object slide moved by hand or, at higher temperatures, with tweezers. This should not, of course, be done just before a temperature reading.

Several devices are available to make possible moving of the preparation with the glass cover plate in place. One of these devices consists of two screws in a metal ring replacing the ordinary ring which supports the upper glass plate. The two screws can be used to manipulate the preparation in any direction. Another device utilizes a single screw with a spherical or cylindrical joint in the metal ring support. With any of these devices it is essential to make certain that the microscope slide carrying the preparation is not lifted from the stage, since this would cause any temperature measurements to be inaccurate. To prevent this the slide is usually placed loosely in a small metal frame connected to the guide screws. Figure 1 shows the hot stage equipped with the device, although in this illustration a metal block is shown rather than a microscope slide. This arrangement has been used by Kofler and Brandstätter for micro molecular weight determinations (page 58).

The Microscopical Preparation

The melting point of a sample is determined on a small sample placed on a microscope slide and covered with the coverslip. Samples consisting of large crystals and aggregates must, however, be finely ground to avoid an erroneously high value for the melting point. This can be done either with a small mortar and pestle or between two object slides. A small amount of the finely powdered sample is then placed on a clean slide, covered with a coverslip and distributed by sliding the coverslip under slight pressure. Usually only 0.1 mg samples should be used, although with some samples, especially highly volatile solids, it may be of advantage to have a larger amount. In general, the amount of sample will depend on the nature of the substance and the purpose of the experiment. More complete discussions on this point will appear later, especially in the Exercises.

For many purposes it is often better to place a coverslip on a microscope slide, place the powdered sample at the edge of the coverslip and heat until the sample melts. The liquid sample will then flow under the coverslip. This technique will usually give a final preparation containing fewer air bubbles. The viscosity of the melt and the cleanness of the glass surfaces will both also affect the number of air bubbles in the melt. In general, of course, the presence of air bubbles is an aesthetic consideration although it may affect the tendency toward nucleation of the melt on cooling since nucleation often occurs more readily at an air-melt interface.

The microscope slide used is about 26 by 38 millimeters by one millimeter, just one-half of the standard microscope slide. The thickness should be approximately 1 mm and slides varying a great deal from this figure should be avoided so that the same rate of heat transfer to the sample can be maintained. In general, the sample need not be specially dried, since the small amount of moisture present in such a small sample will be easily lost in the early stage of heating. The preparation is placed on the hot stage and moved about until a suitable field for observation is found. It is then covered with a "bridge", which is constructed from a glass slide about 50 millimeters long having the ends supported by turning under these edges or with two strips of glass about three millimeters thick (Figure 3). This

bridge completely covers the preparation without touching it. The use of the bridge is not always necessary but since it is so often useful, it is worthwhile to form the habit of using it. The purpose of the bridge is to maintain a more uniform temperature in the preparation especially in the case of high melting points. If the substance has a tendency to sublime, the bridge will prevent sublimation onto the center of the upper glass plate. After the preparation and the bridge are in place the large metal ring and glass cover plate are placed on top of the stage.

With very volatile materials a very slow rise in temperature may cause complete sublimation before the melting point is reached. In these cases the preparation is heated very rapidly up to the immediate region of the melting point.

If this precaution is not sufficient, the preparation can be completely sealed by surrounding the edge of the coverslip with a suitable cement. The cement which is used should harden quickly, not dissolve any of the crystals, and maintain a good seal at the temperatures reached. No single material can be recommended which will fulfill all of these conditions, although picene, rubber cement, carboxy methylcellulose, polyvinyl alcohol, cellulose acetate and even Canada balsam have been used. Cements which melt at high temperatures should usually be avoided since they may dissolve the sample or may lower the melting point of the sample. These cements work best in preparations in which the sample has been fused and then allowed to resolidify. This procedure for sealing preparations is very useful in the thermal studies of volatile materials.

The melting points and the refractive indices of volatile substances can also be determined in Fischer's microbulb (page 17). Melting point determinations in a vacuum or in an inert gas* are also possible in the microbulb or even in a closed capillary tube such as that used in the micro determination of molecular weight (page 58).

(b) Accuracy of the temperature readings

The advantages of microscopical determination of melting points and other constants are becoming better known. Many publications (³⁵⁻⁴⁴) have pointed out the time-saving, simplicity and accuracy of microscopical methods for analytical purposes. For testing of costly materials or for substances available only in small quantities, e.g., hormones and other natural products, the advantages of the microscope are self-evident.

The accuracy of the temperature values using the Kofler hot stage with the same operator on successive operations is usually $1/2^{\circ}\text{C}$, often less and never more than 1°C . The Kofler hot bar, on the other hand, is accurate to $2-3^{\circ}\text{C}$. The limit of error is greater when the data of different operators are compared; this is not, however, the fault of the hot stage but due, instead, to variations in technique. It is essential, in order to obtain good results, that a carefully standardized procedure be used. Variations in slide thickness, amount of test sample, rate at which the temperature rises and presence of the glass cover bridge can cause inaccuracies in the temperature data.

*A special ring fitted with tubing for the introduction of an inert gas is available through A. H. Thomas Company; it is interchangeable with the regular metal ring used to support the glass plate on the hot stage.

Probably the most important variable is the rate at which the hot stage is heated. This is especially true for substances which decompose since the longer the time the sample remains at a high temperature the lower the observed melting point will be. It is also very difficult to obtain check values with an impure compound since melting will not be sharp.

(c) The hot stage microscope

Almost any microscope can be used as a hot stage microscope as long as it has a stage that will not be damaged by heat and as long as the substage can be lowered so that it too will not be damaged. In general, however, most microscopes are more complex than necessary for use with the hot stage.

For this reason, both suppliers (Reichert through H.J. Hacker in New York and A.H. Thomas in Philadelphia) furnish a special low cost microscope for use with the Kofler hot stage. The Reichert instrument (Figure 2) has the hot stage solidly mounted on the microscope, no fine adjustment is used since the magnification seldom exceeds 80-100X. The polarizer and analyzer are Polaroid® with the former mounted below the substage iris diaphragm and the latter mounted in the eyepiece. In general, the simplest and least costly microscope can be used with the Kofler hot stage.

4. Kofler Micro Cold Stage

With increasing use of the microscope hot stage there has arisen a need for a simple apparatus for microscopical studies at low temperatures. Since the beginning of this century a large number of such stages have been described: Boeke⁵, Wahl⁴⁵, Vorländer, Selke and Ereiss⁴⁶, Mason and Rochow⁴⁷, Chamot and Mason⁴⁸, Erk⁴⁹, Leslie and Heuer⁵⁰, Harvalik⁵¹, Tschamler⁵², Hocart and Monier⁵³. Most of these cold stages use either liquid air or carbon dioxide snow as cooling agent. In comparison with observations with the hot stage, observation with the cold stage is considerably more difficult and requires more complex equipment and more careful handling.

The Kofler cold stage⁵⁵ is based on experience with the hot stage^{18,23,54}; it is an instrument similar in general appearance to the hot stage and can be used without special preparation. The general manipulation of sample and cold stage accessories is identical with the above-mentioned use of the hot stage. The cold stage extends the range of observation to low temperatures; however, both the hot stage and the cold stage respond very slowly near room temperature. In order to construct a microscope stage with positive action near room temperature, the stage must include both a heating and cooling unit.

The Kofler cold stage (Figure 3) is fitted to the microscope in the same manner as the Kofler hot stage. It consists of two chambers: one for the preparation and another beneath this chamber for cooling purposes. The heating unit is also placed in the lower chamber. The upper chamber in which the slide-coverslip preparation is placed is covered by a glass plate supported on a metal ring. The distance from the preparation to the top surface of the cover plate is about 6 mm. This is sufficient for the normal optical system giving an enlargement of 80-100X although in special cases enlargement of over 200X is possible even with this working distance. Light is transmitted through both chambers of the stage by a small central opening. Cooling is achieved using cylinders of carbon dioxide. The gas is led to the

stage with pressure tubing and allowed to expand into the lower chamber through a small opening. The temperature is adjusted by controlling the flow of carbon dioxide; temperatures as low as -55°C can be maintained.

The heating element in the lower chamber is so adjusted that the maximum temperature possible is 80°C . This is sufficient to give positive cooling or heating action in the room temperature range. A thermometer is used for temperature measurement in precisely the same way as with the hot stage. The temperature scale is calibrated with known melting points or other known temperature changes. The use of the cold stage corresponds generally to the use of the hot stage. There is, however, the complication of condensate on the cold glass cover plate to contend with. The glass plate should be removed as infrequently as possible to avoid condensation of moisture on the interior of the upper chamber. The tendency of most liquids to supercool on rapid cooling is also a difficulty since all remedies except patience require removal of the cover plate. Pressure on the coverslip or scratching the edge of the preparation are often effective in inducing crystallization. In general, of course, a given preparation may be expected to crystallize most easily when the degree of supercooling is slight since under these conditions the temperature is higher and the mobility of the molecules is greater. It might be worth considering a minute opening in the metal supporting ring through which a long pointed needle might be introduced to scratch or seed the liquid. This would eliminate the necessity of lifting the cover plate.

If the cover plate has to be removed during a low temperature experiment, it is always best to slide it to one side rather than lifting it vertically. Carbon dioxide is heavier than air and tends to remain in the chamber unless convection currents help to displace it. Sliding of the cover plate, then, is designed to prevent such convection currents. Condensation of moisture on the upper surface of the cover plate is not troublesome if a thin layer of glycerin is applied to the area under the objective before cooling is begun. If, for any reason, condensation of moisture within the cooling chamber becomes a problem, the top of the coverslip and the bottom surface of the glass cover plate should both be coated with a thin layer of glycerin.

With the cold stage described above it is possible to closely control the temperature over any desired small temperature interval. The temperature can be raised or lowered in the range below room temperature in the same manner as in the range above room temperature using the hot stage. Analogous observations can be made on aqueous solutions or organic liquids which crystallize in the range down to -55°C . The melting process, polymorphic transformations in either the solution or solid phase, binary liquid systems or binary mixed fusions, in general, can all be studied as discussed in later chapters.

5. Kofler High Temperature Hot Stage (750°C)

The micro determination of melting points is usually restricted to organic compounds melting below about 400°C , thus imposing a restriction so far as inorganic compounds are concerned. The reason for this lies in the fact that most high temperature stages are inconvenient and awkward. Many require special long working distance objectives or an auxiliary lens system. Frequently also a given arrangement was designed for a special purpose and is not, therefore, useful for more general purposes.

The literature includes a variety of hot stages for high temperature use: Burgess⁵⁶, Oberhoffer⁵⁷, Wright⁵⁸, Endell⁵⁹, Fieldner, Selvig and Parker⁶⁰, Stadnichlenko⁶¹, Eitel and Lange⁶², Curtis⁶³, Swinden, Howie and Chesters⁶⁴, Chalmers, King and Shuttleworth⁶⁵, Williams⁶⁶, Cech⁶⁷, Grabar and McCrone⁶⁸.

The Kofler high temperature hot stage (Figure 4) can be used up to temperatures of 700°C and requires no special optics. The temperature range up to 700°C was chosen because a thermometer could still be used at this temperature.

The hot stage proper consists of a plate of heat-treated steel with a diameter of about 55 mm. This is placed in an aluminum container having a 75 mm diameter and about 30 mm high and supported therein at 3 points so that an air space of not less than 7 mm remains between them. The air space acts as a heat insulator and also as a help in uniformly heating the surface. The hot stage is fastened to the stage of the microscope with two binding screws. An aluminum plate extends across about 5.5 mm above the entire hot stage; it possesses a glass window in its center.

Heating is with low voltage current from a variable transformer. The power consumption is no different from the ordinary hot stage. Two thermometers are used with this apparatus: a Supremax glass thermometer for the range from 300-500°C and a quartz thermometer from 500-750°C.

The hot stage has a central opening for passage of transmitted light. Observations are made at 80-100X just as with the ordinary hot stage. Slight displacement of the stage is possible in order to choose the best field of view. To avoid overheating of the objective the bodytube is raised at all times except for actual observation, or the thermal microscope described below may be used. The bodytube on this microscope swings to one side out of the heating zone when not in use.

6. The Thermal Microscope

Work with the hot stage and cold stage has shown the desirability of modifying the usual microscope in order to simplify the manipulation of both the microscope and the stages. The principal change is reversing the microscope base and the bodytube so that these two elements are on opposite sides of the bodytube support. Another disadvantage of the usual microscope is the necessity of raising the bodytube to introduce samples or cooling blocks. By allowing the bodytube to swing to one side, these and other disadvantages are overcome.

The thermal microscope (Figure 5) possesses, in place of the usual base, a small case containing the illuminator assembly in the form of a low voltage lamp, an iris diaphragm, red filter and Polaroid filter. Besides this, the case also includes a variable transformer to feed both the light source and the hot stage. The knob for the variable transformer is placed on the left side and an indicating scale and pointer on the front show the actual voltage.

The support for the bodytube is mounted on the side toward the observer. The bodytube is inclined and can be swung to one side by loosening a set screw at the top of a rotation mount.

Either of the hot stages or the cold stage can be fastened to the thermal microscope. Current connections are made from the top of the illuminator case to the bottom of the stage. There is no further change in either the microscope or the hot and cold stages. To introduce a specimen or to ap-

ply a cooling block the bodytube is quickly swung to one side. By this means the stage is available facing the observer in a convenient position for all necessary manipulations. After swinging the bodytube into position only a slight adjustment of the focus should be necessary.

The use of iris diaphragms, filters and Polaroid is controlled by knobs on the right side of the illuminator case. On the left side, there is also a switch controlling the line voltage; the line voltage enters a plug-in connector at the bottom of the case.

B. Behavior of Substances

1. Melting Point Determination

The melting point can be observed in two ways under the microscope, *i.e.*, either by continuous heating or by an equilibrium method. In the continuous heating method of determining the melting point the temperature is allowed to rise continuously past the melting point of the substance. At the first instant of melting the finest crystals become liquid and they are followed a moment later by the larger crystals. The larger crystals will become rounded at the edges and corners as they gradually melt. One such process is illustrated in Figure 4 for anesthesine. Melting has not yet begun at 89°C, but starts at 90°C, continues at 90.5°C and is complete at 91°C. The value of 90.5°C is chosen as the best value for the melting point.

With the equilibrium method of determining the melting point, heating of the hot stage is stopped before the preparation is completely melted. In this way the partially melted preparation can be maintained at equilibrium at a constant temperature. Slight cooling will cause the crystals to grow whereas slight warming will cause the crystals to melt. Through several repetitions of the process, the equilibrium melting point can be located with great accuracy. The crystals can be made to grow very slowly or to dissolve with temperature changes so slight that they are hardly detectable on the thermometer. Skill in controlling the heating and cooling rate by manipulating the rheostat to give an accurate equilibrium melting point is obtained only with practice.

To make the determination easier, slightly more material is used for the observation of melting points with the equilibrium method than with the continuous heating method.

The observation of crystallization or fusion is also easier when crossed polars are used. In many cases, especially in the formation of very thin platelike crystals, *e.g.*, fatty acids, the difference in refractive indices between the crystals and the melt is so low that the crystals can hardly be distinguished without crossed polars. It is often possible to achieve good visibility of thin crystals or crystals with low contrast by using parallel polars. This may be especially valuable in studying an enantiotropic transformation in a system such as camphor where the high temperature modification is isotropic.

2. Sublimation and Sublimation Temperature

Only a few substances remain unchanged during heating to the melting point. Most compounds show some evidence of sublimation during heating and some substances change so significantly during heating that they possess an entirely different appearance by the time the melting point is reached.

Often the substance sublimes partially or even completely from the object slide to the underside of the coverslip. Sublimation can occur at a temperature just below the melting point or in some cases many degrees below the melting point. For many substances the appearance of the sublimate is characteristic and very valuable for identification. Crystals, droplets or mixtures of the two can be present in the same sublimate. The droplike sublimate is more accurately defined as liquid condensate, and usually forms at a temperature just below the melting point. The crystalline sublimate may have any habit from very fine needles or equant particles to very perfectly formed large crystals. If both droplets and crystalline material appear the droplets are always absent in the immediate neighborhood of the crystals and indeed tend to disappear in other regions with resulting growth of the crystals. Occasionally the droplets crystallize as a result of spontaneous crystallization or through seeding by one of the crystals (Figure 8).

Very volatile compounds such as caffeine and camphor sublime readily to give a continuous patchwork layer of crystals resembling a paving block or honeycomb design. The latter is especially characteristic of the isotropic crystalline phases.

The literature covering the tendency for sublimation of organic substances shows many contradictions and inaccuracies, because most comprehensive works and handbooks give a single sublimation temperature without reference to the method by which that value was obtained. It is now known that the sublimation temperature depends, to a great extent, upon the experimental procedure.

Among the conditions which should be specified are temperature, pressure, time, sublimation distance and nature of the surface from which the material sublimes and on which it collects (Spielen⁷⁰).

In the literature, there is often reference to the sublimation tendency of a substance at a given characteristic temperature, without other experimental conditions being specified. For example, theobromine is reported to sublime at 290°C (in other sources at 295°C). This property is given along with a melting point of 351°C as if such a temperature were a physical constant. The slight tendency for sublimation of theobromine is actually significant, but under suitable experimental conditions theobromine can be sublimed at a much lower temperature. For instance, Kempf⁷⁰ reports sublimation at 91°C and Eder⁷¹ reports sublimation at 125 and 128°C.

Even in handbooks and in comprehensive volumes where statements covering the experimental conditions can be found, these conditions vary considerably so that the sublimation temperature of a compound cannot be found relative to that determined for any other compound. The figures refer partly to normal and partly to reduced pressure as well as various other experimental conditions.

In some cases, only weighable amounts are considered evidence of sublimation while in other cases at random microscopic amounts are sufficient. Naturally only those values which were determined under similar experimental conditions are strictly comparable. However, there are, indeed, a number of works in which the ease with which various substances sublime has been determined under comparable conditions; each author, however, uses a different experimental procedure so that sublimation temperatures by different authors should not be compared.

For this purpose the average sublimation temperature for compounds melting over successive 20°C intervals is plotted against the corresponding melting point. The melting temperature is plotted as abscissa and the subli-

mation temperature as ordinate (Figure 9). The resulting curve is a straight line, the slope of which is less than 45° measured from the abscissa. The difference between the melting point and the sublimation temperature can be easily recognized by drawing in the 45° diagonal. This corresponds to the limiting case when no sublimation is observed before melting. It is also apparent from the diagram that with a rising melting point the difference between the melting point and the sublimation temperature increases steadily. For example, for substances with melting points of 50, 100 and 200°C the average temperatures for the beginning of sublimation are, respectively, 41, 78, 115 and 152°C with correspondingly increasing differences of 9, 22, 35 and 48°C .

The curve in Figure 9 for the average volatility is continued only to 260°C because, in our tables, the number of compounds melting above this temperature is too small to give a reliable average value.

Many substances deviate from these average values. For example, compounds melting between 134 and 135°C show an average sublimation temperature of 104°C according to the diagram. The table, however, shows a deviation from this average value of 44°C in one direction for sorbic acid and 2,6-dimethylpyrone both with a sublimation temperature of 60°C and a deviation of more than 30°C in the opposite direction for ortho-sulfobenzoic acid since no sublimation occurs below the melting point for this compound.

The tendency of a given compound to sublime is very important in microscopical work since small pure samples of such compounds can be quickly prepared by sublimation and used for micro melting point or refractive index determination. The question of purity is too seldom considered. It is of obvious importance in the use of the Identification Tables in Part II of this book to have determined the original data on pure compounds and to have pure samples of unknowns for identification. Few compounds are pure as received and many compounds decompose slowly on standing as a result of action by air or sunlight. Colorless o-aminophenol, for example, assures a dark red surface discoloration on standing for a short time. A small sample of the pure material can often be quickly prepared at any time by sublimation.

As a rule, the best sublimation temperature is about 10° below the melting point. The sublimation may be carried out by subliming from one heated slide to a second slide held above it, from a slide to a coverslip resting on a few milligram of the substance at one end of a slide, from a depression in a slide to a coverslip covering the opening, from a slide to a coverslip supported on a glass or metal ring (10-12 mm in diameter and 2-5 mm high) above the slide, or in a well in a metal block to a coverslip covering the opening.

The greater the evaporating surface the more readily a given substance will sublime, hence each sample should be finely ground before heating.

The use of glass or metal rings as support for the coverslip has the advantage that the thickness of the ring and therefore the sublimation distance can be varied at will. While a short sublimation path is best in general to achieve rapid sublimation, a longer path (thicker ring) is best for separation of mixtures by sublimation.

There is no general rule to cover the choice of temperature of the condensing surface or the temperature difference between the sublimation source and the condensing surface. The conditions chosen depend on the nature of the substance to be sublimed and the purpose of the sublimation. A cool condensing surface is best for a high yield of sublimate, but a warm

surface is better if large well-formed crystals are obtained. It is especially important in the latter case to preheat the condensing surface before sublimation begins to decrease the number of crystal nuclei formed on the condensing surface. Waddington⁷², as early as 1867, discussed the importance of these factors. In some cases it is necessary to seed or scratch the condensing surface to cause condensation as crystals rather than liquid droplets⁷³. This is especially important when sublimation of an unstable polymorphic form is desired.

A cold condensing surface is more likely to lead to condensation of an unstable polymorphic form. Such a surface may be formed using the Kofler sublimation block by placing a wet square of filter paper or a small aluminum block on the upper surface of the coverslip. In this way, the unstable form III of p-oxybenzoic acid methyl ester (m.p. 110°C)⁷⁴ is obtained whereas, without cooling, crystals of the stable form I (m.p. 126°C) are obtained⁷⁵. The stable form is obtained in either case if the coverslip is first seeded with crystals of this form.

The methods described above are quite sufficient for the purpose of microscopical identification of a substance after separation of the desired component from a mixture. The use of the hot bar rather than the hot stage may be more convenient for sublimation since any desired temperature may be more quickly obtained and since several sublimations at different temperatures may be carried out simultaneously in order to find the most suitable conditions. The progress of sublimation may be observed using a band magnifier so that a microscope is not necessary.

There have been many different sublimation arrangements described in the literature, many of which are useful in special cases. Mayrhofer⁷⁶ has described an arrangement for simultaneous determination of the melting point and sublimation temperature. Kempf⁷⁰ has constructed an apparatus useful for slow sublimation over long periods of time and with slow rates of temperature rise. The sublimation apparatus of Fischer⁷⁷ is a large block having many cylindrical depressions of different depths. Tubes containing the substance to be sublimed are placed in these holes and the tubes are closed with coverslips. A vacuum may be applied to each tube and cooling may be used if needed.

Fischer⁷⁸ sublimes the residues after evaporation of extracts in a steplike tube which is about 4 cm high and about 2 cm wide at the top. A step about 3-10 mm from the bottom acts as a support for the condensing surface. The tube is heated in a hot bath whose temperature is measured.

Sublimation on the microscope stage may be performed in the following manner: The substance to be sublimed is placed between two small glass strips which form a support for a coverslip. The sublimate formed during heating can be continuously observed. The sublimation block described by Fischer is a good one for many purposes. It is a low cylinder with a deep depression and a small opening in the center to allow light to pass through for microscopical observation.

Kofler and Dernbach⁷⁹ have constructed a vacuum block for vacuum sublimation under the microscope. The sublimation temperatures observed with this apparatus are naturally lower than the temperatures observed at normal pressures.

Microsublimation is most useful for purification of small samples for micro melting point determination since other purification techniques require larger amounts of material. The sublimation of ferulic acid from Asa-fetida is a good classroom example of microsublimation. Using the hot stage

microscope it was shown that the sublimate from *Asafetida* is umbelliferone⁸⁰. In a similar way the sublimate from *Cetraria Islandica* has been identified as fumaric acid rather than "Lichesterinsaure"⁸¹.

These methods have been applied by a number of authors to the identification of allaloids, narcotics⁸², and preservatives^{83,84}. The greatest number of substances has been studied by Mayrhofer⁷⁶ who used microsublimation for the detection and estimation of the common medical remedies and poisons.

It is not always possible to obtain a well crystallized pure sublimate starting with an impure mixture since two or more components may sublime together. In these cases, it is necessary to use extraction methods or some other means of concentrating the desired component before final sublimation. Markovic⁸⁵ has used solvent extraction and microsublimation as a means of isolating and identifying anthracene derivatives in drugs.

The mechanism of crystal growth from supersaturated vapors can be observed and studied during microsublimation. Unusual spiral layer growths on the surfaces of inorganic crystals have been known for some time⁸⁶. Brandstätter⁸⁷ has observed a similar type of layer growth during sublimation of organic compounds such as 2,3-benzofluorene, carbazol, phenanthrene, benzoic acid, borneol, bornylchloride etc. in the hot stage. Two examples of layer growth are shown in Figures 10 and 11.

3. Crystallization of the Melt

Most substances crystallize spontaneously from the melt on cooling. However, organic melts are characterized by the degree of supercooling before crystallization. As a general rule, compounds crystallizing in a platelike habit (e.g., anthracene, phenanthrene, fluorene, fatty acids, diphenyl, naphthalene, β -naphthol etc.) supercool less readily than compounds that crystallize as needles.

The slide-coverslip preparation for study of thin crystalline films is usually cooled rapidly by placing on a cold surface. With some compounds it is better to cool rapidly on a cold metal surface and with others slow cooling on a nonconducting surface is best. The latter method should be used when rapid cooling gives a large number of crystal nuclei such as nicotinamide or β -acetyl naphthylamine. Rapid cooling with ice is frequently useful especially if an unstable polymorphic form of a compound melting point below 120°C is desired (see page 80).

Spherulitic crystal growth from the melt is very common. These crystal aggregates are made up of needles growing from a single center and may be spherical or disclike; if so large the coverslip and slide limit their growth. In some cases, the needle units may be so small that the spherulitic nature of the aggregate appears only with crossed polars; all spherulites then show a black cross. When crystallization is complete the spherulites, having grown together, show polygonal grain boundaries with the preparation as a whole showing a mosaiclike pattern. Many spherulites show a concentric pattern of shrinkage cracks on cooling.

Although as a rule, the more strongly a substance is supercooled before crystallization the finer the resulting individual crystals; each compound varies in this tendency to form large or small crystals. The slower the rate of crystal growth the larger resulting crystals⁸⁷.

Some substances will not crystallize spontaneously if their melt is cooled too low^{87,88}. The tendency to crystallize spontaneously increases as

the temperature of the melt falls below the melting point, but below a characteristic temperature for each compound the tendency to crystallize decreases. If this optimum temperature is passed rapidly enough the high viscosity of the supercooled melt will prevent nucleation and crystallization. The melt may, in this case, solidify to a glass and may even crack spontaneously like a rapidly chilled glass. Crystallization can then be initiated only by rewarming (e.g., luminal).

4. Melting Point Determination of Highly Volatile Substances

(a) Use of large coverslips (21 x 28 mm)

There are two difficulties in the determination of melting points of compounds which sublime readily, especially if the melting point is above 200°C. In the first place, the sample may volatilize completely before the melting point is reached and, in the second place, the sublimed sample condensing on the glass bridge or cover plate may make it impossible to see the sample.

The simplest remedy for this situation is the use of a large coverslip 21 x 28 mm and a correspondingly larger amount of sample. This method works very well in preventing condensation of material on the cover plate and glass bridge even for compounds such as barneol, camphoric acid imide, p-bromobenzoic acid and 8-naphthylamine hydrochloride. It is also a good idea, however, even with a large coverslip, to place the preparation in the hot stage only 10-15°C below the melting point. This method can often be used even for the determination of refractive index of the melt (see page 28).

(b) Sealing the edge of the coverslip

Another means of preventing loss of sample due to sublimation is to seal the edges of the coverslip using a cement⁵⁴. In some cases the melt of a second substance may be used to enclose the sample. For example, a mannite melt can be used to seal in a preparation of camphor. (Brandstätter, see page 58). Silicone grease is a good inert sealing material for general use⁸⁹. The coverslip may be supported on a metal ring, 3-4 mm high and about 25 mm in diameter (Figure 9). Different ring sizes may be used depending on the purpose and coverslip size. The upper side of the ring has a recess into which the appropriate coverslip just fits. The coverslip takes the place of the glass bridge usually used.

The smallest ring with a 12 mm coverslip is used for highly volatile compounds. The metal ring is sealed to the slide with a thin layer of silicone cement. The sample is placed in the center of the slide, covered with a small coverslip and the ring is sealed to the slide around the preparation.

Since the arrangement here is slightly different from that ordinarily used with the hot stage, it was necessary to compare the melting points obtained by these methods on pure standard compounds. The results show that there is no difference between the results obtained with the glass bridge and those obtained with the silicone chamber up to a temperature of about 200°C. However, at higher temperatures there is a difference amounting to about 1-2°C at 215°C and about 2-3°C at 240°C. It is desirable to determine this correction as a function of temperature for the system to be used. In the

determination of refractive index of the melt the calibration may be neglected since the method is reproducible with high accuracy and since the method is, in any case, a relative one.

The silicone chamber is especially useful for the examination of mixtures of two components of high volatility. The mixture is rapidly melted between the slide and coverslip and a large size metal ring (18 mm) is sealed around it. All observations of the primary freezing process and subsequent crystallization phenomena can be observed without interference due to sublimation. (see Figure 12).

The use of silicone cement as a direct seal without the use of the metal ring is not possible because many substances are soluble in this cement at higher temperatures. The silicone cement used is that sold as stopcock grease. In some cases, it is an advantage to replace the silicone cement with a fused salt which melts at 141°C (55 percent KNO₃ - 45 percent NaNO₂). The salt mixture is fused on the microscope slide as a large drop and the coverslip with the crystalline sample on it is pressed quickly into the fused salt drop. The crystals of the sample must be entirely surrounded by the salt melt. This method is very satisfactory for all substances where the vapor pressure does not exceed one atmosphere at the melting point. This seldom happens; even camphor has a vapor pressure of only 354 mm Hg at 170°C⁹⁰. β -Hexachlorocyclohexane which melts at 310°C and is highly volatile can be studied by this means⁹¹. Although most organic compounds are insoluble in this salt mixture, a few compounds such as veronal, luminal and other barbituric acid derivatives dissolve in this mixture at high temperatures and cannot be studied in the salt chamber.

5. Fischer's Micro Bulb⁹²

The micro bulb is made from a thin-walled glass tube 2 mm in diameter and 6 cm long by flattening the tube over one-half its length and then sealing the end by fusion. The flattened half is about 3 mm wide and 1 mm thick. The interior of this portion is 2 mm wide and 0.3 mm high. This tube is easily made in the laboratory when needed. It should be made from chemically resistant glass, preferably Pyrex[®]. These tubes can also be obtained ready-made from the firm of Wagner and Munz, Munich.

To fill the bulb its open end is inserted into a small amount of the substance and then the bulb is allowed to fall several times to the bottom of a glass tube 1 meter long in order to pack the substance in the flattened portion of the bulb. The micro bulb is then sealed in a small flame at the junction between the tube and the flat portion. It is then withdrawn quickly as soon as it is sealed to avoid distortion through further heating. Care should be taken to remove all particles of the test substance from the fused portion of the bulb before sealing. For low melting compounds it is recommended that during sealing the flat portion of the tube be held with moist filter paper to prevent heating. If it is desirable to evacuate the micro bulb, the free end is connected to a vacuum pump during sealing. With the help of a three-way stopcock and a vacuum pump the micro bulb can be filled with nitrogen or with any other gas.

The sealed micro bulb is then placed in the micro melting point apparatus. A rectangular plate of metal 1.5 by 26 by 38 mm is used to hold the micro bulb for better and more uniform heat transfer. A slot about 3 mm wide and 24 mm long running parallel to the long direction is cut in the plate. During the determination of the melting point, the metal plate and the micro

bulb lie in the center of the micro melting point apparatus with the micro bulb in the slot of the metal plate.

The micro bulb is useful for the micro melting point determination of substances which are so readily volatile that they escape completely from between the coverslip and the slide before the melting point is reached. The micro bulb serves further for determination of melting points either in vacuum or in an inert gas. Also, according to Fischer, melting point determinations can be made on precipitates which are formed in the micro bulb. Critical solution temperatures can be determined and, finally, it is useful for the determination of refractive indices of volatile organic liquids (see page 55). This will be discussed below.

6. Melting with Decomposition

Substances which decompose at the melting point cause the same difficulties in micro determination of the melting point as in macro determinations. As is well known, the temperature at which organic substances decompose depends to a large extent on the rate of heating; it is therefore essential when giving the decomposition temperature also to mention the rate of heating. The decomposition temperature measured with high heating rates is almost always higher and can be reproduced more closely than that obtained by the usual heating rate in macro determinations.

In the micro method the rate of heating is more rapid than that used in the macro procedure. Therefore, the decomposition temperatures obtained are usually higher than the macro values reported in the literature. If the rate of heating were the same, both methods should give identical values for the decomposition temperature. It is obvious, of course, that the process of decomposition can be detected more readily and followed more accurately under the microscope than in a capillary tube.

It is often of value to determine whether the substance melts intact or with decomposition. If the same preparation shows the same equilibrium melting point on repeated determination, then no decomposition has occurred, but if the equilibrium melting point becomes progressively lower, decomposition is occurring.

Further information on decomposition of the melt is covered in the section on the determination of the refractive index of the melt.

For the determination of purity of substances which decompose, see page 28. The hot bar is very useful for the estimation of melting points of decomposable substances since it utilizes a maximum heating rate (see page 21).

7. Hydrates

Compounds which contain water of crystallization may behave in different ways during heating under the microscope. Usually the water of crystallization is lost before the anhydrous material melts. Other compounds may melt as the hydrate, and with further rise in temperature resolidify as the anhydrous material which then melts on additional heating.

In a crystalline hydrate, dehydration causes a change in appearance due to a physical breakdown of the lattice into a micro crystalline aggregate of the anhydrous material. In most cases, however, the external shape of the original crystals is maintained. The original clear transparent crystals become either brown or black due to light scattering by the tiny crystal sur-

faces developed during dehydration. This process is illustrated in Figure 13 with photomicrographs of phloroglucinol. This substance loses its water of crystallization between 55 to 90°C, and the anhydrous crystals then melt between 215 to 220°C with decomposition. Figure 13a was taken at 55°C and shows a single crystal beginning to darken and a small rectangular crystal at the lower right which is completely dark. In Figure 13b (at 65°C) the process has progressed further and in Figure 13c (at 90°C) is complete. The transformation to the anhydrous form frequently proceeds by inoculation of one crystal by another as shown in Figure 13b.

It is not always possible to observe the change to the anhydrous form clearly. When the original material is in the form of fine dust or small needles then the escape of water of crystallization can be noticed, if at all, only by the physical movements of the crystals. In such cases, or when we have very thin platelike crystals, the formation of the anhydrous form can be detected better between crossed polars.

It should be mentioned that cloudiness or darkening of the crystal is, in itself, not sufficient indication of a dehydration process since the change of a clear transparent crystal to a dark finely crystalline aggregate without change in the external crystalline form is also observed during a polymorphic transformation. The occurrence of dehydration can usually be confirmed, however, by detecting the presence of escaping liquid of crystallization made visible by allowing a drop of paraffin oil to run under the coverslip and around the crystals before heating.

Although the dehydration temperatures given in the literature for most substances are usually from 100° to 110°C, it is possible to observe this loss at a lower temperature under the microscope. Under the heating conditions maintained during the micro determination of melting point, the crystals usually become dark between 60 and 90°C. If, however, the rate of heating is somewhat slower, or if the temperature is maintained at 50 to 55°C, phloroglucinol will be completely dehydrated within 10 minutes (provided that the sample does not exceed about 0.1 mg).

Crystals of many hydrates remain unchanged up to the melting point of the hydrate, but on further heating the anhydrous material or a lower hydrate may crystallize from the melt. This is true of those hydrates having congruent melting points such as $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ or $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and especially of those hydrates melting considerably below 100°C, for example, $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$, which melts at 34°C. A hydrate is actually, of course, a molecular compound of the substance with water and it may melt sharply or with decomposition.

In most cases, hydrates are unstable since they melt, in general, incongruently^{93,94} (see page 95). As the hydrate dissolves, therefore, crystals of the anhydrous form or of a lower hydrate usually appear in a saturated aqueous solution. The six-sided monoclinic crystals of $\text{NaBr} \cdot 2\text{H}_2\text{O}$ melt, as an example, at 51°C with simultaneous formation of anhydrous crystals of NaBr (Figure 14).

The holding of a three-phase equilibrium between hydrate, anhydrous phase and melt is often difficult not only with organic hydrates but with inorganic hydrates. This depends on the difference between the incongruently melting hydrates and the otherwise unstable but congruently melting hydrates⁸⁵⁻⁹⁷. Because of this the melting points of hydrates are not sharp but occur over an interval of several degrees, sometimes as much as 10°C.

In general, loss of water from droplets of the hydrate melt can be observed only at temperatures above 100°C and it appears then as vapor bub-

bles escaping from the melt. After the water of crystallization has volatilized the melt is composed of pure anhydrous material. In many cases resolidification of the anhydrous phase takes place immediately. How rapidly this recrystallization occurs, however, depends on the nature of the substance, the amount of material etc.

Occasionally some of the crystals in a preparation may melt as hydrate while at the same time other crystals may darken and lose water of crystallization. Such a preparation can then show three different anhydrous phases: dark crystals which have lost their water of crystallization; resolidified droplets; and, finally, supercooled droplets (e.g., codeine hydrate at 80°C, Figure 15).

The behavior of hydrates is dependent, to a large extent, on the experimental conditions, such as crystal size and number, and the rate of heating. Examples of this are covered in the Exercises. The different experimental conditions explain why different melting points are obtained with the macro and micro methods. With many substances the macro method in the capillary tube gives the melting point of the hydrate while the micro method gives the melting point of the anhydrous substance. In the latter cases the hydrate loses its water of crystallization before reaching its melting point, e.g., oxalic acid. The melting point of anhydrous codeine itself is quoted in the literature as 155°C and its hydrate as 153°C. The latter value is, however, incorrect^{95,97}. This value was obtained in the capillary tube in which the same processes are apparently taking place that can be observed under the microscope. However, in the capillary tube, the liberated water cannot escape completely and the melting point observed around 153°C is the melting point of somewhat moist anhydrous codeine rather than the hydrate. Codeine hydrate melts incongruently but without decomposition at 80°C^{95,98}. This again emphasizes that the experimental conditions should be recorded with each melting point.

If it is difficult to determine whether a substance contains water of crystallization, e.g., with finely divided crystals, the question can be unequivocally answered by immersing the substance in paraffin oil between a coverslip and slide. On heating, any water of crystallization is released as very fine liquid droplets suspended in the mineral oil⁹⁹. The process is definite and easy to observe even without the microscope. The crystals themselves show the same changes in appearance on heating in paraffin oil as in air.

The use of paraffin oil around hydrate crystals during melting not only permits differentiation between dehydration and polymorphic transformations, but also makes possible the differentiation between congruent or incongruent melting of a given hydrate⁹⁷. Paraffin oil prevents the premature loss of water of crystallization below the melting point. During heating under these conditions a congruent melting point is illustrated by melting of the hydrate to a clear drop of melt whereas a hydrate melting incongruently will melt with simultaneous crystallization of the anhydrous phase or of a lower hydrate.

When $\text{NaBr} \cdot 2\text{H}_2\text{O}$ is heated in air the crystals lose the water of crystallization and become cloudy without melting. When heated in paraffin oil the same hydrate melts at 51°C with simultaneous separation of cubic crystals of NaBr (Figure 14). Oxalic acid dihydrate behaves similarly when heated in air and melts congruently at 100.5°C; the hydrate may also melt incongruently at 100°C. On further heating the water is lost as vapor bubbles and the droplets of melt crystallize as anhydrous oxalic acid. Other sol-

vates of organic compounds behave similarly although it may be necessary to use another liquid in place of paraffin oil if the latter acts as a solvent for the test substance.

Chloreton (chlorbutols) shows a peculiar behavior due to its very low solubility in water^{100,101}. It can be melted under water since it is more soluble in paraffin oil than in water.

Hydrates will, as a rule, sublime to give the anhydrous crystals, and it is only when sublimation begins below the dehydration temperature that there is a possibility of obtaining sublimed crystals of hydrate. In the determination of the micro melting point of oxalic acid containing water of crystallization the sublimate will include, in addition to many anhydrous crystals, thin plates and kernels which then become dark at about 90°C as they lose water of crystallization. A large proportion of oxalic acid dihydrate will sublime as such when a very large quantity of oxalic acid is used. Above about 70°C transparent kernels and rods of the hydrate sublime; these melt along with the unsublimed hydrate between 100 and 105°C. Almost immediately after melting, however, the melt crystallizes as anhydrous oxalic acid.

Substances which crystallize with organic liquids of crystallization behave similarly. Exhaustive observations on this phenomena made by A. Kofler⁵⁴ include various argot alkaloids containing ethanol, methanol, acetone, benzene, toluene and dichlorethylene as liquids of crystallization.

To distinguish between water and other liquids of crystallization, Fischer¹⁰³ has combined the paraffin oil method with the method given by Piltz¹⁰⁴ for detecting traces of moisture through the use of a mixture of potassium iodide and lead nitrate. A small amount of these two salts is ground into the paraffin oil used in the preparation. Water of crystallization from the crystals then precipitates lead iodide which gives a yellow color or even the formation of yellow crystals in the neighborhood of the hydrate crystals. Fischer emphasizes that for the detection of water the yellow color of lead iodide is not sufficient, but that the evolution of gas bubbles must also be observed.

C. Kofler Hot Bars

1. The Normal Hot Bar

The hot bar¹⁰⁵ is useful for heating of organic substances to give rapid determination of melting points, mixed melting points and eutectic temperatures. It can also be used for the detection and observation of behavior of water of crystallization, observation of decomposition and estimation of the degree of volatility. The use of the hot bar gives the organic chemist a good idea of the behavior of a given sample and suggests how to carry out the desired experiment with the hot stage quickly and to the best advantage.

The normal hot bar (Figure 16) consists of a chrome-nickel bar, 38 x 370 mm heated at one end. The temperature at the hot end is about 260°C and falls gradually to about 50°C on the opposite end. This range includes the melting points of most organic substances.

The bar itself is constructed in such a way that the gradient is nearly linear. This is accomplished by using two different metals of different conductivities and by using the proper shapes of each metal. The upper portion of the bar is chrome steel and the under portion is made up of several aluminum strips of different lengths so that they form as a whole a wedge. For a short range near the cool end the steel conducts the heat without the

under layer of aluminum. At the cool end itself there are a few strips of aluminum, again to conduct the heat away. The bar is fastened at each end to supports. An extension of the left end of the bar downward at right angles contains the electrical heating element.

The temperature of the upper surface of the bar is influenced by external conditions, especially by the voltage used and room temperature.

The current is stabilized by a special iron wire resistance bulb developed by Dr. L. Kahovec of Graz and manufactured by the firm of K. Bartelt of Graz. This bulb fits into a socket at the rear of the hot bar. The normal variation in room temperature (10-30°C) is taken care of by a monograph plot on the temperature scale furnished with the hot bar. The actual temperature at a given point on the hot bar is determined at different room temperatures by determining the melting points of a series of standard compounds at different room temperatures. The results are plotted to give the lines shown on the temperature scale. A sliding pointer is used to read the temperature of the hot bar at any point corresponding to the room temperature existing at that time (Figure 14). The following substances are a convenient series to use for calibration purposes: azobenzene, 68°C; benzil, 95°C; acetanilide, 115°C; phenacetin, 135°C; benzanilide, 163°C; salophen, 190°C and saccharin, 228°C.

It should be remembered that the hot bar requires 40 minutes to come to equilibrium. The bar may be left on for long periods of time without harm.

A melting point determination will usually require 1-2 minutes and only as much material as is usually used for a capillary melting point. A hand magnifier is often used for accurate setting of the melting point with small quantities of material. This test method is a great advantage for control analyses in a plant where a sample can be compared with a plant standard to check the relative melting points. This saves time and material.

The behavior of a hydrate on a hot bar may be evident in one of several ways. Many hydrates melt as such but recrystallize subsequently at the same temperatures as anhydrous crystals as the water of crystallization is lost. In other cases, crystals of the hydrate may be observed to become cloudy below the melting point as the water of crystallization is driven off. The rate at which this cloudiness develops depends on the temperature. At higher temperatures it occurs quickly but at lower temperatures it occurs only gradually. In general, an accurate measure of the melting point of a hydrate cannot be obtained. It depends on the size of the crystals and on the quantity of material used. Often a few crystals of the sample become cloudy immediately adjacent to other crystals which melt just as observed microscopically with the hot stage.

Oxalic acid dihydrate, for example, melts completely at 140°C but recrystallizes again after evaporation of the water. Between about 125°C and 140°C the crystals partly melt and partly become cloudy. Between 65°C and 125°C the crystals become cloudy almost immediately on touching the hot bar; below 65°C clouding occurs gradually. All droplets of melt formed recrystallize sooner or later as cloudy anhydrous crystals. The melting point of the anhydrous material can also often be determined if it is not too high.

Substances which decompose on melting often show a higher melting point on the hot bar than that reported in the literature. This illustrates the known fact that the melting point of a decomposable compound depends on the heating rate as pointed out for sugar osazones by E. Fischer¹⁰⁶. Kempf and Kutter¹⁰⁷ found in compiling their melting point tables that the litera-

ture seldom gave any information on the heating rate used for the determination of a given melting point. For this reason the literature values for melting points of decomposable substances varies considerably^{106,107}. The best values of the melting point of such compounds (i.e., highest and most reproducible values) are found when the heating rate is very rapid.

The usual methods of determining melting points do not heat the sample rapidly enough. The hot bar, on the other hand, overcomes this difficulty since the sample is heated to the melting point in a few seconds. The procedure is reproducible so that the melting point of a substance melting without decomposition remains fixed on the hot bar. However, the melting point of a decomposable substance appears to decrease more or less rapidly as it remains on the hot bar.

As expected from the above discussion, the melting points as measured on the hot bar are, in general, higher than those determined by other methods where the heating rate is lower¹⁰⁸. As an example, aspirin (acetylsalicylic acid) is reported in the literature to have various melting points ranging from 118°C to 141°C. The melting point observed on the hot bar is 143°C after which rapid decomposition occurs¹¹⁰.

The time at which the melting point is read after sprinkling the powdered sample on the hot bar is very important if good reproducibility is to be obtained. In general, a finely powdered sample will be at equilibrium with the hot bar in 4-5 seconds after sprinkling on the hot bar and the melting point may be determined after that time. With a compound which decomposes it is important not to wait more than a few seconds beyond this lower time limit of 4-5 seconds. The sample should be finely divided and sprinkled carefully over the hot bar to avoid piling.

If the melting point of an anhydrous phase of a given hydrate is to be determined, it is desirable to heat the hydrate first on the hot bar to drive off the water of crystallization. This is done by placing the powdered hydrate on a half slide at about 60°C on the hot bar and gradually moving it to higher temperatures. Most hydrates of organic substances will be completely dehydrated at about 130°C. Some compounds, such as lactose, require a temperature of about 170°C.

If there is any question as to whether the sample consists of the hydrate or the anhydrous phase, a hot stage is the best means of determining the answer⁵⁴. The determination can usually also be made using the hot bar. In either case, the sample is placed on a half slide, a drop of paraffin oil is added and the preparation is covered with a coverslip. On heating to 100°C or slightly higher the sample will show gas evolution due to the water of crystallization if it is a hydrate.

In certain cases where good reproducibility is desired and where the substance decomposes strongly, it may be helpful to set a standard time of 10 seconds after placing the sample on the hot bar at which time the melting point is determined. In these cases the substance usually melts over a range and the pointer is set at the lowest temperature giving a clear, completely melted droplet¹⁰⁸. Table 1 shows the melting point of a few substances determined in this way.

The compounds listed in this table have been chosen from the Pharmacopias of Germany, Switzerland, U.S.A. and Denmark. In spite of the fact that the melting point is included as a means of identification and determination of purity it is the capillary melting point. Furthermore, some important compounds have no values listed because they decompose so badly.

TABLE 1

Melting Points of Substances Melting with Decomposition

Substance	Hot Bar	Melting Point, °C	
		Hot Stage	Capillary
Chloral hydrate	76	50-63	49-53 ¹⁰⁹ , 52-57 ¹¹¹
Aspirin	143	130-136	>135 ¹⁰⁹ , 134-141 ¹¹¹ , 134-136 ¹¹²
Glucose	150	146-148.5	146 ¹¹³
Sucrose	189	185-190	-160 ¹¹⁴ , 170 ¹¹⁵ , 180 ¹¹⁶ , 185 ¹¹³
Ascorbic Acid	191	185-190	-190 ¹¹⁷ , 189-194 ¹¹¹ , 192 ¹¹³
Doryl	209	203-206	200-203 ¹¹⁷ , 206-210 ¹¹¹
Lactose	218	206-216	201.6 ¹¹³
Phloroglucinol	222	205-220	217-219 ¹¹³ , 200-209 and 217-219 ¹⁰⁷
Acetophenolisatin	251	240-246	241-248 ¹¹¹
c-Arginine	260	220-230	207 ¹¹³
Morphine	260	245-255	254 with decomposition ⁹⁰
Sulfathiasole	266	257-259	252-256 ¹¹⁷ , 254-259 ¹¹¹

The Swiss and U.S. Pharmacopias give no melting point for chloral hydrate. The German and Danish Pharmacopias give values which differ from each other, differ from the microscope value and differ most of all from the reproducible value obtained using the hot bar.

It should be mentioned again that the reproducibility of the hot bar melting points depends most of all on the care with which the heating time is controlled. If the melting point is ready exactly 10 seconds after the finely powdered sample is sprinkled on the bar, values within $\pm 1^\circ\text{C}$ can be obtained.

Dennis and Shelton¹¹⁸ have reported values for several compounds measured with their hot bar using a thermocouple and a copper bar. The values given differ, however, from the values determined with the Kofler bar according to the above procedure. For example, phthalic acid 228.5°C (D and S¹¹⁶) against 234°C , glycine 297.2°C against 277°C and barbituric acid 250°C against 234°C . Since Dennis and Shelton give no experimental details, it is not possible to say what causes these differences.

It is also possible with the hot bar to follow the course of decomposition and to find the temperature limit below which a given compound can be expected to remain chemically stable. This is done by sprinkling a strip of finely divided sample from a point above the melting point to a point at

which decomposition should be negligible. The melting point is then determined as a function of time. This has been carried out for cane sugar¹⁰⁸ with the following results:

<u>Substance time (minutes)</u>		<u>m.p.(°C)</u>	<u>Time (minutes)</u>	<u>m.p.(°C)</u>
Cane sugar	1/6	189	50	172
	5	188	60	170
	10	185	120	163
	20	182	180	160
	30	178	240	159
	40	176		
Ascorbic acid	1/6	191	20	186 (152)
	5	190 (174)*	30	184 (144)
	10	188 (159)	40	183 (140)

*The figures in parentheses indicate the temperature at which the solid substance shows discoloration.

The melting points of substances which sublime can usually be determined using the hot bar since they reach the melting point so quickly they do not have time to volatilize. Only with very highly volatile compounds such as hexachloroethane and borneol is the determination difficult or impossible.

The hot bar is especially useful for the preparation of sublimates since all of the possible temperatures between 50°C and 260°C are immediately available. The substance to be sublimed is placed either in a glass cup about 1 cm in diameter and 3-5 mm high, or on a slide with a glass or metal ring around it. In either case the cup or ring is then covered with a preheated clean coverslip to collect the sublimate. If a finely divided sample is desired for any reason, the coverslip should be cold and perhaps kept cold with an aluminum block or drop of water. The metal sublimation block as sold for use with the Kofler hot stage may also be used.

2. Special Hot Bar

(a) Hot bar with a limited temperature range, 80-180°C

The accuracy of the melting point data determined with the common Kofler hot bar¹⁰⁵ is usually sufficient ($\pm 1^\circ\text{C}$). A greater accuracy in terms of melting point differences is, however, necessary when the melting points of two samples of a given compound are compared as a test for relative purity or when the possibility of melting point lowering by a second added component is being tested. To serve this need a special hot bar with a temperature gradient of only about 100°C (80-180°C) has been constructed¹¹⁹. The length of each degree interval is roughly double that of the normal hot bar with the 220°C gradient. Of course, the actual temperature range covered could be varied as desired. The scale is graduated in single degrees so that fractions of a degree may be estimated.

To attain the high accuracy possible with this special hot bar it

is especially important to avoid clumping of the sample during spreading on the bar. It has been advantageous in this connection to apply the sample with a small sieve cut from a 60 mesh screen about 15 mm in diameter. A small amount of substance on the sieve is applied to the bar by holding the sieve about 2-3 cm above the bar and tapping it gently as it is moved through the desired temperature range. The powder should not be sprinkled very densely but so that the crystals are well separated.

Compounds found useful for calibration purposes include naphthalene, 80.2°C; pyrocatechol, 104°C; β -naphthol, 122.5°C; cholesterol, 148.5°C; and noctal 183.5°C as well as those compounds listed above (see page 22). The accuracy with which melting points can be determined depends on the care with which the calibration is carried out and the difference between the melting point of the test substance and the standard of nearest melting point. The error may be as low as 0.3°C and usually is about $\pm 0.5^\circ\text{C}$.

With this procedure and the special hot bar the melting point difference between phenacetin and urea have been checked. In the first place, the literature agrees very well on these two melting points — phenacetin, 135°C and urea, 132.7°C^{113,120}. However, even on the normal hot bar, it is evident that there is hardly any difference between the melting points of these two compounds. On the special hot bar a melting point difference of 0.3°C has been measured. Since the value of 135°C for phenacetin is believed to be correct, the melting point of urea must be 134.7°C rather than 132.7°C. Since the melting point of urea slowly decreases on further heating (134.2°C, 10 min; 133.7°C, 20 min) and since the melting range increases as the melting point is lowered, the discrepancy in the literature can probably be attributed to decomposition resulting from long heating of the sample during the melting point determination. The hot stage microscope had previously given a melting point for urea of 134.5-135°C⁵⁴.

The determination of melting point differences is a useful and highly accurate means of obtaining melting points relative to a standard compound. The accuracy, of course, depends on the accuracy with which the melting point of the standard is known. This method is very useful in evaluating the purity of different commercial samples of a given compound and in following the purification steps.

The special hot bar may be used with advantage for the determination of composition diagrams since the lower limit of clear melt represents the point of primary crystallization for that mixture. The same holds also for the determination of the eutectic temperature (see page 39) since the lowest temperature at which any melting occurs can be easily seen, especially with mixtures near the eutectic composition. The mixed samples used in these cases are first melted, then powdered and sifted onto the hot bar.

(b) Hot bar for lower temperatures, 10-210°C

The normal hot bar is satisfactory for most uses but occasionally a bar suitable for lower melting compounds such as fats and waxes is desired. This is accomplished by a suitable modification of the normal hot bar such that the cold end is immersed in an ice-water bath of constant water level (Figure 18). It is useless to attempt to cool the end of the hot bar further since even at 10°C that end of the bar is coated with a condensed film of moisture. This bar, like the normal one, is graduated in 2°C intervals.

β -Naphthylethylether (m.p. 35°C) is used for calibration of the lower range of this hot bar. This hot bar may be used without sacrifice of

accuracy in the range above about 95°C without filling the container with ice-water.

Most operations with this hot bar are unchanged from those discussed above for the normal hot bar. The determination of eutectic temperature in the range between 10°C and room temperature is different but very simple. The first component is sprinkled on the bar and covered with the second component. The mixture thus formed will show melted droplets down to the melting point of the eutectic.

II. REFRACTIVE INDICES OF THE MELT

A. Introduction

The determination of the principal refractive indices of crystalline substances is tedious and requires a knowledge of crystallography. Various schemes have been proposed to obtain useful refractive index data without the extensive training and time-consuming work involved in measuring the several principal indices for a given compound. Schroeder van der Kolk determined the maximum and minimum refractive indices by mounting a number of small ground fragments in successive mounting media of known refractive index. Kley¹²¹ and Mayrhofer¹²² have also applied this procedure. Tables are then prepared in which the compounds are listed either by the higher of the indices found or the difference between the two indices (birefringence). Great care must be exercised in applying these methods if errors are to be avoided.⁷³

Many authors are now beginning, as a result, to give the principal indices to avoid error.¹²³ In any case, however, the measurement of refractive indices presupposes a background in crystallography so that variations in crystal habit and polymorphic form can be handled properly. Behrens¹²⁴ has gone so far as to say that the morphology of crystals cannot safely be used to identify substances. This has been illustrated for the identification of opium alkaloids⁷³. Polymorphism is much more common than ordinarily suspected and even crystals prepared by sublimation may exist in two or more crystalline forms. Examples of polymorphism in toxicology are given by Fischer^{125,126}.

In spite of these difficulties some authors still publish identification schemes based on crystal morphology. Recently Turfitt¹²⁷ published a scheme for the identification of the clinically important barbituric acid derivatives based on evaporation of an ether solution or precipitation from an ammoniacal solution by dilute sulfuric acid. Brandstätter has checked this procedure and found that the crystals shown by Turfitt are not generally characteristic of the compounds involved.

B. Immersion Method of Determining Refractive Indices of Crystals

If a solid particle is immersed in a liquid medium it will disappear if the two media have the same refractive index. In general, the particle will appear more or less strongly visible depending on the difference between the two refractive indices.

To determine in a given case whether the refractive index of the particle is higher or lower than the liquid, the Becke line test is used. This test is based on the "lens" effect of the crystal in the liquid and is apparent as a clear white outline of the particle which moves in or out on focusing above and below the position of best focus. The line always moves toward the higher refractive index on focusing higher. The use of this method for the identification of fine crystals is in general use.¹²⁹⁻¹³⁵

The determination of the refractive index of the unfortunately rare cubic crystals is very simple since only one refractive index is present and orientation of the crystal is immaterial. Anisotropic crystals, on the other hand, must be properly oriented before estimation of the refractive index.

C. The Glass Powder Method for Determining Refractive Indices of Isotropic Melts

As a means of simulating the isotropic nature of anisotropic substances L. Kofler¹³⁶ suggested the use of refractive index of the melt for organic substances. This procedure involves the use of the hot stage microscope for preparation of the melt phase and a series of standard glass powders for checking the refractive indices. To measure a refractive index by this method a small amount of glass powder of known refractive index is added to the substance to be tested. The mixture is then fused in the hot stage and the refractive index of the melt is compared with that of the glass powder (L. Kofler¹³⁶).

Linck and Koehler¹³⁷ first used a series of glass powders in order to measure the refractive index of various liquids by immersion methods when no refractometer was available. Their series consisted of 16 glass powders whose refractive indices differed by an average of 0.02.

Inasmuch as the differences between the different glass powders were too high for the present purposes, a more complete set has been assembled with the cooperation of Linck and the Jena Glass Works. This series has an average difference of 0.01^{138,139}. The powders in this series* have the following refractive indices (measured with sodium light at room temperature):

1.3400	1.4842	1.5204	1.5611	1.6011	1.6483
1.4339	1.4936	1.5299	1.5700	1.6126	1.6598
1.4584	1.5000	1.5403	1.5795	1.6231	1.6715
1.4683	1.5101	1.5502	1.5897	1.6353	1.6877

The upper limit of the previous series was taken as 1.6718 because in over 1,200 substances investigated only a few were found with a higher index; a new powder of 1.6877 has, however, been added recently.

The powders with low indices were much more difficult to obtain since the glasses with lower indices are not resistant to moisture. Four powders from our original series had to be eliminated and replaced with more resistant materials (Kofler and Ruess⁴⁷, A. and L. Kofler⁴⁸).

There are no suitable moisture resistant glasses available below 1.4584 which was prepared from vitreous silica. Fortunately, however, crystalline fluorite with a refractive index of 1.4339 is quite suitable. Efforts are still being made to find materials to cover the gap of 0.0245 between vitreous silica and fluorite.

Other powders to fill in the range below fluorite are also very desirable, but it has been impossible to find such materials (major efforts notwithstanding). Cryolite, with a refractive index 1.3400, is being used as the lowest member in this series. For molten organic substances this low index is seldom required except for fluorinated compounds. It is useful, however, for the determination of refractive index of organic liquids in the micro bulb of Fischer⁹². Fischer^{140,141} uses sodium fluoride (1.3255) and

* Available through A. H. Thomas Company, Philadelphia. The actual values for the postwar set available have been substituted for the values originally published for the prewar set. In most cases, the difference is less than 0.00004.

lithium fluoride (1.3918) in the form of previously fused isotropic glasses for this purpose.

An object is visible in a surrounding liquid medium only when the refractive indices of both materials are different. The particles appear then with a sharp dark line surrounding them. When the microscope bodytube is moved upward a bright line (Becke line) will appear immediately near the boundary of the particle but on the side of the higher refractive index medium. This bright line appears to move toward the medium of higher index of refraction and away from the boundary between the particle and the liquid. When the microscope bodytube is moved downward, the Becke line moves from the boundary into the medium of lower refractive index. This behavior can be stated simply: when the microscope bodytube is moved higher, the Becke line goes to the medium of higher refractive index (Figure 19). This line is more distinct the greater the difference in refractive index between the two media. When the two refractive indices are identical, the Becke line will completely disappear and the object then becomes completely invisible in the surrounding liquid. Becke¹⁴² explains the appearance of this light line on the basis of different refraction of the two media and total reflection at the interface. Spangenberg¹⁴³ explains the phenomenon as a difference in refraction. Recently a new general theory has been given by Berek (see Burri¹⁴⁴).

The Becke line will appear much more distinctly at the boundary between two media, when parallel or axial illumination is used. The plane mirror should be used with no condenser or with the condenser iris diaphragm almost closed. The more strictly these conditions are observed the smaller the difference in refractive index that can be detected by this procedure. Furthermore, the use of monochromatic light is also considered essential; the reason for this will be explained more fully later.

More material (one to two milligrams) is required to obtain a large molten drop for the refractive index determination than is required for the melting point determination. This large amount of material requires that the preparation be heated somewhat above the melting point to obtain complete melting. With an unknown compound on which the refractive index as well as the melting point are to be determined, a small amount of material should be used to obtain the melting point and a second determination should be made with a larger amount of substance containing the glass powder in order to determine the refractive index of the melt. This determination can, of course, be carried out immediately without cooling the apparatus after determination of the melting point.

The substance is examined in successive preparations with the various glass powders until two adjacent powders in the series show for one, a higher, and for the other, a lower refractive index than the melt. After some practice this usually requires no more than three trials. The powder, 1.5204, is usually chosen first and then, depending on that result, a higher or lower refractive index powder is tried.

A still more accurate determination is made possible by the fact that the index of refraction of the melt decreases with increasing temperature while that of the glass remains practically unchanged. A glass is therefore chosen so that immediately after melting the refractive index of the melt is higher than the refractive index of the glass particles. However, on further heating, the refractive index of the melt decreases further and the Becke line, and therefore the glass particle, become less and less visible until at some higher temperature the glass particle will become com-

pletely invisible. On still further rise in temperature the glass particles again become visible, but with the Becke line reversed, since the refractive index of the melt is now lower than that of the glass. The results are expressed, for example, as follows: 1.5403 at 121-122°C, which means that at 121°C the glass powder is still visible and has a lower refractive index than the melt. Between 121 and 122°C the indices become identical and the powder become invisible; at 122°C and above the refractive index of the glass is greater than that of the liquid.

The lowering of refractive indices on heating has been proposed by Gaubert¹⁴⁵ and put into practice by Emmons¹⁴⁶ for the measurement of refractive indices of crystalline materials; in other words, just the reverse of the method described above.

Often as a result of dispersion the glass particles will not completely disappear at any single temperature. In these cases, however, the Becke line, always becomes progressively less distinct with rising temperature and finally reverses its direction suddenly at a definite temperature. Above that temperature the glass particles has the higher index. This reversal can be very sharp even within a single degree centigrade.

The boundary between two media can completely disappear in white light only when the dispersion is the same or approximately the same (e.g., see Exercises: Mannite). In the event of large differences in dispersion, a colored border will appear at the boundary between the two media, even when the refractive indices are identical for one particular wavelength. Instead of a clear, colorless Becke line a double band of green and orange is observed (Christiansen effect). The appearance of these colors has been used by various authors for at least the approximate determination of refractive index (Maschke¹⁴⁷, Ebner¹⁴⁸, Schroeder van der Kolk¹⁴⁹).

This colored edge does not move uniformly as the microscope bodytube is raised upward and downward; but rather each color behaves exactly the reverse of the other. When the bodytube is raised the orange or reddish border nearly always moves toward the center and the greenish band to the exterior of the glass particle. The process is reversed when the bodytube is lowered. The reason for this consistent appearance is that the dispersion of liquids is nearly always greater than that of solid bodies. The colors and the intensity of the dispersion borders are naturally dependent on the wavelength for which identity of refractive indices in the two media is achieved. A change in temperature causes a change in refractive index in this region; hence, the colors in the border will also change. The original clear green band changes on heating of the melt to a blue of lower intensity while the red band moves toward the center of the glass particle and changes to an intense yellow. There is, however, an intermediate point where the two colors have the same intensity insofar as it is possible to judge the relative intensities of two different colors. On further warming the relative intensities of the two colored edges are reversed. Differences in dispersion are especially pronounced with higher refractive index organic compounds.

The appearance of the colored borders complicates the analysis somewhat since comparison of the intensity of the various colors is not easy and also since the reversal of the light line occurs with an intermediate stage of invisibility and without disturbance by the colored borders present with white light. This reversal is, as a rule, distinctly visible over a temperature interval of 1 to 2°C. The use of a filter also makes it much easier to examine colored melts.

In order to eliminate errors introduced through use of different filters, the following values are given for a 1 mm thick red filter RG 1 of the Jena Glass Works, melt number 34904. A filter having these characteristics is furnished with the set of glass powders available through Wagner and Muns in Munich, or through A. H. Thomas Company in Philadelphia. The transmission values of this filter furnished by the company for 1 mm thicknesses are:

<u>Wavelength (mμ)</u>	<u>Transmission</u>
644	0.985
630	0.97
620	0.90
610	0.18
590	0.01

As a result of this work a large number of filters are available from this same melt.

Sodium light or a monochromator can also be used in place of the red filter. In general, however, the red filter is preferable because it is readily available and simple to use.

The refractive indices of the glass powders in this series are furnished by the Jena Glass Works and are the average refractive indices measured with sodium light. With the red filter the refractive index of each glass is naturally somewhat different. Furthermore, the refractive index is also altered somewhat by temperature changes. The original values of the refractive indices of the glass were determined at room temperature while those of molten substances were often taken above 200°C. The temperature effect results, however, in a difference of only one to two units in the fourth decimal place since a temperature coefficient of 0.000001 is characteristic of most glasses. Therefore, if the refractive index of the melt of a certain substance is given as 1.5403 at 121 to 122°C, this does not mean that the melt at 121-1/2°C actually possesses the average index of 1.5403. This statement means, rather, that by this experimental procedure the melt appears to have a still higher index at 121°C with a rising temperature than does the glass powder with the average refractive index at room temperature with sodium light of 1.5403. At 122°C the melt shows a lower refractive index than the glass powder and at temperatures between 121°C and 122°C no difference can be observed between glass and melt.

This restriction is of little importance in the identification of organic compounds, since in practice the reproducibility of the method is the important factor. The reproducibility is, in general, very good although not the same in all cases since it depends on so many different factors. Often the difference in dispersion between glass and melt is an influence; again the fact that the compound decomposes must be considered. The shape of the glass particles is also significant. With some particles the determination is easy, with others difficult, depending on their shape. If all of the glass particles had the form of parallelopipeds (that is, with edges perpendicular to the object slide) the determination of the temperature at which the refractive index of the glass and the melt are identical could be much more accurate. Irregular particles with more or less random conchoidal fracture influence to a certain extent the Becke effect, and hence affect the accuracy of the determination. Furthermore, a single particle of glass pow-

der may show a small variation from the average value. The cause lies in irregularities in refractive index which cannot be completely eliminated even in optical glass¹⁵¹. However, all these factors taken together contribute, in general, no deviation greater than plus or minus one degree. This variation is also no larger than that which would be obtained with the refractometer when the temperature varies by plus or minus one degree, which difference corresponds to a refractive index variation of about plus or minus 0.0004.

The excellent reproducibility of the method is illustrated by the values obtained independently on various preparations by Reimers¹⁵⁰ in Copenhagen, and by us. The following are a few examples.

Substance	Melting point (°C)		Refractive index of the glass	Temperature (°C)	
	Reimers	Kofler		Reimers	Kofler
Benzoic acid	122	122.5	1.5000	133-134	134-135
			1.4937	147-148	148-149
p-Hydroxybenzoic acid methyl ester	126	127	1.5203	133-134	132-133
p-Hydroxybenzoic acid ethyl ester	117.5	116	1.5101	117-119	117-118
			1.5000	138-139	139-140
p-Hydroxybenzoic acid propyl ester	97.5	97	1.5101	98-100	98-100
			1.5000	120.5-121.5	122-123

The refractive indices of many substances can be compared with two or more different glasses in the series. First, the temperature is determined with the glass having the index reached first on additional heating after melting. Then, in a second experiment the refractive index is determined with the glass having the next lowest refractive index. In the case of novatophan (2-phenyl-6-methyl cinchoninate), for example, in addition to the melting point of 59°C, refractive indices of 1.6482 at 63-64°C and 1.6353 at 91-93°C are obtained. From these values it is possible to calculate approximately the temperature coefficient of refractive index, that is, the average change in refractive index per degree rise in temperature. Thus, novatophan shows a decrease in refractive index of 0.0130 in 28.5°C. This gives, therefore, a value of 0.00045 for the temperature coefficient of refractive index.

The temperature coefficients determined in this way show considerable differences from substance to substance. For the compounds studied to date, the values lie between 0.0002 (guanidine hydrochloride) and 0.0009 (9,10-dihydronaphthacene). (Other substances with large temperature coefficients of refractive index are 6-chlorometacresol with 0.0089, p-bromotoluene with 0.00083, and chloral formamid with 0.0008.) The temperature coefficient, in spite of the restrictions mentioned, is valuable in the identification and characterization of substances¹⁵². If the temperature coefficient was in all cases identical, then the determination of refractive index with the second glass would have no value because the result could be calculated from the result obtained from the glass having the higher refractive index. Since, however, the temperature coefficient may vary from substance to substance by a factor of almost five, the determination of refractive index with a second powder makes the identification of the substance just that much more certain.

According to Reimers¹⁵⁰ the temperature coefficients are quite similar within certain groups of compounds. They lie, for example, for a large number of sulfanilamide derivatives between 0.00029 and 0.00040; for barbituric acid derivatives between 0.00033 and 0.00050; and for benzoic acid derivatives between 0.00040 and 0.00057.

The determination of the refractive index with a second, lower refractive index glass is only possible when the substance can be heated far above the melting point without decomposition or vaporizing. Some substances can be supercooled, after melting, without crystallization.* In these cases the refractive indices of the melt can be determined at temperatures below the melting point (e.g., arecoline hydrobromide melts at 177°C and has a refractive index equal to 1.5299 at 139-140°C). This should always be attempted whenever the substance volatilizes or decomposes rapidly above the melting point.

It is not necessary as a rule in the identification of organic substances to exploit the above methods to the limit of accuracy. Furthermore, in most cases, for purposes of identification the use of a single refractive index determination will be sufficient and use of a second glass powder can be eliminated.

The determination of refractive index of the melt is a very useful means of identification (see Part II; Identification Tables). Barbituric acid derivatives can, for example, be quickly identified by means of melting point and refractive index of the melt¹²⁸. In this way, the uncertain morphological characteristics of a given compound are not depended upon for identification.

For substances that melt with decomposition or which decompose soon after melting an exact determination of refractive index is impossible. There are many intermediate gradations between those compounds for which the refractive index can be determined with two or more glasses, and those for which because of decomposition an index cannot be determined. With some substances the refractive index of a melt can be determined sharply to $\pm 1^\circ\text{C}$ in a single experiment. However, when a second determination is made using the same melt, the temperature at which the refractive indices of the glass and the melt are identical may be somewhat different. This indicates gradual decomposition of the substance. The temperature at which the refractive indices of the glass and melt become equal can either increase or decrease on decomposition.

Decomposition frequently occurs to a different extent in different parts of the preparation. This is shown by the fact that the glass particles in different parts of the preparation behave differently. In one part of the preparation the glass particles will disappear completely at a given temperature, while in another part of the same preparation other glass particles can be seen distinctly. Sandoptal (isobutyl-barbituric acid) with powdered vitreous silica (1.4584) gives, as the first temperature at which the refractive indices become identical, 175-177°C. If the preparation is heated somewhat longer at this temperature and the experiment repeated the equality of refractive indices occurs now at a higher temperature. However, the change

* Translator's note: It might be added that a given compound will more readily supercool if it can be held in the molten state for several minutes before cooling.

does not appear uniformly throughout the entire preparation. Specific differences can be observed in the same molten preparation; for example, one glass particle may disappear between 178-180°C and another only between 181-183°C. If heating is continued for longer periods sandoptal decomposes very rapidly, so that the temperature at which the refractive index of the melt is equal to 1.4594 soon rises above 200°C rather than 175-177°C.

The refractive index data in the tables shows a temperature range of several degrees centigrade in certain cases where the compounds decompose. This does not mean that the glass powder remains invisible over a wider temperature range than usual, but that successive experiments may give different temperatures within the indicated range. Even with substances that decompose very rapidly the two glasses in the series having refractive indices on either side of the just melted preparation can still be determined. This means that immediately after melting the refractive index of the substance lies between those two glass powders, but that a more accurate determination is not possible.

If the substance becomes strongly colored on melting or if it melts with the evolution of gas bubbles, this naturally makes the determination of refractive indices much more difficult.

High volatility of the substance near the melting point also makes the determination of refractive indices between coverslip and slide impossible. In such cases the micro bulb of Fischer⁹² may be used or the preparation may be cemented into the silicone chamber⁴⁰. One or the other of these methods makes it possible to work with such compounds as anthracene, bromocinnamic acid, diisobutyramide, camphoric acid imide and m-nitrobenzoic acid.

The more work done with this method of identifying organic compounds by means of glass powders, the more unfortunate it appears that the method cannot be applied to substances which decompose. Such a method for the identification of decomposable substances would be particularly valuable since the melting point of these compounds is not sharp and since the eutectic temperatures are usually difficult to determine and not very reproducible. This difficulty has been overcome by Lennartz¹⁵³ by mixing the test substance with a standard quantity of a known second component thus lowering the melting point below the decomposition temperature so that the refractive index of the undecomposed melt can be measured. This method was first used to determine the quantitative content of mixtures of decomposable substances; this will be discussed more fully later. It has, however, been useful for the identification of decomposable substances (e.g., alkaloids, Kofler and Lennartz¹⁵⁴).

A material is chosen for the second component that will lower the melting point of the unstable compound as much as possible and which will not itself decompose or volatilize at the melting point. Some suggestions as to appropriate second components can be found in the Identification Tables starting on page 253. The amount of the second component used depends on the decomposability of the compound being examined. In general, a 1:1 mixture of the two components is made for qualitative analysis.

Equal amounts of the two substances (about 0.05 to 0.1 gram) are weighed and carefully ground with a small mortar and pestle. It is a good idea to wet the mixture with a little ether or other volatile solvent and then grind it until all of the solvent is evaporated. The resulting melt should cover about half the area between coverslip and slide. The microscopical preparation is then heated on a hot plate to a temperature above the melting point of the mixture and the coverslip is pressed down with tweezers.

During melting, the coverslip should be moved back and forth for the best possible mixture.

In all cases the preparation must be examined microscopically in order to make certain that no unmelted crystals remain. As soon as the mixture is completely melted it is cooled about 10 to 40°C by placing the cooling block on the hot stage next to the preparation. Such mixtures, in general, supercool very readily. As a result the temperature is lowered even farther below the decomposition range of the unstable substance.

The preparations of volatile substances which are still somewhat volatile even at the melting point of the two component mixture must be melted very rapidly and cooled immediately. The observation of the melting point for such a preparation must also be carried out in the center of the preparation since here the possibility of change in composition of the mixture by sublimation is small. The reproducibility of refractive index values on two component mixtures is good if the preparation is carefully weighed, well mixed, melted at as low as temperature as possible and then cooled as rapidly as possible after melting.

Kofler and Lennartz¹⁵³ have investigated all available alkaloids in the form of their bases and their therapeutically valuable salts by the method just described. Of the alkaloids studied, only the highest melting, theobromine, could not be determined by this method.

This procedure is also useful for the determination of refractive indices of the few substances having indices above 1.6877, the highest index glass in the present series as well as those whose indices lie between 1.3400 and 1.4339.

Up to this point only the determination of refractive indices of molten organic substances has been discussed. In addition, the microscopical method can often be used to advantage in those cases where the refractive index is usually determined with the refractometer, e.g., fats and oils (Kaufmann and Lund¹⁵⁵). According to Kofler and Opfer-Schaum¹⁵⁶ it is possible with the help of the powdered glass series to determine the refractive indices of the unsaponifiable fractions which, in general, show greater differences in refractive indices than the oils themselves. It is especially easy to differentiate olive oil from other oils in this way. This method for the determination of liquids has the advantage that the dependence of the refractometer on the temperature of its surroundings can be avoided. For very volatile organic substances, (methyl alcohol, ether etc.) Fischer recommends determination of the refractive index in the micro bulb^{89,157}.

III. MIXTURES

A. General Behavior of Mixtures

1. Simple Melting Point Diagram

To understand the following section it is desirable to study the behavior of substances in the hot stage with the help of the temperature-composition diagram^{93,94}. Figure 20 shows the composition diagram for a simple 2-component system in which the two components are soluble in all proportions in the melt and at the same time form neither mixed crystals nor an addition compound. The addition of component B to the melt of A lowers the temperature at which crystals of A are in equilibrium with the melt. The same situation holds for the addition of A to liquid B. Therefore, the equilibrium curve starts at the melting point of the pure components and decreases in terms of temperature as either component is added to the other. These curves may be regarded either as melting point or solubility curves.

The curves intersect at the eutectic point, E, and at this point the solution of A is saturated with B and vice versa. Below this temperature the liquid state is unstable and both components crystallize independently as a mixture. A mixture of two materials prepared as a fine powder in the proper amounts to form a eutectic mixture will melt at the temperature, E. With very small crystals this melting point is very sharp even though there are two separate phases present. All other mixtures of the two components will melt and also crystallize over a definite temperature range. Although the pure substance, A, melts at a temperature, A_1 , and the pure substance, B, at a temperature, B_1 , all mixtures of the two begin to melt at the lower temperature, E. Therefore, if a mixture of A and B having the composition x_1 (Figure 20) is heated, the eutectic portion of the mixture will melt at the temperature, E, and the excess component A will remain crystalline. As the temperature rises the excess A will dissolve in the eutectic melt until at the temperature a , the last crystals of A will disappear. A mixture having the composition x_2 will show the same behavior except that component B is in excess and solution or final melting of B is complete at the temperature, b .

The amount of molten eutectic changes naturally as the composition changes; i.e., the closer the composition of the mixture corresponds to x the larger the amount of material which will melt at the temperature E. A mixture x_2 will show much less melt at the temperature E than the mixture x_3 .

Solidification of the molten mixture x_2 proceeds in the following way. At the temperature, a , separation of crystals of component A will begin. The melt, therefore, becomes richer in B. On further cooling the composition of the melt changes along the curve until it reaches the composition x at point E. At this temperature A and B crystallize together as eutectic. The crystallization behavior of the mixture x_2 is analogous.

In the diagram, curves CE and DE, as well as the horizontal line FEG define the borders of four different phases. Above CDE only a liquid phase containing the two components is possible, below FEG only solid phases, while in the two regions CEF and DEG solid and liquid phases can coexist.

It is important to remember in microscopical observations that each mixture of two materials will begin to melt at the eutectic temperature. The excess component will remain crystalline at this temperature as already discussed above. With rising temperature the amount of solid phase will gradually decrease. The composition of the melt during heating will change for

mixtures such as x_1 in Figure 10 from the first formed eutectic composition to successively richer mixtures of A. At each temperature along this curve the equilibrium state between the solid and the liquid phases can be stabilized. The difference lies only in the relative amounts of the two phases. The upper melting point, i.e., the point of primary crystallization is the temperature at which the last crystals disappear (page 11). The above discussion assumes, of course, that no change in concentration (e.g., sublimation) occurs during the experiment. Different concentrations can also occur in the preparation since the first material to melt flows to one side of the coverslip while the larger part of the remaining solid phase remains in one spot. In this case a lower temperature for the end melting point will be obtained in one portion of the melt than in other portions of the melt.

2. Mixed Melting Points

The determination of the mixed melting point under the microscope in order to determine whether two substances are identical is very much better than the usual capillary method. As a rule, the macro method indicates only whether the melting point of the mixture is as high and as sharp as that of the single substance. The sharpness or lack of sharpness of the melting point can be observed much more readily under the microscope. At the same time the relative shapes of the two substances and their behavior during heating can be noted. For example, their tendency to sublime, the form of the sublimate etc. can be compared.

If sufficient material is available about 0.1 mg of the mixture is used for a mixed melting point. When only a small amount of material is available, an amount as small as 0.5 to 1 gamma can be used. (Crystals of organic materials which can still be seen distinctly with the naked eye have a weight of approximately 1 gamma.) The procedure is as follows¹⁵⁸: a few crystals of each material are placed close to each other on the object slide and covered with a coverslip. The coverslip is then pressed down and moved from side to side in order to break up the crystals and mix them together.

The preparation is now placed on the hot stage and heated so that in the region of the melting point the temperature rises at a rate of about 4°C per minute. When the mixed substances are identical, they will melt at the same temperature and with the same appearance during melting. If the two substances are different one part of the mixture will melt earlier while the remaining original crystals will gradually melt or dissolve in the melt during further heating.

This procedure is, however, possible only for substances which show little or no sublimation at the melting point. A number of substances will sublime completely before reaching the melting point if present in the amount of one to two gamma. This premature volatilization cannot be avoided with such small amounts of material in the customary manner such as cementing in the edges of the coverslip or use of Fischer's micro bulb. In these cases larger amounts of the mixture must be used. If for this purpose a large amount of one component is used in order to increase the amount of the sample, the possibility then arises that the material melting within the microscopic field of vision may show an unchanged melting point. This danger can be avoided if the known component is brought in the form of a micro sublimate into contact with the unknown material.

The micro sublimate can be prepared in the following ways: a small amount of the known component is placed on an object slide and covered with a

coverslip supported by two glass strips about the thickness of an object slide. This preparation is then heated on a hot bar until the underside of the coverslip is covered with a definite sublimate. For most organic substances this will occur at temperatures below the melting point. (Information of the tendency for sublimation of many components can be found in the tables in Part II.) Through repeated changing of the coverslip additional samples of sublimate may be obtained. A small crystal of the test substance is now placed on the object slide and covered with one of the coverslips holding a sublimate of the known component on the underside. The mixture is then prepared by crushing the crystals into contact. The irregular crystals of the test substance are then still easily differentiated from the crystalline sublimate of the known component (Figure 21a). The form of the micro sublimate is very different and depends on the nature of the substance and the experimental conditions during sublimation. It may appear as needles, kernels, prisms or plates. The sublimate may be made up of single crystals or aggregates, but it will always crystallize on contact with the coverslip in some regular fashion so that it can be easily differentiated from the irregular crystals of the test substances¹⁵⁸.

If the coverslip with the sublimate is placed on the object slide in such a way that the second component lies in the center of the sublimate, then sublimation of the second component in the region of the melting point is prevented even for such volatile compounds as naphthalene. When the unknown substance and the sublimate of the known component are identical, both melt at the same time and with identical appearance. If they are different, melting will begin at the eutectic temperature. The appearance is very clearly different (Figure 21, b and c) since the crystals of the test substance melt gradually and the sublimate in contact with this melt gradually becomes dissolved or melted while the crystals of sublimate not in the melt remain completely intact.

3. Eutectic Temperature

Determination of the mixed melting point under the microscope¹⁵⁹ not only tells whether the two components are identical but, in addition, gives a method for further characterization and systematic identification. This method is based on determination of the eutectic temperature of each compound with several known standard compounds.

To determine the eutectic temperature approximately equal amounts of the two components are ground together with a mortar and pestle or between two object slides. A small amount of the mixture is then placed on another object slide and covered with a coverslip. The microscopical preparation is then heated on the hot stage so that the temperature rise in the region of the expected eutectic temperature is about 4°C per minute. When the eutectic temperature is reached a part of the substance will melt and form tiny droplets which, however, are not clear but still contain crystals of the unmelted excess component. The relative amounts of eutectic melt and excess crystalline component will vary depending on the eutectic composition of the system and on the relative amounts of the two components used. In most cases as little as, and often less than, one percent of molten material in the preparation can be observed.

The most diverse substances can be used as standard components. However, those with melting points not too far from those of the test substances are usually used. The standard component must not sublime easily and

must be very pure. Furthermore, it must not form a solid solution with the test substance (page 88), and must not be used in the form of an unstable polymorphic modification (page 97).

This method is almost unlimited in its application to the characterization and identification of organic compounds. Among other things, substances with identical or closely similar melting points can be differentiated if the eutectic temperature is determined on a mixture of the unknown with a known standard. This is illustrated in Table 2 for twelve substances melting at 135 or 136°C. While these compounds cannot be differentiated from each other by melting point alone, their eutectic temperatures with acetanilide lie between 62 and 109°C, and with phenacetin between 71 and 128°C.

TABLE 2

Eutectic Melting Points of Closely Melting Compounds

Melting Point, °C	Substance	Eutectic temperature (°C) of Mixtures with:	
		Acetanilide	Phenacetin
135	Urea	102	122
135	Cinnamic acid	84	98
135	Cinnamaldehyde	79	96
135	Phenacetin	90	135
135	2,6-Dimethylpyrone	68	91
135-136	Malonic acid	62	84
136	1,4-Isobutylphentidine	85	104
136	Phenylpropionic acid	53	71
136	d-Galactonic acid-γ-lactone	109	128
136	Allyl-isobutylbatbituric acid	89	105
136	Sitosterol	105	115
136	Resorcylaldehyde	70	84

The determination of eutectic temperatures of one or more mixtures makes the use of the often unsatisfactory color reactions used for the characterization of substances quite unnecessary. It also frequently eliminates the necessity of preparing derivatives.

The method is also valuable for the identification and characterization of substances which because of decomposition show no sharp melting point. A sharp eutectic temperature can frequently be determined with a suitable second component. For example, acetyl salicylic acid (aspirin) melts with decomposition over the wide range of 130 to 136°C (or 143°C; see page 23), but shows a sharp eutectic temperature of 81°C with acetanilide and 97°C with phenacetin.

The reproducibility of the eutectic temperature is as a rule very good with an error of not more than plus or minus one degree centigrade. Large variations may be observed, however, if the test substance or the known component shows polymorphism. Different modifications of the same substance will generally show different eutectic temperatures with a given test material. In practice several different possibilities may be encountered, the most important of which are the following: (1) the substance exists as an unstable modification, (2) the substance is present as the stable modification, but through sublimation forms an unstable modification during determination of the eutectic temperature.

During ordinary crystallization and purification, many substances crystallize in an unstable polymorphic form. For example, commercial preparations of veronal, theophylline and sulfathiazole always appear in the unstable modification (page 80). During determination of eutectic temperature, the substance changes partially into the stable modification. The stability of the unstable modification determines whether the behavior of the system follows (1) or (2). Many substances always follow (1), others always follow (2). Some substances lie between, following either (1) or (2) depending on the experimental conditions.

Under different conditions different eutectic temperatures can sometimes be observed with the same test substance. For example, by rapid heating or by putting the preparation in a preheated hot stage, the eutectic temperature with the unstable modification may be determined, while with long heating below the eutectic melting point, complete transformation to the stable form may be obtained. In the latter case, a different eutectic temperature characteristic of the stable modification will be obtained.

When the transformation proceeds only part way, as in (3), the eutectic temperature observed will be that of the unstable form as in case (1). If the transformation has progressed almost to completion at the melting point, the eutectic temperature will not be sharp. A small part of the mixture will begin to melt at the eutectic temperature of the unstable modification, and a larger part will follow at the temperature of the stable modification. The appearance in this case would indicate that the eutectic temperature does not have a single value. Melting does not occur sharply but appears to be sluggish in contrast to the usual appearance during melting of a eutectic of two components.

Sulfathiazole shows many of these phenomena. The stable modification melts at 201°C, and unstable form at 174°C^{160,161}. All commercial preparations (elendron or cibazol) examined to date have crystallized as the unstable modification. In the micro determination of the melting point all commercial samples of sulfathiazole begin to melt at 174°C. However, only part of the material melts at this temperature since a portion has already transformed into the stable modification which then melts at 201°C. The eutectic temperatures of the two modifications with salophen and dicyandiamide are quite different.

<u>Sulfathiazole</u>	<u>M.P. (°C)</u>	<u>Eutectic temperature (°C) with:</u>	
		<u>Salophen</u>	<u>Dicyandiamide</u>
Mod. I	201	172	167
Mod. II	174	159	152

The use of sulfathiazole in the original mixture permits determination of the eutectic temperature of the unstable modification. If sulfathiazole is heated for about ten minutes at 170°C, it will be completely transformed and the eutectic temperature of the stable modification can be observed.

Even substances which are present ordinarily as stable modifications may form unstable modifications by sublimation during determination of the eutectic temperature, so that the eutectic temperature of the unstable modification is observed. These cases are rare since the crystals of the original stable form usually prevent formation of an unstable modification in the immediate neighborhood. This can occur only when the unstable modification is relatively stable in the presence of the stable modification. When

the original stable crystals give only a small amount of the unstable modification during sublimation, then the eutectic temperature will not be sharp. This happens to a small extent with nipagin (p-methyl hydroxybenzoate). The eutectic temperature of the stable form of nipagin with acetanilide is 75°C. With a slow rate of heating numerous small droplets are observed on the underside of the coverslip at a temperature many degrees below the melting point. These are formed by sublimation of the low melting unstable modification before melting of the eutectic.

Impurities are often the cause of sluggish melting of the eutectic. If either one of the two components contains a large amount of a single impurity, the beginning of melting of the ternary eutectic can be observed sharply. If the impurity is present only in small amounts (under 1 percent), or if many impurities are present in small amounts, the beginning of melting will not be sharp. In this latter case a sluggish melting of the eutectic is observed.

Sluggishness of melting will naturally influence the reproducibility of the eutectic temperature. Data on more than 1,200 substances in the tables in Part II have shown that, in general, the reproducibility of the eutectic temperature is better than that of the melting point of the single component. It is worth repeating in this connection that some substances, although they show no sharp melting point because of decomposition, frequently give easily reproducible eutectic temperatures.

Another reason for the appearance of different eutectics between two substances is the formation of molecular compounds. With such systems the eutectic between the molecular compound and either of the two components can be observed, depending on the composition of the mixture and the experimental conditions. Investigation, however, has shown that during warming on the hot stage these mixtures in powdered form show, as a rule, the lower eutectic melting point unless the composition lies very near that of the higher of the two eutectics.

Isomorphous substances with identical or similar melting points give similar eutectic temperatures in combination with the standard substance. Identification, in these cases, is based on determination of the refractive index of the melt or, depending on the type of isomorphism, by the fusion method.

Most hydrates give the same eutectic temperature as their corresponding anhydrous component (Kofler and Brandstätter¹⁶⁴), since the hydrate usually loses its water of crystallization at comparatively low temperatures (page 18), so that only the anhydrous material is present by the time melting occurs. Hence, morphine (melting point, 247-254°C), phloroglucinol (melting point, 215-220°C), and oxalic acid (melting point, 190-191°C) give the same eutectic with salophen (melting point, 190°C) whether they are used as hydrate or as anhydrous crystals (respectively 156, 159 and 164°C). If a lower melting material is chosen as standard substance, the eutectic temperature of the hydrate may be observed. For example, phloroglucinol as the hydrate gives a eutectic with acetanilide (melting point, 115°C) which melts at 64°C; anhydrous phloroglucinol gives a eutectic with acetanilide which melts at 84°C.

Occasionally the mixture of a hydrate with a standard substance will melt partially, followed by crystallization of the anhydrous compound from this melt. On further heating a melt is formed consisting of the eutectic of the anhydrous substance with the standard. As an example of this, a mixture of oxalic acid dihydrate (melting point, 100.5°C) with dicyandiamide

(melting point, 210°C) melts partially at 85°C, although the eutectic with anhydrous oxalic acid (melting point, 190-191°C) is formed only at 177°C.

In a few cases no eutectic, *i.e.*, no depression of the melting point, occurs with either the hydrate or the anhydrous substance. For example, 4-oxypyridine as the hydrate begins to melt in a mixture with azobenzene (melting point, 68°C) at 55°C, the melting point of the hydrate. The same system but using anhydrous 4-oxypyridine (m.p. 150°C) begins to melt at 67°C, the melting point of azobenzene. Similar behavior is shown with both phloroglucinol and oxalic acid, which in mixtures with azobenzene begin to melt at 67°C whether the hydrate or the anhydrous crystals are used. This behavior is due to low solubility of the two components in the liquid phase. On the other hand, phloroglucinol hydrate gives a eutectic melting at 50°C with antipyrine (melting point, 111.5°C). In this case antipyrine and phloroglucinol are mutually soluble in the liquid state. Oxalic acid dihydrate loses its water of crystallization even on mixing with antipyrine at room temperature.

When two hydrates are mixed together a eutectic between the hydrates is often obtained; in other cases, the eutectic between the anhydrous phases results. A mixture of the hydrates of phloroglucinol with oxalic acid, for example, shows darkening of the crystals as the water of crystallization is lost and finally gives a eutectic between the anhydrous phases melting at 165°C. Melting occurs at room temperature as 4-oxypyridine is mixed with arecoline hydrobromide (melting point of the hydrate, 49°C; melting point of the anhydrous crystals, 177°C). The eutectic temperature for the two anhydrous phases is 92°C.

Test substances are always used for the identification of organic substances with the help of eutectic temperatures anhydrous. It is immaterial, as shown above, whether a hydrate or the anhydrous material of the unknown component is used since in most cases the same eutectic temperature will be obtained. Errors can, however, occur when the hydrate melts below the eutectic temperature and if this melting of the hydrate is considered to be the eutectic temperature. In spite of this, no confusion should result during the identification procedure since the simple melting point determination would show the presence of hydrates.

Under some circumstances highly volatile substances may sublime at a relatively low temperature, so that the eutectic temperature cannot be determined or, at least, only with difficulty (*e.g.*, camphor). For such substances the test is carried out by subliming a heavy deposit of the unknown onto the under surface of the coverslip. This coverslip is then placed over a small particle of the reference substance on the microscope slide. This is the same method described previously (page 39) for determination of mixed melting points when only small amounts of material are available.

4. Purity Test

The presence of impurities is, often, first observed during the micro determination of the melting point by the premature melting of some particles. It is self-evident that smaller amounts of impurity can be observed under magnification of 80 to 100X with the microscope than with the naked eye or hand magnifier in a capillary tube. As a rule about one-quarter percent impurity can be observed under the microscope as a result of early melting. The macro methods, in general, show a corresponding limit of about 2 percent.

If a considerable amount of a single compound is present as impurity, the beginning of melting of the eutectic will be noted. As a rule about 1 percent impurity can be detected in this manner¹⁶². In amounts less than 1 percent the distribution of the impurity in the preparation will play a part (page 46). Larger amounts of impurity will cause a very sharp beginning of melting at the eutectic temperature, while with smaller amounts of impurity the beginning of melting is not sharp at the eutectic temperature, although melting becomes more pronounced as the temperature increases. Gradual melting of the material will be observed when more than one impurity is present in small amounts. This is, of course, the most common situation.

Often single particles of impurity will be observed in the microscopic field of view, if the impurity is distributed unevenly. The opposite extreme is a so-called molecular distribution, obtained through melting of the mixture followed by rapid cooling and pulverizing. During melting of such a powder about two to three percent of impurity are required to show the beginning of melting at the eutectic temperature. There is, indeed, an optimum distribution of small amounts of impurity for best recognition of the eutectic temperature. Most laboratory and commercial preparations are crystallized from a solvent and, in general, contain easily discernible crystals of the impurity. In these cases, the beginning of melting can be recognized earlier in the original powder than in a preparation which has been previously melted and then rapidly cooled and pulverized, provided that the amount of impurity does not exceed a certain amount. A commercial sample of diphenyl, for example, begins to melt in its original condition at 55°C but after melting, cooling and pulverizing it begins to melt between 62 and 65°C. In both cases melting is complete at 69°C¹⁶².

In the determination of purity under the microscope the preparation must be observed continuously during heating in order that the beginning of melting not be overlooked. With small amounts of impurities, it is sometimes possible to observe a decrease in the amount of melt rather than an increase during the early stages of melting. This may occur as a result of volatility. The few small droplets containing the principal component, the impurity, or both may evaporate so that with rising temperature the droplets of melt may entirely disappear.

The presence of inorganic impurities can be detected, since they will remain unmelted and undissolved after most of the material is melted and even after further heating. The sensitivity of the method depends upon the amount of sample used for the determination. If enough sample is used so that the melt completely fills the region beneath the coverslip, as little as one-quarter percent of sodium chloride can be detected in the melt of, for example, acetanilide¹⁶³.

In a similar way, it is possible to recognize very high melting organic purities, those which dissolve only slightly in the melt or, finally, those showing very little melting point lowering. Sucrose or lactose, for example, behaves in this way. These sugars would remain unmelted and undissolved in the melt of most organic substances melting below their melting points, respectively 185 and 210°C.

Soluble impurities will naturally lower the equilibrium melting point of a substance. Frequently, nonreproducible equilibrium temperatures are observed during successive determinations of the micro melting point of impure substances. This is due to sampling difficulties on a microscale since, in a single determination, different crystals or particles containing different amounts of impurity will be observed. This difference is not ob-

served in the macro determination of melting point in capillary tubes since the large amount of material used represents a better average of the composition of the substance.

If many solid phases are present in the preparation, the equilibrium temperature will naturally be lower than when only a few solid phases are present. The most accurate and reproducible value for the melting point is represented by the equilibrium with the last crystalline residue to melt. It is important when using the melting point range as an indication of purity not to use a preparation made up of a large quantity of large crystals since a preparation requires a long time to melt.

A further help in the test for purity is the determination of the eutectic temperature with a known second component¹⁶⁴. The substance to be examined is mixed with a reference compound and the eutectic temperature is determined. If this sample is pure it will begin to melt at the eutectic temperature characteristic of that pair of substances; if impure, melting will begin at a lower temperature. For example, the eutectic temperature of acetanilide with anesthesin is 67°C. If the acetanilide contains antipyrine or phenacetin, the eutectic temperature of the mixtures with anesthesin is 67°C. If the acetanilide contains antipyrine or phenacetin, the eutectic temperature of the mixtures with anesthesin will be 30 and 62°C, respectively. One percent of antipyrine or phenacetin can be recognized in acetanilide by this means. Antipyrine is very useful as a reference component since it gives low melting eutectics with a large number of organic compounds. The presence of m-aminophenol in p-aminobenzoic acid is easily shown by the addition of antipyrine and determination of the melting point¹⁶⁵.

Although, in general, this determination gives no more information than a simple melting point determination, it has advantages for many isomorphous and thermally unstable compounds. An example of the first type is β -naphthol (melting point, 122°C) with naphthalene (melting point, 80.5°C) as impurity¹⁶⁴. Since both substances are isomorphous (Type I of Roozeboom, page 94) a small amount of naphthalene causes no noticeable lowering of the melting point of β -naphthol. In addition, the high volatility of naphthalene also makes the lowering of the melting point more difficult to observe. The presence of less than 5 percent naphthalene in β -naphthol cannot be determined with certainty by a simple micro melting point determination. However, the determination of the eutectic temperature of such mixtures with a standard reference substance will show smaller amounts of impurity. The eutectic temperature of β -naphthol with anesthesine is 54°C. The addition of 2 percent naphthalene lowers this to 45°C.

Equal amounts of the sample and the reference material are usually mixed for the determination of eutectic temperatures. However, for the determination of purity it is desirable to use less of the reference compound (about one-quarter of the final preparation) so that the sample will not be unnecessarily diluted.

The greatest value of the eutectic method of determining purity is for substances which decompose on melting since, in this case, the simple melting point of the substance gives little or no information concerning the degree of purity¹⁶⁴.

Impure substances can often be recognized by melting with a reference substance even though the eutectic temperature of the binary mixture is unknown. If, in a three-component mixture, one substance is present in small amount, then at the ternary eutectic temperature only a small amount of the mixture will melt. With further heating little or no increase in the amount

of melt will occur. However, as soon as the eutectic temperature of the two principal components is reached, rapid melting of a large part of the sample will occur.

An especially sensitive purity test is based on observation of the melting process in the solidified melt¹⁵⁸. This depends on the slow rate of solidification in the neighborhood of the melting point with separation of the substances dissolved in the melt (Tammann). This procedure is suitable only for substances that melt without decomposition. A large amount of the sample is melted between coverslip and slide so that small crystals are left unmelted. Heating is then stopped and the hot stage is allowed to cool slowly. The unmelted crystals will then grow to large crystals. The gas bubbles present in the melt become larger and during the latter stages of the crystallization process become distorted. Many small new gas bubbles also appear during solidification, many of them flat and stretched along the interstices and cracks in the crystal mass. They naturally appear in the last portion of the melt to solidify and lie between the crystals and the coverslip or the slide, or, in the case of platelike crystals, between the crystal plates. In order to obtain complete crystallization it is best to allow the preparation to stand undisturbed for several hours before further investigation. On reheating such a preparation, but still many degrees below the melting point, the boundaries of the irregular gas bubbles will begin to move throughout the crystal mass, thus indicating the presence of a liquid. On further heating the movement of the now more circular gas bubbles becomes still more active. They become smaller and smaller, however, and may dissolve completely before melting is complete.

The temperature at which movement of the gas bubbles becomes apparent depends on the nature of the mixture and is not a direct measure of the purity of different substances. With the same major component and impurity the temperature at which the gas bubbles begin to move can be used as an approximate measure of purity¹⁵⁸.

In judging the purity of a substance from melting points polymorphism must always be kept in mind (page 68). Some polymorphic substances crystallize from solution or from the melt in an unstable modification. A few substances, e.g., atophane (2-phenylquinoline-4-carboxylic acid) and volantal, exist as unstable modifications and the commercial preparations, which up to now had been considered as stable modifications are actually metastable (page 41). If, on recrystallization, such a substance suddenly appears from one or another of the usual solvents as the stable modification, the resulting higher melting point might be assumed to indicate higher purity.

Polymorphism can simulate the appearance of impurities when a stable and an unstable modification exist in contact and when the unstable modification is somewhat stable under these conditions. An example of this is atophane which is reported in the literature to have a melting point of 213°C but which can crystallize in four modifications. Only the stable I (melting point, 218°C) and II (melting point, 213°C) are of importance, however. Modification II behaves as if it were very stable. When atophane is precipitated from an aqueous solution of the alkali salt through addition of mineral acid, Modification I containing varying amounts of II is obtained. The product melts over the range 213-218°C. A second recrystallization from organic solvents gives Modification II with the melting point 213°C. As a result of recrystallization the melting point, therefore, becomes sharper but lower.

Another example is sulfathiazole which is used as a drug under the names eleudron and cibazol (page 41). This substance is dimorphous: I,

201°C; II, 174°C. The industrial preparation exists in the form of Modification II. During the melting point determination, the transformation of II to I begins at about 160°C although it is usually not complete before the temperature reaches the melting point of II¹⁶⁰. Therefore, melting of the unstable form begins at 174°C and is complete only at the melting point of the stable form (201°C). Careful observation shows that many of the droplets formed at 174°C resolidify, especially when in contact with solid substance. Both commercial preparations, eleudron and cibazol, behave similarly. However, another preparation of sulfathiazole transformed completely to I during the melting point determination before the temperature reached 174°C so that melting was first observed at 201°C.

B. Purification Procedures

1. Separation and Purification Through Absorption of the Eutectic Melt

Purification of a substance, i.e., separation of a substance from a mixture is carried out in the following way (Kofler and Wannenmacher¹⁶⁷: First, the beginning of melting, i.e., the eutectic temperature, page 39, is determined on a small sample. Second, a small piece of hardened filter paper (Schleicher and Schull, No. 575) is placed on an object slide and covered with a thin layer of the substance to be purified. A second object slide is then placed over this preparation and the whole assembly is then heated on the hot stage beneath the microscope to a temperature slightly above the eutectic temperature. When this temperature range is reached, the top object slide is pressed down firmly so that the resulting melt is absorbed by the filter paper. The unmelted portion remains in contact with the upper object slide. The upper object slide is then removed with the adhering solid; the filter paper is replaced and the operation is repeated. The filter paper may be changed several times and the operation continued as the temperature of the hot stage rises slowly. In this way a pure substance in an amount large enough for identification by micro methods is usually obtained in about 10-15 minutes (see Exercises).

The filter paper will become colored or blackened if heated to too high a temperature. For higher temperature ranges clay plates about 18 mm square and 1.5 mm thick can be used. Clay plates are just as effective in absorbing the eutectic melt as the filter paper but have the disadvantage that the residual pure compound may become contaminated with fine particles of clay. It was believed at first that the upper limit of usefulness of the filter paper was about 200°C¹⁶⁷. Later, however, it was found that, with rapid heating, filter paper could be used for substances with melting points up to 300°C so that it is almost never necessary to use the clay plate. Braun¹⁶⁸ and later Lindner¹⁶⁹ have, however, used the clay plate method with great success in the separation of quinoline and quinaldine derivatives.

2. Purification by Chromatographic Analysis

Recrystallization from solvents or absorption of the eutectic melt are sometimes not too satisfactory due to high volatility of the compound or close solubility relationships between impurity and major component. In many of these cases an adsorption material such as charcoal can be used to adsorb the impurity Tswett¹⁷⁰ originated chromatographic adsorption which applies

this same principal but uses a column of adsorption agent through which the solution of impurity and major component is passed. (For a few comprehensive works on chromatography see references 171-177).

Activated charcoal, aluminum oxide and lactose have been chosen from the long list of possible adsorbents as most satisfactory for use on a micro scale. Petroleum ether, benzene, chloroform, ether, acetone, ethanol, water and methanol have been chosen as solvents. These solvents are listed in decreasing order of affinity for the solute. In other words, a given solute molecule would, in general, be more easily adsorbed from a petroleum ether solution than from a methanol solution^{173,174}. Usually the intermediate solvents acetone, ethanol or water will be found most useful.

The simplest experimental arrangement is to use a glass column 10 cm long, 0.5 cm in internal diameter and drawn to a tip at the lower end. The bottom opening is plugged with a wad of glass wool and the adsorbent, usually aluminum oxide, is mixed with the solvent to be used to form a paste and this paste is drawn by suction into the column so that the packed height of adsorbent is about 6 cm long. A few ml of solvent are then added immediately and drawn through to wash down the walls and complete the packing of the adsorbent. The solution is then added to the top of the column and slowly percolated through the column by very slight suction. Single drops of the effluent solution are placed on a slide and evaporated by slight heating. To avoid spreading of the drop over the slide heating is carried out on a metal block having an opening in the center over which the drop is centered (Behrens-Kley¹⁷⁸). The solution evaporates quickly and concentrates at the coolest point on the slide (over the center of the opening in the block). It may, in some cases, be necessary to seed the residue to initiate crystallization.

Many compounds can be isolated as molecular addition compounds as, for example, aromatic hydrocarbons which give well-crystallized addition compounds with picric acid or trinitrobenzene. These compounds are best purified by chromatography in benzene or benzene-cyclohexane solution since the excess picric acid or trinitrobenzene are more strongly adsorbed and thereby removed.

3. Adsorption During Sublimation

Adsorption from the vapor state during sublimation is often a useful purification method¹⁷⁹. Air or some other carrier gas saturated with the substance to be purified is passed through a preheated adsorption column. By varying the pressure, temperature, and gas velocity suitable conditions can usually be found to ensure good separation.

A well known example of this method is the treatment of the vapors of subliming camphor with coal, sand, lime and iron powder. The method to be described is related to the method for adsorption during distillation of Hesso and Eilbrecht¹⁸⁰ but differs in that one is based on distillation and the other on sublimation. The usefulness of the sublimation-adsorption procedure is based on the case of sublimation of the compound in question. Information on this can be obtained in the Identification Tables, where it is stated which compounds sublime during micro determination of the melting point. About 80 per cent of all organic compounds sublime sufficiently to be purified by sublimation-adsorption. The compounds that do not sublime sufficiently may be purified by the same procedure but only by raising the temperature above the melting point in order to raise the vapor pressure.

Adsorption-sublimation is carried out in a glass tube 15 cm long and 5 mm inside diameter (Figure 22). A glass tube (2) about 6 cm long and 10-12 mm inside diameter is sealed to the upper end of the 5 mm tube. The adsorbent (3) is held between two asbestos plugs, (4) and (5). The upper plug is located at the junction between the narrow and wide diameter tubes. The sample (6) is placed below the adsorbent and held with an asbestos plug (7). The sublimate collects on a copper rod (8) in the upper wide portion of the column. The copper rod extends through a rubber stopper into the upper half of the column and is cooled externally with a water-cooled glass jacket (9). A connection for a vacuum pump is also present in the upper part of the column. Heating is carried out with a 15 cm glass tube electrically wrapped (10) which may be moved up or down the adsorption column. A thermometer (11) in a glass tube (12) is placed so that it registers the temperature of the adsorption column at equilibrium. A stream of air or carrier gas is passed through the column at a rate of 0.1-0.5 l/min by means of a stopcock.

The apparatus is made ready for use by placing the upper asbestos plug in position first and compacting it by pressure from both ends. The inverted column is next filled with dry adsorbent which is held in place by an asbestos plug.

Larger adsorption columns may be constructed and one 15 mm inside diameter and 20 cm long has been found to be very useful. At this larger diameter the plugs of asbestos fiber must be replaced with filter paper or asbestos paper. These plugs may be held in place by slip rings of aluminum, copper or other soft metals. The column must be packed carefully to eliminate the possibility of channeling. The upper portion of the tube need not be flared with this larger column and the condensing surface of copper and cooling of this rod are carried out as outlined for the smaller apparatus. Fritted glass discs may be used in place of the filter paper discs at higher temperatures.

The adsorbents ordinarily used are too strongly adsorbing in many cases since compounds in the vapor state are strongly adsorbed and, in a sense, show a lowered vapor pressure. As a result, very coarse silica gel, floridin XS and charcoal are generally used for sublimation-adsorption. Finely powdered adsorbents should not be used.

Silica gel is used in the form usually furnished for drying purposes (about 1/8" grains). Different grades and types of silica gel vary widely in their adsorbing power. All samples used should be ground and sieved to give a size fraction averaging about 0.5 mm. Smaller grains adsorb more strongly but hinder the gas flow. Silica gel is suitable as an adsorbent at temperatures up to about 150°C.

Floridin XS has small pores and a grain size of about 0.5 mm. The fines are removed before use. This adsorbent adsorbs more strongly than silica gel and may be used at temperatures up to about 200°C.

Charcoal is used in the form manufactured for use in gas masks. It is very strongly adsorbing and may be used at higher temperatures and for compounds that are weakly adsorbed. If temperatures above about 250°C are to be used air cannot be used as a carrier gas during the adsorption since at this temperature charcoal begins to burn. An inert gas must therefore be used with charcoal above about 250°C.

The choice of an adsorbent is usually based on the melting point of the sample to be purified. The higher the melting point the more strongly adsorbing the adsorbent must be. The adsorbent should be chosen so that the compound to be purified passes through the column at a temperature not more

than 20-30°C above its melting point. The upper limit is, to some extent, determined by the temperature at which the substance decomposes.

The vacuum used during adsorption-sublimation often affects the results obtained. When, for example, a mixture of azobenzene and salicylic acid are sublimed in this apparatus at atmospheric pressure azobenzene is less strongly adsorbed and leaves the column first. On raising the temperature, however, salicylic acid is eluted from the column. If now a vacuum of 20 mm of mercury is applied to the column again a small quantity of azobenzene will be seen condensing on the copper rod. This additional amount of azobenzene had presumably been held by capillary condensation.

The column of adsorbent often shows colored bands during a separation. These bands may be more intense in color or even different in color from the substances in the normal solid state. For example, p-nitroaniline adsorbed on floridin XS shows a dark yellow band and m-nitroaniline a brown band whereas both are ordinarily yellow. Even normally colorless compounds may show on the column as a different shade of color than the adsorbent itself. The normally colorless 2-methyl-2,4-naphthaquinone is red when adsorbed. This phenomenon is more strongly pronounced with floridin XS, hence this adsorbent is often used in order to be able to see the adsorbed substance during the adsorption. For the same reason charcoal is seldom used and only when high melting compounds are involved.

A disadvantage of this technique is that in some cases the adsorbent catalyses decomposition of the adsorbed substance. Some compounds normally stable 40-50°C above their melting point may decompose on the adsorption column at temperatures even below their melting point. It is often possible, however, to avoid decomposition of a given compound by choosing a different adsorbent. For example, 1,2-benzanthracene, α -cinnamylbenzylcyanide, α - and β -naphthol and α -naphthylamine decompose on silica gel but remain unchanged on floridin XS at corresponding temperatures. It has not yet been possible, however, to find suitable adsorbents for some compounds. Methylolbenzanide, 1,8-dioxyanthraquinone, α -naphthylaminothiourea and veratrylcyanide decompose completely on silica gel, floridin and charcoal. In some cases one of the decomposition products may be isolated in a pure form on the condensing surface; for example, pure benzamide is isolated from methylolbenzamide.

The weight ratio of adsorbent to sample depends on the extent of purification necessary. A final purification may be carried out with a weight ratio of 10 parts of adsorbent to 1 part of sample. A ratio of 20:1 or greater is required for separation of a major component of a mixture.

Elution of the desired component from the column is carried out by passing air or an inert gas through the column as the temperature is slowly raised. The rate at which the gas passes through the column is very important. If too fast, there is insufficient time for equilibrium in the column; there may be channeling and the eluted substance may be carried past the condensing surface and out the exhaust. If too slow, the time of the experiment is unnecessarily prolonged. With the proper flow rate a complete experiment generally requires 2-3 hours. A flow rate of about 0.1 l/min and a vacuum of 20-30 mm of mercury is recommended.

The condensing substance appears on the copper rod as fine crystals which may be removed at will to check the purity by means of the micro melting rod or cleaning at intervals.

Even technical grade samples may be obtained completely pure by one adsorption in this apparatus, e.g., o-, m- and p-nitroaniline, anaesthesin,

m-aminophenol, p-nitrophenol, anthraquinone, p-nitrobenzene, p-nitroanisole, and 2-methyl-1,4-naphthoquinone. The crude brown "Acidum Benzoicum e Resina" used by pharmacists can be purified quickly and easily in this apparatus to give pure benzoic acid in spite of the fact that repeated sublimation of this same material does not give a pure product.

Mixtures of benzoic acid-cinnamic acid can be completely separated by adsorption-sublimation in spite of the fact that repeated fractional sublimation alone fails to make this separation¹⁸¹. Similar o-, m- and p-nitroaniline may be separated and in the same order found by Karrer and Nielson¹⁸² for chromatography of a solution of these compounds. Isomorphous substances such as 2,4,6-tribromophenol and 2,4,6-trichlorophenol may also be separated.

The following tables (3 and 4) give the results of purification experiments (3) and separation experiments (4). All of the experiments were carried out with the small apparatus with a vacuum of 20 mm of mercury and an air velocity of 0.1-0.2 l/min.

TABLE 3

a. Purification of Substances Adsorbed Without Decomposition

Substance	Adsorbent	Melting point (°C)		Color of band on column
		Before	After	
o-nitroaniline	Floridin XS	70-73	73	red
m-nitroaniline	Floridin XS	112-113	114	brown
p-nitroaniline	Floridin XS	146-148	148	dark yellow
anaesthasin	silica gel	84-88	90.5	colorless
p-nitrophenol	Floridin XS	112-114	114	colorless
benzoic acid resin	Floridin XS	110-121	122	colorless

b. Substances that may be adsorbed only on some adsorbents without decomposition

Substance	Adsorbent	Melting point (°C)		Remarks
		Before	After	
1,2-benzanthracene	silica gel	154-156	-	decomposes; column black
1,2-benzanthracene	Floridin XS	154-156	158.5	column dark blue
α-naphthylamine	silica gel	47-49	-	decomposes; column dark purple
α-naphthylamine	Floridin XS	47-49	49.5	column dark purple

TABLE 4

Separation

1:1 mixtures; adsorbent, Floridin XS

Mixture	Melting point of fraction (°C)				
	I	II	III	IV	V
benzoic acid-cinnamic acid	122	85-91	135	-	-
azobenzene-salicylic acid	68	115-156	156	-	-
o- and p-nitroaniline	73	73	73	59-67	148
m- and p-nitroaniline	114	114	91-100	148	-
2,4,6-tribromophenol and 2,4,6-trichlorophenol	66	66	66	62-64	94

IV. PROCEDURE FOR THE IDENTIFICATION OF SOLID ORGANIC SUBSTANCES

A. Identification of Solid Organic Substances

The physical constants which can be determined microscopically and which are useful for identification of solid organic substances are listed in the Identification Tables (Part II). About 1200 compounds are now included in these tables listed in order of increasing melting point. The melting points of eutectic mixture with two standard compounds are also listed as well as the refractive index of the melt by the glass powder method. These four constants are sufficient for rapid and certain identification of any pure compound covered in the tables. In another column of the tables are listed a group of special characteristics of each compound during heating; these may be used to confirm the identification and, in some cases, to speed the identification. Since many compounds normally liquid at room temperature can be identified microscopically as derivatives, the tables list a large number of such derivatives. Alkaloids can be identified by the melting points of the styphnates¹⁸³⁻¹⁸⁵.

The use of these tables presupposes that the unknown is pure. Usually a pure sample can be obtained in a few minutes by micro sublimation although in certain cases absorption of the eutectic melt using filter paper may be necessary. In cases where recrystallization from solvents is necessary activated charcoal should be added to the hot solution to adsorb impurities. The precipitated crystals may be isolated by filtration or by centrifuging (Lieb and Schoniger^{186,187}). In special cases, absorption methods must be used (see page 47). The procedures for purification are given in the Explanation to the Identification Tables (page 187).

B. Identification of Organometallic Compounds

Organometallic compounds such as drugs may be identified by micro methods. The general procedure is to liberate the organic portion of the compound, purify it and identify it using the tables. A few experiments of this type carried out by Brandstätter and Thaler¹⁰³ follow:

Tribromophenyl bismuth

About 100 mg of this substance is heated to 50°C on a microscope slide with 2 drops of concentrated hydrochloric acid. After the acid has completely evaporated the residue is sublimed at 70-90°C. Needles of tribromophenyl melting at 94°C condense.

Calcium phanodorm

About 200 mg of this material is heated to 100°C on a microscope slide with 3 drops of concentrated hydrochloric acid until the acid is completely evaporated. The residue is sublimed at 160°C. The sublimate consists of supercooled droplets of phanodorm (173°C).

Calcium oxalate

Oxalic acid is liberated from its calcium salt by heating about 200 mg of the salt with 5 drops of concentrated hydrochloric acid on a slide at

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100°C. After the acid has evaporated the treatment is repeated once, then the residue is sublimed at 180°C. Anhydrous oxalic acid crystals are obtained.

Saccharine (sodium o-benzoic acid sulfimide)

About 100 mg of saccharine is dissolved in a drop of water on a microscope slide and 2 drops of hydrochloric acid are added to decompose the salt. Free saccharine precipitates after which it is dried at 100°C and sublimed at 190-200°C.

Zinc salicylate

Salicylic acid can be sublimed at 120-130°C from the residue after treatment of about 200 mg with 3 drops of concentrated hydrochloric acid.

Theophyllino-sodium salicylate

Three drops of concentrated hydrochloric acid are used to decompose about 200 mg of this substance. The residue after drying at 100°C is sublimed at 125°C to remove the salicylic acid and finally at 200°C to obtain theophyllin.

Quinosol (equi-molecular compound of o-oxyquinolinsulfate and potassium sulfate)

This compound (about 200 mg) is dissolved in about 1 cc of water and 3 cc of saturated bromine water is added. After shaking, the excess bromine is destroyed with sodium sulfite and the precipitate is filtered off and sublimed 170-180°C. The sublimate melts at 200°C (5,7-dibromo-8-oxyquinoline).

Since the micro melting point apparatus has been calibrated by melting substances between coverslip and slide (see page 58) the values obtained in a capillary tube in a metal block must be corrected to give the true melting point. However, since the molecular weight determination depends on a difference in melting point between the pure solvent and the solution of the compound in bornyl chloride determined in identical fashions, no correction is necessary.

Sodium sulfosalicylate

This compound (0.2 g) is dissolved with heat in 1 cc of water; to this is then added 1 cc of a hot 15 percent alcoholic solution of S-benzyl thiuronium chloride. After cooling crystallization is initiated by scratching the walls of the flask. Five cc of water are then added and the salt precipitates in large amounts. The dried crystals are recrystallized with activated charcoal from 10 cc benzene and 4 cc alcohol given S-benzyl thiuronium sulfosalicylate (m.p. 198-200°C).

Sodium di-iodo-p-phenolsulfonate

The procedure here is the same as above; 0.1 g of the substance is dissolved in 0.5 cm of water on a watch glass by warming on a watch glass.

To this is added 0.3 cc 15 per cent alcoholic S-benzyl thiuronium chloride solution and after 0.5 minutes the watch glass is removed and allowed to cool. The precipitate is recrystallized from 5 cc benzene and 1-2 cc alcohol with activated charcoal. The dried product melts at 190-192°C.

Melubrin

Melubrin (0.1 g) is dissolved in 2.5 cc of water and heated formaldehyde and SO₂ are driven off. After cooling 0.5 g of sodium acetate is dissolved in the solution and 1.5 cc of alcoholic benzaldehyde is added (0.5 g benzaldehyde in 12.5 cc alcohol). A yellow precipitate of 4-benzamide-antipyrene (m.p. 176°C) results on shaking.

Sodium p-aminosalicylate

Three drops of water on a microscope slide are sufficient to dissolve about 200 mg of this substance. Then 5 drops of concentrated hydrochloric acid are added and heated to dryness. The residue sublimes at 170°C to give good crystals of the hydrochloride of p-aminosalicylic acid (m.p. 215-220°C with slight darkening in color).

C. Identification and Analysis of Organic Liquids

1. Indirect Detection Using Solid Derivatives

The preparation of solid derivatives of organic liquids is done directly by precipitation or by vaporizing the liquid so that it comes into contact as a vapor with the liquid or solid reagent in a hanging test drop above the test substance (hanging drop method). An example of the latter procedure is given by Griebel^{188,189} for the detection of aldehydes and ketones.

The liquid to be identified is placed in a micro beaker which is covered with the coverslip on the underside of which is the reagent drop. The beaker is then slightly heated in order to vaporize the aldehyde or ketone. Griebel used several different reagents (substituted phenylhydrazine, semicarbazide etc.) to obtain positive or negative results and paid a great deal of attention to the appearance of the crystals of the reaction product. Fischer and coworkers¹⁹⁰ showed with photomicrographs that most of the precipitates were made up of needles, and so similar in appearance that they were difficult to differentiate. They introduced, therefore, the micro melting point for the identification of precipitates and in this way put this method on a sound basis. Fischer and Paulus¹⁹¹ used the procedure for detection of the volatile alkaloids, coniine and nicotine. For additional applications of the hanging drop method see Opfar-Schaum¹⁹² and Markovic¹⁹³.

Mathiesen and Hagadorn¹⁹⁴ identify aldehydes and ketones as 2,4-dinitrophenylzones. The glass powder method for the determination of refractive index of the melt has been used by Brandstätter¹⁹⁵ to analyze quantitatively binary mixtures of such derivatives. Brandstätter and Thaler¹⁹⁶ have performed a wide variety of experiments of this type on alkaloids, phenols, esters, aliphatic acids and benzene derivatives. Primary alcohols form 3,5 dinitrobenzoic acid esters¹⁹⁷ and phenols the p-nitrobenzoic acid esters. In both cases the final identification is based on the Identification Tables. Esters are first saponified and the acids and alco-

hols are identified separately. The acids are identified as such or as hydrazones with confirmation as the S-benzyl thiouronium salt¹⁹⁸. Aniline, nitrobenzene, benzene and similar compounds form tribromoanilides; pyridine, quinoline etc. form good picrates.

2. Detection by Micro Boiling Points, Refractive Indices or Critical Temperature

The micro boiling point method as used by Fischer¹⁹⁹ is a modification of a method used by Emich²⁰⁰ and by Alber and Bryant²⁰¹. Brandstätter and Thaler have used the method of Siwoloboff²⁰² which is simpler than the Emich method. With this procedure the boiling point capillary is placed in the metal block designed for micro molecular weight determination (page 59).

Fischer¹⁹⁹ uses a glass cell for the determination of the refractive index of the liquid. The cell is filled with the liquid and a suitable glass powder. The refractive index at 80°C is calculated from the temperature coefficient determined using two glass powders²⁰³.

The determination of the critical temperature is carried out by Fischer and Reichel²⁰⁴ in a capillary under the microscope. It is a very useful constant in some cases.

3. The Critical Solution Temperature as Means of Identification and Analysis

Fischer and his co-workers²⁰⁵ have used a glass capillary 30 mm and 0.7-0.8 mm inside diameter for the micro determination of critical solution temperatures. The two components are sealed in the capillary and heated on the hot stage. The disappearance and reappearance of the meniscus can be observed usually at a single temperature and with only 1-2 mg of each component. The critical solution temperature (MT_c) may be used as a characteristic constant for identification, for quantitative analysis and for testing purity.

Fischer and his co-workers^{205,206} recommend the following test substances for identification and testing purity:

- (a) Aniline, benzyl alcohol and tricresyl phosphate for aliphatic hydrocarbons.
- (b) Performic acid for halogen derivatives of lower aliphatic hydrocarbons.
- (c) Methanol and aniline for naphthene.
- (d) Paraffin oil for lower aliphatic alcohols.
- (e) Water for intermediate aliphatic alcohols.
- (f) Glycerin for aliphatic aldehydes and ketones.
- (g) Paraffin oil for fatty acids.
- (h) Glycol or ethylcyanide²⁰⁷ for benzene and its homologues.
- (i) Ethylene cyanide or glycol for halogen derivatives.
- (j) Water for phenols.
- (k) Glycol for essential oils²⁰⁵.
- (l) Butanol or phenol for aqueous solutions of inorganic substances.

The following test substances are given²⁰⁵ for the analysis of bi-

mixtures: Phenol and n-butanol for inorganic compounds; ethylene cyanide, water, glycol, acetophenone, acetone and paraffin oil for organic systems. The quantitative determination of binary mixtures by this procedure is illustrated in Figures 23 and 24. Ethylene cyanide is used for the pairs benzene:xylene and benzene:toluene (Figure 23). The accuracy is, of course, greater at the MT_k of the benzene mixtures. Figure 24 shows the systems phenol:aniline and phenol:cyclohexanol. Water is used to the test substance; it possesses an MT_k of 66°C with phenol, 167°C with aniline and 182.5°C with cyclohexanol.

4. Identification Using the Cold Stage

Using the cold stage discussed on page 8 temperatures down to -55°C may be reached. The determination of the micro melting point on the cold stage has the advantage that the degree of purity may be judged. The refractive indices of the melt can also be determined with the cold stage. A value above and one below 20°C should be determined so that the temperature coefficient may be used to calculate the refractive index at 20°C for comparison with the literature. Solubilities in various other liquids may be tested and eutectic temperatures determined by mixing 2 drops or in a mixed fusion. Finally if the amount of sample permits, a molecular weight determination may also be carried out.

V. MOLECULAR WEIGHT DETERMINATION

With the increasing use of microscopical methods for the determination of melting points and other physical constants, the question repeatedly arose as to whether molecular weights could be determined with the micro melting point apparatus. Above all it seems most natural to expect that the method of Rast²⁰⁸ might be used with microscopic observation of melting of the last crystal residue in the hope that this would be easier than observation with the naked eye or hand magnifier. According to the investigations of Kofler and Brandstätter²⁰⁹, two different methods can be used. The mixture can be melted on the hot stage beneath the microscope either between object slide and coverslip or in a sealed capillary tube.

1. Between Object Slide and Coverslip

In general, the object slide and coverslip preparation is preferred for molecular weight determinations with the hot stage since all changes can be followed more readily. With some substances this is not, however, possible due to high volatility, e.g., camphor. Even during melting of the mixture between coverslip and slide and finally through heating of the crystalline film evaporation of the camphor causes a high loss, so that no reproducible values are obtained even after cementing the edges of the coverslip. A number of substances proposed by Pirsch²¹⁰ have been tried in place of camphor for molecular weight determination.

The bornyl chloride used by Kofler and Brandstätter has a melting point of 134°C and a molar depression of 45°C. The melting point and molar depression must be determined, however, on the actual material used since both values and especially the latter, depend on the degree of purity. Bornyl chloride is not as volatile as camphor, although it is still necessary to seal the edges of the coverslip in order to avoid loss during determination of the melting point.

The molecular weight determination between coverslip and slide using bornyl chloride is carried out in the following way: A carefully weighed mixture of bornyl chloride and the substance to be investigated are mixed in a mortar in the approximate ratio of 10:1. The substances should be wetted with a few drops of pure ether during the grinding in order to obtain thorough mixing. A small portion of this mixture is placed on an object slide, covered with a coverslip, and placed on the hot stage previously warmed to 150°C or on another hot plate warmed to 150°C. As soon as the mixture is completely molten the preparation is immediately removed from the hot plate and cooled on a cold metal block. The coverslip is then sealed with a cement (for example, nitrocellulose lacquer or Duco®) and dried for a short time at 30 to 50°C. Several identical preparations are made at the same time, and the melting operation should be as rapid as possible in order to avoid sublimation of bornyl chloride. The preparation is then placed on the hot stage which has been previously heated to 80°C. Heating is continued with the expectation that the melting point will lie in the region of 100 to 110°C. A portion in the center of the coverslip is used for observation. The crystals of bornyl chloride remaining after melting begin from a fine network which can be observed best using axial illumination (condenser diaphragm nearly closed). During the melting operation the fine focus adjustment is used continuously in order to follow the solution process. Toward the end of melting a few rounded crystals will remain floating in the melt, and the

temperature at which the last crystal residue disappears is noted. The melting point is determined on three to four preparations with only 5 to 10°C cooling of the melting point apparatus between each determination. The variable resistance is set so that the temperature rises two degrees per minute in the region of the melting point. The average of these determinations is used for calculation of the molecular weight.

Only substances melting under about 130°C can be determined using bornyl chloride. No suitable solvents have been found for higher melting substances. This represents, then, the upper limit restricting the use of molecular weight determinations in object slide-coverslip preparations.

2. In Small Capillary Tubes

A second very useful method of determining molecular weight is based on measurement of the capillary melting point on the microscope hot stage as developed by Kofler and Brandstätter²¹¹ and Brandstätter²¹². If a small sealed capillary tube about 1.8 mm inside diameter containing a mixture of camphor and the compound to be determined is heated in the hot stage, the camphor will sublime to the upper end of the capillary. This difficulty can be eliminated, if the capillary tube is enclosed in a flat metal block which has a small window for observation at the lower end of the capillary. This metal block can be fastened into the micro melting point apparatus in any way but preferably so that it can be moved from the outside (Figure 24). The metal block (corresponding to the space between the moving arms) is 33 x 26 x 5 mm and has an opening of 3 mm in diameter bored from the small side of the block. The window for observation of the specimen is in the center of the metal block and has a diameter of 3 mm. The metal block is fastened on the hot stage by means of the movable arms so that the window falls directly above the round opening in the hot plate.

The freezing point as well as the melting point are determined. The calculations of temperature difference are generally based on the melting points. The temperature for spontaneous crystallization lies 1-1.5°C lower than the melting point and for 10 percent mixtures in camphor lies 2-2.5°C lower. The determination of the freezing point (Figure 21) is a reproducible control point for subsequent reheating for the melting point determination. In an actual determination the capillary preparation is completely melted and allowed to cool slowly (1°C per minute) until crystallization begins. The temperature is then raised slowly (2°C per minute) to determine the melting point of the last crystals to disappear. The temperature is read to the nearest 0.5°C. Checking the melting points thus obtained with an Anschütz thermometer graduated to 0.1°C has shown that the 0.5°C accuracy is correct and sufficient.

The following table shows the melting points of a camphor mixture and of camphor determined with four different Anschütz thermometers and four ordinary laboratory thermometers with 1°C graduations. The temperature differences calculated from the two sets of data are almost identical.

Anschutz Thermometers
Melting Point

Camphor	Mixture
178.8	166.7
178.6	166.8
178.8	166.5
<u>178.4</u>	<u>166.7</u>

$$\Delta T = 11.95$$

Laboratory Thermometers
Melting Point

Camphor	Mixture
179	166.5
178.5	166.5
178.5	166.5
<u>178.5</u>	<u>166</u>

$$\Delta T = 13$$

Wherever possible crossed polars should be used for the observation of the melting point. However, in many cases, excellent solvents for use in molecular weight determination are isotropic and cannot be observed with crossed polars. This is true for camphor which, although it possesses a birefringent modification, usually exists in an isotropic form with a transition between 80°C and 125°C so that the isotropic form is stable throughout the range of practical use of this compound. Table 5 lists the possible solvents and their behavior with crossed polars. Only two of the nine are birefringent in the temperature range of interest.

TABLE 5

<u>Substance</u>	<u>Micro m.p.(°C)</u>	<u>Optical Behavior</u>
Perylene	276	Anisotropic
Borneol	204	Isotropic
Hexachloroethane	186	I isotropic; II anisotropic; $T_T^* \quad 72^\circ\text{C}$
Camphor	178	I isotropic; II anisotropic; $T_T \quad 80-125^\circ\text{C}$
Bornylchloride	130	Isotropic
Tetrabromomethane	93	I isotropic; II anisotropic; $T_T \quad 46.5^\circ\text{C}$
2,4,6-Trinitrotoluene	82	Anisotropic
Cyclopentadecanone	65	Anisotropic
Camphene	49.5	Isotropic

* T_T transition temperature

The literature²¹³ reports that the use of camphor is limited to those cases where an addition compound is not formed. Many experiments using compounds that are reported to form addition compounds with camphor showed no error due to compound formation. Furthermore, Rast originally calculated the molar depression for camphor using a system later shown by Le Fèvre²¹⁴ to

possess an addition compound. Even with salol and salicylic acid which are reported to form addition compounds the correct molecular weights are obtained. Obviously in most cases the addition compound dissociates at the temperature of melting so that very few, if any, molecules of the undissociated compounds remain.

A much more common difficulty is the insolubility of the test substance in camphor. To quickly determine whether the substance is soluble in camphor and similar compounds are highly volatile, this solubility test is modified slightly. A few crystals of camphor are melted under the center of the coverslip on a microscope slide and cooled quickly on a cold metal block. The second component is then melted at the edge of the coverslip so that it runs under the coverslip into contact with and completely surrounding the camphor. The camphor is thereby sealed in so that it cannot readily volatilize and the degree of solubility is estimated by observation of the zone of mixing. In those cases where only small quantities of test substance are available the preparation is sealed in with a third component such as mannite.

Where the two components are mutually soluble in the melt a eutectic will be observed. The other extreme indicating insolubility is the formation of two liquid layers on melting. Other possible solvents can, of course, be tested in a similar manner although sealing is not necessary for solvents of lower volatility such as 2,4,6-trinitrotoluene.

Only solvents having a high molar depression of the melting point (Beckmann constant) can be used for the melting point determination. Pirsch associates a nearly spherical molecular shape with the low heat of fusion characteristic of such compounds. Rast²¹⁵ has also pointed out that these compounds also usually sublime readily (*i.e.*, have a narrow liquid range). Rast, however, points out that some substances have a high Beckmann constant (cyclohexanol, cyclopentanone) but do not sublime readily and, conversely, bromocamphor sublimates readily but has a low Beckmann constant. There is probably no simple correlation to cover this situation.

Ekborn²¹⁶ has also reported use of the micro hot stage for the determination of molecular weight by the Rast method. He noted by his procedure either the melting point of the last camphor crystals or the initial freezing point on slow cooling of the melt. In the first case he obtained less accurate values although this was undoubtedly due to the fact that he raised the temperature too rapidly, 5°C per minute. Furthermore, he rested the capillary tube horizontally on the hot stage; this has been found to produce less accurate results than when the tube is inclined since the latter method concentrates the excess camphor crystals at the bottom of the tube in the field of view. The fact that Ekborn observed accurate results by determining the freezing point is probably due to the slow cooling rate, 2°C per minute. There is always, however, a possibility of supercooling with this procedure.

Ekborn mentioned that it is advantageous with some mixtures to use polarized light. It should be noted, however, that camphor crystallizes at high temperatures as a cubic and, therefore, isotropic modification so that observation with crossed nicols is impossible. The transformation temperature of cubic camphor to the birefringent hexagonal form lies close to the melting point according to Tammann, however, it is usually observed in these preparations at about 78°C. It is obvious, therefore, that polarized light should not be used for the micro molecular weight determination by the Rast method under the microscope unless the melting points to be determined are below the transformation temperature.

The transition point of camphor and its dependence on purity has, unfortunately, never been accurately determined. (Translator's note: molecular weight calculations using camphor will be different depending on the polymorphic form obtained since the melting points of the two forms are different.)

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VI. MICRO THERMAL ANALYSIS

A composition diagram in an average inorganic system is often based on the results of about one year's investigation. It is frequently possible to clear up the very complicated behavior of many practically important two-component systems of metals or silicates only through compilation and consideration of partial data from many investigations.

The knowledge of composition diagrams for organic systems has been based almost exclusively on the results of thermal analysis. In the usual procedure the temperatures of "primary crystallization" have been determined and the melting point curve is then obtained by joining these points. The inevitable results of this type of determination is the failure to recognize many of the characteristics of that system related to the crystal phases which appear, and thus the synthesis of the diagram for a two-component system of organic compounds has been regarded as simple. It appeared as if each organic two-component system should be placed in one of the theoretical basic types. This followed particularly for systems showing mixed crystal formation in which each composition diagram appeared to fall in one of the five Roozeboom types. Only in a few cases was determination of the relation between substances supplemented by petrographic methods or by x-ray diffraction and in these cases only to differentiate between pure substances. For this reason, the crystal chemical investigation of organic two-component system based, on the one hand, on thermal analysis and, on the other hand, on morphological and internal structural studies led to repeated contradictions.

New insight into the nature of organic two-component systems is given by the use of micro thermal analysis which essentially consists of making microscopical observations during thermal analysis. Contradictions and differences in opinion could, in many cases, be easily resolved by these techniques. In one series of cases, unknown crystal phases corresponding either to molecular compounds or to a definite mixed phase were found within certain composition intervals. The latter is related to polymorphism, in that an unstable polymorph is stabilized by solid solution formation (isopolymorphism). These components arise only partly from the stable phases and would not be detected by macro thermal analysis, since the curve deviates too little from the simple curves. Partly, however, new phases result from unstable modifications which become stable in the mixed state. These hitherto unknown organic phases characterized as stabilized intermediate phases deserve special interest because of their analogy with the intermediate phases in metallic systems.

It can be concluded that micro thermal analysis of organic two-component systems is neither as simple nor typical as assumed earlier from the results of macro thermal analysis. In particular, the observation of isodimorphism or isopolymorphism which has been specially emphasized by Bruni and Groth is indispensable in the evaluation of the isomorphous behavior of organic materials. The errors which occur repeatedly even in recent literature can frequently be traced to neglect of isopolymorphism in the behavior of crystal mixtures.

Micro thermal analysis as described in the following sections covers the techniques available for determining all crystal phases appearing in a given system and offers, therefore, a basis for further procedures leading to morphological structure investigations. In systems involving isopolymorphism, an analysis of these phases is not always possible because of their instability. Microscopic thermal analysis is usually the only way in which the correct composition and relation between all phases can be determined.

Before discussing the microscopical methods for investigation of two- and three-component mixtures and the information that can be obtained from them, a preliminary discussion of polymorphism and isomorphism is necessary. This is necessary since without an accurate knowledge of these phenomena, an understanding of the crystal-chemical relationships in two- and three-component systems is not possible.

Polymorphism and isomorphism must be discussed before micro thermal analysis for still another reason. In both cases the procedure is the same; hence frequently the discovery of a new modification of an organic substance is an unexpected result of the determination of a two-component phase diagram.

A. Polymorphism

1. General

The term polymorphism signifies the property of many substances to crystallize in different crystal forms or modifications depending on the experimental conditions.

Mitscherlich¹ in 1821 first reported two different crystal forms of the same substance (monosodium phosphate) and soon he was also able to report monoclinic and rhombic crystal forms of sulfur². In 1832 Wohler and Liebig³ reported the dimorphism of benzamide, the first example of polymorphism for an organic compound. There followed then the discovery of two modifications of p-nitrophenol⁴ and of 1,2,4-chlorodinitrobenzene⁵. The observation, first reported by Lehmann⁶ for sulfur, that either of two crystal forms may be stable depending on the temperature and that a transition temperature exists separating the two stability zones was later reported for many organic substances. With increasing use of the microscope the recurrence of different crystal forms has been shown to be very common for both organic and inorganic substances.

The early view concerning polymorphism expressed by Küster⁷ held that different polymorphic forms were made up of identical ions, atoms or molecules. Tammann⁸, however, felt that there should be two basically different mechanisms, one involving identical molecules arranged differently but another involving slightly different molecules arranged more or less the same. The theory of Smits⁹ that the different crystal forms are mixed crystals of different molecular types is denied by Tammann.

The phenomenon of polymorphism is still not clear. The use of x-ray diffraction to locate the exact positions of the atoms in the crystal lattice will help to explain many of the things still unknown about the relation between crystal form and chemical constitution. To some extent this has been done for some inorganic compounds. Assume for discussion purposes a single cation surrounded by eight somewhat larger anions. This arrangement (Figure 26) is limited by the relative sizes of the anion and cation as given by

$$R_A^2 + 2R_A^2 = (R_A + R_C)^2$$

and:

$$R_A = \sqrt{3} = R_A + R_C$$

and the radius ratio, R_C/R_A , must be smaller than about $\sqrt{3}-1 = 1.732$ since, if the anions are larger they will be forced apart and away from the cation, thereby loosening the anion-cation bond and causing instability of that lattice. Another lattice then with a lower coordination number for the cation (say 6 as in Figure 27) becomes the stable form. In this form the limiting R_C/R_A ratio is 0.41. If the anions become larger this form will, in turn, be unstable and a coordination number of four with a R_C/R_A ratio of 0.22 becomes stable. With further increase in anion size relative to the cation three and two coordination numbers only are possible.

The atomic radius and therefore the radius ratios depend on the temperature and pressure. If a given lattice has radius ratios near the limiting values for the coordination number shown a temperature change could cause a change in ratio such that a transformation to a system of different coordination number would be possible or perhaps necessary. For example, CaCl_2 and NH_4Cl each have two enantiotropic modifications. The low temperature forms have a coordination number of eight and the high temperature forms six with transition temperatures at 445°C (CaCl_2) and 184°C (NH_4Cl). As a rule the lattices with higher coordination numbers tend to be stable at lower temperatures and higher pressures (M. Buerger¹⁰).

In the carbonate series (Mg, Ca, Sr and Ba) and in the nitrate series (Li, Na and K) the radius ratios change in such a way that the lower members of both series show a trigonal lattice while the higher numbers show a rhombic lattice. Both CaCO_3 and KNO_3 , however, have radius ratios near the limiting value and can crystallize in either lattice depending on the temperature. These two are, therefore, dimorphous.

The number of organic compounds showing polymorphism that have been studied from a lattice structure point of view is very small. Crystals of organic compounds are more complex from a molecular standpoint and usually possess lower symmetry than inorganic compounds. Investigations of the relationship between the polymorphic forms of organic substances has been studied for picrylbromide¹¹ and for some long chain molecules (page 76).

The lattice forces in crystals of organic substances are not ionic or atomic as in most inorganic compounds but molecular (i.e., Van der Waals or, in many cases, hydrogen bonding) (Nowacki¹²). The hypothesis that different polymorphic forms of a given organic substance are made up of identical molecules is gradually being abandoned. Rather, it is now believed that the molecule is modified slightly (not necessarily chemically but at least sterically) during the transition from one polymorphic form to another.

All polymorphic forms of a given compound dissolve or melt to give liquids having identical physical properties. This fact forms the basis for the difference between isomorphism and polymorphism¹³ since in the former case there is a molecular difference which persists in the liquid state. Zincke¹⁴ described as polymorphs two crystal forms of benzophenone; however, Schaum¹⁵ by careful work has found small differences in refractive index and viscosity due to lattice differences alone. Similar behavior has been found for such compounds as pyrazolone¹⁶, nitrostilbene¹⁷, the three allocinnamic acids¹⁸, chalcone^{19,20} and derivatives which contain the benzylidene nucleus²¹⁻²⁴. Hantzsch²⁵ has shown that it is very difficult to draw a clear line of demarcation between isomorphism and polymorphism. In investigations on nitro compounds Hantzsch has reported chromoisomorphism and homochromo-isomorphism. The former involves colored forms of a nitro compound either heteropolar or homopolar. In the latter case the two forms are identical in color but differ in solubility and melting point. Hantzsch believes these differences to be due to small differences in arrangement of the atoms in the unit-cell. The structure of the nitro group according to Hantzsch is similar to that of ammonia with nitrogen at one apex of an almost regular tetrahe-

dron. The other apexes are occupied by two oxygen atoms and the rest of the molecule¹³. Different forms of the nitro compounds then involve variations in the bond distances and in chromo-isomerism the oxygen atoms take a larger share of the bonding. Chromo-isomerism and homochromo-isomerism are regarded by Hantzsch as isomerism but by Billmann²⁶ as polymorphism.

Roozeboom²⁷ regards these phenomena as parts of a single over-all phenomenon. According to the most recent ideas²⁸⁻³¹ polymorphic forms are regarded as slightly different stereoisomers in which the difference is restricted to the crystalline state. Rapid solution phase or melt phase transformations to the stable form at that temperature should be expected on this basis. There are, however, all gradations in stability between polymorphic and isomorphous phases¹³.

Since there are more possibilities for varying the arrangement of a molecule in a molecular lattice^{31,32} there are usually more chances for polymorphism with organic substances. It is probably a safe assumption that all organic substances are polymorphic given a sufficiently wide temperature or pressure variation.

2. Enantiotropism and Monotropism

The modifications behave like different states of aggregation of one and the same substance. They show differences in their physical properties, e.g., melting points, heats of fusion, specific heats, solubilities, densities, crystal structures, optical properties, electric conductivities etc. At one definite pressure and temperature only one form is stable, the other unstable, unless these conditions happen to coincide with the transition temperature. In general, the unstable modification transforms at a more or less rapid rate into the stable form.

Two modifications can be separated at a definite temperature, since the unstable phase is more volatile and possesses a higher solubility. Both the vapor pressure and solubility increase with increasing temperature; this relation can be shown as vapor pressure curves which would be different for the two modifications²⁷⁻³⁴. In Figure 25 curve I-I shows the vapor pressure curve for one modification, II-II for the other modification, and f-f for the liquid phase. Curves I-I and II-II intersect each other at point u. Therefore, if room temperature lies above point u, form II will possess the higher vapor pressure, will be unstable, and will be transformed to modification I which melts at F_1 . If, however, form II can be held without transformation, melting will then occur at F_2 , a lower temperature than the melting point of I. If the preparation is cooled, the melt should crystallize as Form I which would then, on reaching point u, transform into form II. This type of behavior of polymorphic forms is described as enantiotropic³⁵. If the melt can be supercooled to point x, the unstable form II may solidify spontaneously according to Ostwald's law of successive states which states that an unstable phase will change to the phase involving the smallest possible loss of free energy.

The transformation temperature, like the melting point, is a definite constant for the system at a constant pressure and the transformation of II to I at u evolves heat. Modification I possesses as a rule a higher specific heat and a lower density than II. The vapor pressure curves can also lie so that the transition temperature lies above the melting point and is, therefore, inaccessible (Figure 29). With such materials (which are more common than those exhibiting enantiotropism) modification II, with the higher vapor pressure, is unstable while modification I with the lower vapor pres-

sure is stable at all temperatures below the melting point. When heated, modification I melts before the transformation point, u , is reached. All transformations in such systems progress only in one direction, from II to I and not vice versa. Such modifications are called monotropic (Lehmann³³).

The limiting case occurs when the vapor pressure curve of the melt f - f passes through the transition temperature. Under these conditions the melting points of both modifications and the transition temperature are all identical (e.g., β -naphthol³⁶). The two forms of γ -hexachlorocyclohexane melt at almost identical temperatures (about 0.1°C apart). The presence of a small percentage of impurity causes the two forms to melt at the same temperature³⁷.

It is usually very easy to determine the melting point of form II in an enantiotropic system especially of an organic compound (Figure 28). This melting point can also be calculated from the equation of Le Chatelier-van't Hoff. Verstrete³⁸ used this

$$\ln \frac{C_2}{C_1} = \frac{Q}{R} \left(\frac{1}{T_1} - \frac{1}{T_2} \right)$$

equation to calculate the melting point of the unstable form of CCl_4 (-39.6°C¹³).

The transition temperature, u , (Figure 29) can never be measured for a monotropic system although according to Schenk³⁹ it can be calculated using the following equation:

$$T_u = \frac{F_1 \Delta_2 - F_2 \Delta_1}{\Delta_1 - \Delta_2}$$

where T_u is the transition temperature, F_1 and F_2 the respective melting points and Δ_1 and Δ_2 the molar depression of the melting points for each form.

Tammann⁴⁰ has pointed out that classification as enantiotropic and monotropic is not unequivocal since in the composition diagram the position of u above or below f - f in Figures 28 and 29 depends on the pressure, so that, for example, under high pressures an enantiotropic system may become monotropic and vice versa. Therefore, it is only through the determination of the melting point curves at different pressures that the stability of a given modification can be decided. If a definite stable modification shows no transformation even at higher pressures, this form is designated by Tammann as totally stable. For example, the stable form of benzophenone (48.5°C) shows no transformation point up to pressures of 3500 Kg/cm²; this form is, therefore, described as totally stable. There are no systems known at this time which change at high pressure from monotropic to enantiotropic. It seems instead that the degree of instability seems to increase with increasing pressure so that the triple point of it exists at lower pressures^{41,42}.

Tammann⁴³, Bridgeman⁴⁴ and Deffet^{45,46} have investigated the behavior of organic polymorphic substances under high pressures.

For organic materials the behavior is usually determined at only one pressure, i.e., atmospheric pressure, and the results in terms of enantiotropic and monotropic polymorphic forms are based on these observations alone. The expressions "stable" and "unstable" are so used in the following

discussion that the stable form always possesses the higher melting point regardless of the monotropic or enantiotropic behavior of the system. In order to avoid errors it is best to use Roman numerals to designate the various forms; Form I has the highest melting point and the numbers decrease as the melting point decreases. Since additional polymorphic forms of intermediate melting point may be found at a later date, it is best to refer to the melting point after each Roman numeral. For example, 2,4,6-tribromophenol⁴⁷, I (94°C) $\rightleftharpoons$ II (92°C) $T_t = 84^\circ\text{C}$ or Veronal⁴⁸, I (190°C), II (183°C), III (181°C) $\rightleftharpoons$ IV (176°C) $T_t = 135^\circ\text{C}$. The double arrows indicate a transformation at the temperature, T_t .

The behavior of many organic systems showing polymorphism has been compiled by J. Timmermans and L. Deffet³⁰.

3. Transition Processes

Unstable modifications which, under ordinary conditions, are difficult to transform into the more stable forms are called "metastable". It might be mentioned that the metastability of many unstable forms may be increased by the presence of small amounts of impurities (usually under one percent). Often pure substances crystallize only with great difficulty in the unstable form and change readily to the stable form, while impure preparation of the same compound in the unstable state can be heated to the melting point without transformation.

This phenomenon is also known for alloys and here the stabilization is due to impurity ions. For example, the bronze, Cu-Sn, is stable only in the presence of a slight excess of copper⁴⁹. Some silicates also show stabilization by impurities⁵⁰.

The existence of polymorphism in a given system can be determined in various ways. Lemann³⁵ first used the hot stage microscope for this purpose. In this way the different melting points and the transition itself can be observed. Other properties that may be used are x-ray structure determination⁵¹⁻⁵³, density, dielectric constant etc. In some cases, the melting point is of no value because the two melting points are identical or nearly so (e.g., 8-naphthol³⁶, diphenylamine⁵⁵, voluntal⁵⁶ and γ -hexachlorocyclohexane⁴⁸ etc.). In other cases, the transition occurs with no observable optical effect (e.g., GeH⁵⁷). Kruis and Kaischew⁵⁸ found transitions in HBr and HI using the polarizing microscope that could not be found by x-ray diffraction. Rinne⁵⁹ pointed out a similar case and H₂S has also been shown to behave in this same manner⁶⁰.

The transformation from the unstable to the stable state can take place in two ways, abruptly or gradually. In the first case it takes place very rapidly, with entire regions of the lattice changing orientation almost instantaneously to that of the stable lattice. This transformation does not take place through temperature changes in the crystal, but is due to internal stresses. According to Dehlinger and Kochendörfer⁶¹ this is a sort of chain reaction mechanism. In this way there usually arises from a single unstable crystal, not a single stable crystal but, instead, numerous individual small stable crystals. The progress of the transformation takes place not through increase in size of the first crystals but as a result of new transformations. The resulting crystals are often lamellar systems of parallel crystals arranged in a twin relationship; the transformation always proceeds enantiotropically. This can be readily observed with metals^{62,63}, with potassium dichromate⁶⁴, suberic acid⁶⁵ and methylbutylketone-2,4-dinitrophenylhydrazon⁶⁶. This type of transformation requires a very close relationship be-

tween the modifications of sustinite and martensite shown by Kurdjumow and Sachs⁶⁷. Such transformations have been designated as $\alpha \rightleftharpoons \beta$ transitions.

A second type of transformation described as the reorientation transformation⁶⁸ does not take place at the same time throughout an entire uniform lattice orientation but, rather, through gradual growth of one orientation at the expense of another. This takes place, therefore, with a measurable rate dependent on the temperature and because it depends on diffusion it can be brought to a halt at lower temperatures. The new modification from an aggregate of small crystals often maintains the external form of the original crystals, and such formations are called paramorphs as distinguished from pseudomorphs which involves a chemical change as well. It often occurs, especially with enantiotropic transformations, that the aggregate of the primary phase will change to a large single crystal of the secondary phase. Frequently there is a relationship between the orientations of the two modifications such that a given orientation of one form will give a corresponding definite modification of the second form. Enantiotropic transformations have also been investigated by x-ray diffraction. The change at 128°C for KNO_3 has been studied by Borchert and Leonhardt⁶⁹ who found the original lattice is deformed at the transition temperature and the small crystals of the rhombic lattice form from the deformed trigonal form on cooling.

Wyrobhoff⁷⁰ described two types of transformation, direct and indirect, depending on the extent of lattice change, *i.e.*, whether there is a small lattice change or whether complete destruction of the primary lattice occurs; Friedel⁷¹ described these two types as paramorphs and non-paramorphs. Barth⁷² proposes these differentiations dependent upon the type of polymorphic transformation, polytype, polysynony and polytropy. The reactivity of a system shows an increase at the transition temperature as shown by Hedval⁷³ for sulfur. TiO_2 (anastase and rutile) also shows special properties in the region of transition⁷⁴.

Observations of polymorphic transformations with organic compounds using the polarizing microscope are described by Lehmann³⁵, Schwartz⁷⁵, Tammann⁴⁰, Schaum^{70,77}, Schaeling⁷⁸, Müller⁷⁹, Lautz⁸⁰, Weygand²⁰, Kofler⁸¹ and many others. Deffet⁴⁶ published a monograph in 1941 covering the complete literature up to that time on organic substances showing polymorphism.

If the nature of the substance is neglected for the moment, the linear rate of transformation depends upon the temperature and on the position of the transition temperature. At lower temperatures the rate decreases practically to zero in agreement with the expected decrease in rate of diffusion.

The rate of an enantiotropic transformation generally increases with a rise in temperature, reaches a maximum and falls to zero again on reaching the transition temperature. At the transition temperature both crystal phases are stable in contact with each other. On further heating the rate of the reverse transformation increases. Work in this field has been carried out on sulfur by Gernez⁸² and on organic systems by Lehmann³⁵, Schwartz⁷⁵, Lautz⁸⁰ etc.

There is also the possibility that the transition point can be determined by studying the behavior of the corresponding modifications in a two-component system⁸⁰. This depends on the fact that the transition temperature does not change on addition of a second component unless solid solution occurs. The second component must naturally be chosen very carefully so that the eutectic temperature lies below the transition temperature. In this case the melting point curve is made up of two partial curves, one for each of the

enantiotropes, and these curves intersect at the transition temperature^{65,80}.

The transition temperature of enantiotropic substances can be determined by various other methods in which a discontinuous change in a given property is noted (e.g., dilatometric methods⁸³ measure the volume change; dynamic methods⁸⁹ determine the rest point in the heating or cooling curves; x-ray diffraction methods⁸⁵ the change in lattice structure; thermal goniometry⁸⁶, the change in crystal angle; and thermal optics, the optical constants before and after the transformation⁸⁸ etc.). The position of the transition point, u , depends on the pressure (Figures 28 and 29). The experimentally observed fact that the transition temperature increases with increasing pressure is a confirmation of Ostwald's theory. The transition point is also changed by impurities if solid solutions are formed⁸⁹ but, in this case, the transition temperature may either rise or fall. This suggests the possibility of changing a given monotropic system to an enantiotropic system by the addition of a proper amount of the proper impurity. This has been observed in a few cases, e.g., cinnamic acid, hydrocinnamic acid⁹¹, etc. (page 101).

With monotropic systems the rate of transformation usually increases with the temperature because of the higher diffusion rate. This behavior is, however, not essential, since the rate also depends on the position of the two vapor pressure curves. The behavior of the polymorphic forms of a given substance depends on the relative position of the equilibrium curves (vapor pressure and sublimation curves), which can be either parallel or intersect each other. Modifications of the first type are classified as thermal crystal groups or form groups⁹² (Figure 30). Those of the second type are called singular forms⁹². Only with a form group or a singular form whose vapor pressure curves do not converge too strongly is the temperature an important factor in the transformation. If, however, the vapor pressure curves converge strongly so that the transition temperature in Figure 29 comes very near the vapor pressure curve for the melt (f-f), the transformation behavior is similar to that of an enantiotropic substance near the transition point; that is to say, when the melting point is approached the rate of transformation will decrease as, for example, with trans-cinnamic acid⁹¹, p-nitrophenol⁹³, diphenylamine⁶⁵.

4. Nucleation

The formation of a given polymorphic form is a question of nucleation⁹⁴ in the melt or vapor phase or even in a solid crystalline substance in the case of a transition.

In crystallization from the melt the number of nuclei first increases with the degree of supercooling, and after reaching a maximum again decreases⁴⁶. Impurities may increase or decrease the number of nuclei⁴⁶. The rate of nucleation decreases with overheating of the melt^{45,95}; a phenomenon common also in metals systems⁹⁶⁻⁹⁸. A greater degree of supercooling usually results in the formation of an unstable form⁹⁹. Microscopic studies especially by Schaum¹⁰⁰ have shown that nucleation often occurs on foreign particles in the melt or on the glass surfaces. An indication of this is the observation that a carefully filtered or centrifuged preparation can be maintained in a supercooled state for a longer time¹⁰¹⁻¹⁰⁴. Pressure, mechanical shock and magnetic fields are also known to have an effect on nucleation.

The action of foreign particles is still not completely clear. Particles of almost any type seem to have an effect⁹⁴ but especially so if there is any structural relationship between the particles and the crystal-

lizing phase. Before nucleation, adsorption on the solid surface occurs¹⁰⁵ giving a two-dimensional crystal¹⁰⁶ from which a nucleus can more readily form¹⁰⁷. It is essentially a process of heterogeneous catalysis¹⁰⁶. Seifert¹⁰⁵ has pointed out that only a one-dimensional structure analogy is necessary for absorption and nucleation. An obvious chemical composition relationship is therefore not necessary in order to obtain nucleation.

That the chemical effect is not the important factor is indicated by the fact that nucleation by the foreign material often reproducibly produces an unstable polymorph. Stilbene in a trional melt results inevitably in formation of Trional II (m.p. 56°C) on cooling. This does not occur in the absence of stilbene¹⁰⁸. The effect of seeding with isomorphous substances is discussed on page 100.

At this point only the partial seeding mechanism as discussed repeatedly by Weygand⁹⁹ will be covered. These include temperature effects or the influence of volume relationships as observed by Schwab¹⁰⁹ for oriented overgrowths of AgCl on AgBr (page 97; for discussion of oriented overgrowths see Chapter VII, page 124).

There are usually fewer nuclei formed during crystallization from solution than from the melt. Unstable forms are usually formed easiest on rapid crystallization. This is achieved by rapid cooling of a hot saturated solution or by rapid evaporation of the solvent. A number of organic compounds customarily crystallize from solution as monotropic unstable polymorphs which, however, transform on heating so that they give the melting point of the stable form (veronal, sulfa-thiazole etc.). In certain cases the nature of the solvent influences a particular polymorphic form to appear, e.g., follicle hormones¹¹⁰.

Similarly atophane always crystallizes from organic solvents as modification II (m.p. 213°C) which, however, does not transform during heating. The stable form is prepared only in a special way¹¹¹. In enantiotropic systems, in general, the unstable form crystallizes from solution but Form I results on spontaneous crystallization of the melt (this depends a great deal on the transition temperature).

Sublimation either at normal or reduced pressure is an important method for the preparation of unstable polymorphs. This is more obvious when it is remembered that 80% of all organic compounds probably can be sublimed even at atmospheric pressure (page 11). The degree of volatility varies considerably; some compounds sublime at very low temperatures and some only near the melting point. In general, lower temperatures are more likely to result in unstable polymorphic forms. Reduced pressure is not, in general, an advantage in trying to form an unstable modification. An unstable modification formed by sublimation has, because of its existence as isolated crystals, a good chance for surviving during a melting point determination although its higher vapor pressure must be considered in this connection. By subliming a thicker layer of crystals, by more rapid heating or by sealing of the coverslip a melting point can usually be obtained (page 85).

Nucleation of one crystal form in a crystalline mass of a second modification depends also on the presence of foreign particles in the solid or on the glass surfaces. Shrinkage cracks and solid-gas interfaces, in general, are also important¹¹¹⁻¹¹⁶. The relationship between the two structures helps also to determine the ease of nucleation as well as the nature and rate of subsequent transformation. As examples, both β -naphthol and dibenzyl crystallize from the melt as Form II, but on cooling many nuclei of form II soon appear (page 134).

5. Reasons for Polymorphism of Organic Substances

The general idea has been expressed that substances that form molecular compounds are most apt to possess polymorphic forms. This idea is based on the feeling that the residual valence forces contributing to compound formation can be used as well to bind molecules of the substance itself in different ways.

Schaum¹⁵ believes that two general types of polymorphism must be explained: (1) association polymorphism, and (2) configuration polymorphism.

The possibility of association as a mechanism for polymorphism was suggested by Tammann. Hertel and Schneider¹¹⁵ regard p-cyano-o-nitro-p'-methoxystilbene as an example of this type. This compound forms a stable red, triclinic modification with one molecule per unit cell and an unstable yellow rhombic modification with two molecules per cell presumably due to dipolar association (Figure 31).

Configuration polymorphism can be subdivided further.

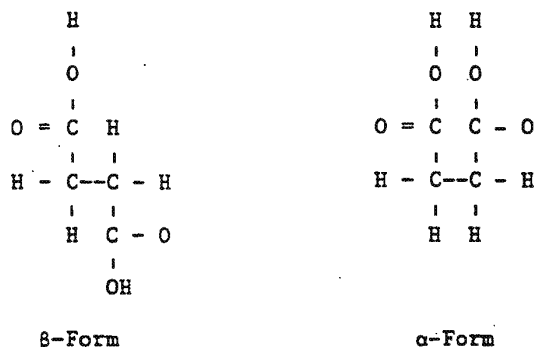
(a) Orientation polymorphism; aliphatic hydrocarbons are an example of this type. Polymorphism of organic compounds having long chain molecules¹¹⁶⁻¹²⁵ has been more extensively studied than for any other type of compound. This is no accident but is due to the availability of homologous series of compounds of this type which permits extensive experimentation (Müller¹²⁶). In the rhombic form the chains lie parallel to each other and perpendicular to the basal plane (Figure 32). A transformation occurs with the chains sliding one or more carbon atoms parallel to their length but maintaining the same distances between (Figure 32, b and c).

Schoon⁵² has calculated the crystal angles of these unstable polymorphs assuming a movement of chains relative to their neighbors of one, two, three etc. carbon atoms (90°, 70°, 61°, 54°, respectively). The calculated and measured angles agree very well except for compounds having long side chains.

(b) Rotation polymorphism; the relative positions of parts of the same molecule depend on the possibility or extent of free rotation about the constituent bonds. Dibenzyl is a good example. X-ray diffraction shows a non-planar configuration for this molecule¹²⁸ (Figure 33). However, the unstable form (m.p. 50°C) must have a planar form¹²⁹ since it is isomorphous¹³¹ with stilbene and azobenzene which have been shown by x-ray diffraction to be planar molecules¹³⁰ (Figure 34). The lattice of dibenzyl analogous to that of planar stilbene (Figure 34) is very unstable and crystallizes spontaneously only on rapid cooling to 0°C. Small amounts of stilbene, however, stabilize the planar dibenzyl lattice. The composition diagram is Type IV Roozeboom with 3% of stilbene sufficient to stabilize the planar dibenzyl¹³¹. This explains why the literature¹³² reported Type I Roozeboom for this composition diagram.

The dimorphism of succinic acid is based (Dupre la Tour¹³²) on the fact that in the α -form the two functional groups point away from each other and in the β -form (stable above 137°C) they lie on the same side. The transition involves the 180° rotation about the central C-C bond.

(c) Distortion polymorphism; this type of polymorphism occurs as a result of change in valence angles. The observed polymorphism of diamethylsulfide and diphenylsulfide is probably of this type¹³⁴.



(d) Motion polymorphism; the polymorphic transformation can, in these cases, be attributed to a change from no rotation to free rotation about one of the chemical bonds¹³⁵. Many cubic high temperature forms of spherical or symmetrical planar molecules are included here¹³⁶; likewise, the hexagonal high temperature forms of long chain hydrocarbons and fatty acid salts. High temperature forms in such systems are always of high symmetry, cubic or hexagonal. The heats of fusion of the high temperature forms of this type are very low¹³⁷⁻¹³⁹ while the heats of transition are quite large¹⁴⁰⁻¹⁴².

Walden's rule¹⁴³ which states that the entropy of melting of non-associated, molecular lattice organic compounds is constant and equal to 13.5 does not hold for spherical molecules. Instead the total energy involved in conversion of the stable polymorph to the melt seems to be a constant and equal to about 13.5¹⁴⁴. In other words, the sum of the entropy (or entropies) of transition and the entropy of melting is about 13.5. In the series of normal paraffin hydrocarbons from 24-44 carbon atoms there is a transformation 5-10°C below the melting point in each case due to a change to free rotation¹⁴⁵. Even with the shorter carbon chains Bernal¹⁴⁶ found a hexagonal high temperature form of C₁₂H₂₅OH with freely rotating zigzag carbon chains. Hendricks¹⁴⁷ found similar behavior in the series of primary aliphatic amines with 3-8 carbon atoms.

The fact that free rotation does indeed occur was shown by Bonhoeffer and Harbeck¹⁴⁸ with ortho and para hydrogen and almost simultaneously by Clusins and Hiller¹⁴⁹ with methane. The phenomenon has been treated by Pauling¹⁵⁰ from a quantum mechanics point of view. Schaeffer¹⁵¹ has developed a theory for rotation transformations based on experimental evidence. The problem has also been treated theoretically by Eichhorn¹⁵², Smits¹⁵³, Kruis and Kaischew¹⁵⁴, and Eucken¹⁴⁴.

The change from hindered to free rotation is a gradual one and may or may not result in a lattice change with formation of a new phase. A few molecules in the lattice change first to free rotation and as the temperature rises more and more molecules change. With NaNO₃ the rotation of the anion occurs gradually over the range 150-275°C without any lattice change¹⁵⁵. NH₄NO₃ shows complete rotation of the anion above 125°C¹⁵⁶.

There may be a second mechanism for transformation of spherical shape molecules to a high temperature cubic form¹⁵⁷⁻¹⁵⁸ involving a statisti-

cal disorientation of a given molecular axis with respect to the lattice. Quinualidine possesses a spherical molecule with trigonal symmetry.

This is, for example, possible with long chain molecules since the dimensions of such molecules differ in only one direction so that the basic structure of these molecules is preserved over wide molecular weight variations and even with many derivatives so long as excessively long side chains are not formed¹⁶⁰.

6. Polymorphism of Organic Molecular Compounds

The occurrence of polymorphs of organic molecular compounds¹⁶¹⁻¹⁶² may be due to "stereoisomerism" or to different types of bonds between the two component molecules. Since molecular compounds vary so that no one explanation suffices, three different groups can be discussed¹⁶³.

(1) The different modifications of the molecular compound show similarity to the corresponding modifications of one of the components of the molecular compound.

(2) The polymorphic transformation may be due to a change in bonding of the two components of the molecular compound (complex isomerism¹⁶⁴).

(3) Modifications which do not show any relationship in the sense of (1) or (2).

p-Trinitrobenzene and fluorene form an equi-molecular addition compound having three polymorphs which behave in many respects like the three high-temperature forms of p-trinitrobenzene itself (Group 1, above). It must be assumed that the mechanism of transformation in the complex is related to the three corresponding transformations of pure p-trinitrobenzene. Hertel¹⁶⁵ has studied this compound by x-ray diffraction.

The molecular compounds included in group 2 are often characterized by a color change during transformation. For example, the molecular compound between 4-dimethylamine-2-hydrodistyrylketone and 4-dimethylaminobenzaldehyde occurs either in a red or a yellow form¹⁶⁶. Hertel¹⁶⁷ after studying many such systems and after consideration of Werner's coordination theory¹⁶⁸ and the work of Pfeiffer¹⁶¹ has decided to call this "Complex Isomerism". He has included here the molecular compounds of the nitrated phenols which have two points of attachment; one, on the hydroxyl and the other, on the nitro group. When the nitro group forms the bond with an aromatic ring system a deeply colored complex is formed. In the other case the bond is formed by the hydroxyl group and the complex is not highly colored. Whether the nitro group or the hydroxyl group forms the complexing bond depends on the system. For example, p-trinitroanisole forms a deeply colored 1:1 complex with α - or β -naphthylamine, hence it is assumed that the nitro group is the bond-forming group. On the other hand, dimethylamine forms a 1:1 complex with trinitroanisole with its light colored, hence the hydroxyl group must be the bond-forming group. In this case the formation of hydroxyl-bonded complexes seems to be related to the basicity of the amine. Hertel¹⁶⁷ was able to obtain both types of complex with some compounds in which he had modified the basicity of the amines by halogenation. o-Bromoaniline and picric acid, for example, give both a yellow and an orange-red complex. "Complex isomerism" of which this is an example must be regarded as intermediate between polymorphism and true isomerism (page 69).

Most molecular compounds fall in group 3. However, a systematic study of many examples would undoubtedly permit reclassification of many of them in groups 1 and 2.

7. Liquid Crystals

Liquid crystals represent a state intermediate between the liquid and the solid state. In a sense, they are very soft crystals although they possess all other characteristics of a well-crystallized solid.

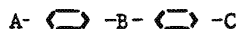
Reinitzer¹⁶⁹ first reported the peculiar iridescent turbid melts of cholesterol derivations. Lehmann¹⁷⁰ examined similar materials (e.g., ammonium oleate and silver iodide) and came to the conclusion that they are "liquid crystals" or crystalline liquids.

Liquid crystals are made up of a parallel and orderly arrangement of molecules but without the lattice forces necessary to maintain the solid condition considered necessary for a crystal. The internal order causes the optical anisotropy of the liquid crystals which are usually uniaxial. According to Vörländer¹⁷¹, crystalline liquid phases are often characteristic of long-chain molecules and aromatic substances.

In the microscopical preparation, between the slide and the cover-slip, the liquid crystals appear in a variety of formations. Most often, perhaps, they form parallel layers in which the axes of the molecules are perpendicular to the slide and coverslip. The layers appear dark between crossed polars; their liquid crystalline nature is detected by their distorted centered uniaxial interference figures. Often droplike liquid crystals appear, which are similar to the spherulites of solid phases; in other cases long rods with parallel extinction are formed; these move during growth and fuse on contact into larger crystals. At the edge of air bubbles they orient themselves normal to the surface and form hollow, liquid "spherocrystals".

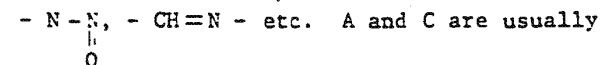
p-Azoxyanisole mixed with Canada balsam or rosin is excellent as a preparation for microscopical demonstration since it dissolves on heating and precipitates on cooling. The "drops" show a black cross between crossed polars. Molecular compounds also often form liquid crystalline phases; in a mixed fusion between cholesterol and cetyl alcohol they are easy to recognize and observe (see Exercises).

One basic type of compound showing liquid crystalline phases is:



Where B may be a symmetrical group such as:

- N=N - , - C≡C - , - CH=CH - , - CH₂ - CH₂ - etc., or unsymmetrical such as:



alkyl groups, CH₃ -(CH₂)_n.

The forms of liquid crystals are distinguished by Vörländer¹⁷¹ depending on their relationship as Pl-forms (for phenolethers) and as Bz-forms (for benzoic acid esters).

The Pl-forms show birefringent drops under crossed polars which

grow and flow together. They do not, however, show an x-ray pattern.

The Bz-forms also show birefringent drops with a spherulitic black cross. These drops grow and form finally a mosaic structure. Bz-forms always show an x-ray pattern. It may crystallize from the melt or from a Pl-form. Weygand and Gabler¹⁷² found two different Bz-forms for p-n-monoxy-benzylphenetidine.

8. Microscopical Investigation of Polymorphism

(a) The occurrence of unstable modifications

Although it is generally known that many substances show polymorphism, the chemist does not often encounter the different modifications in his normal experience. It is shown by the micro determination of melting points that all substances possess the possibility of formation of several modifications. Many contradictions and errors in the literature on melting points could be traced to lack of recognition of polymorphic forms.

A melting point of 209-213°C is given in the literature and in the drug tables for atophane (2-phenyl quinoline-4-carboxylic acid). In the industrial preparation of atophane the material precipitated from an aqueous solution of a salt by mineral acid shows a melting point of 217-218°C. After purification and recrystallization from organic solvents a melting point of 209-213°C is obtained. Microscopical investigation gives an explanation of this unusual behavior. Under certain conditions the atophane forms a previously unknown, high melting modification which crystallizes from organic solvents as modification II with a melting point of 213°C¹¹¹. The difference in literature values for the melting points of the sulfonamides can also be traced to the existence of several polymorphic forms (page 46).

Further microscopical investigation⁵⁶ would show a number of errors in the literature because the melting point of so many substances has been described without crystallographic or x-ray investigation of the crystal type and since an unstable modification with lower melting point was actually obtained. This occurs, for example, with cholesteryl acetate¹⁷³, resorcinol¹⁷⁴, thiosinamine¹⁷⁵, veronal^{176,177} etc. This occurs in enantiotropic systems as well as in monotropic systems (e.g., veronal¹⁷⁸).

Unstable forms of organic compounds can be crystallized by all of the various techniques, i.e., crystallization from the melt, sublimation, and from solution (page 74).

An unstable modification is especially likely to be formed from the melt by rapid crystallization after supercooling. Some low melting compounds should be supercooled with ice although, in no case, should a preparation be supercooled to the point where the melt solidifies to a glass since nucleation cannot occur under these conditions. Luminal (melting point = 174°C) supercools to a glass on rapid cooling but on rewarming to about 100°C several crystal nuclei soon form, often of different modifications. Such a preparation (Figure 35) may be held without change at room temperature. Figure 36 shows two modifications of benzalazine; a central spherulite is surrounded by a rapidly growing unstable modification. On heating the unstable form is gradually transformed (Figure 37).

For microscopical investigation the material is melted under the coverslip, the slide is placed on the cold microscope stage, and the solidification process is observed. In a system showing polymorphs, spontaneous crystallization frequently gives rise to different modifications, often in the form of spherulites, but occasionally single crystals or coarse crystal-

line aggregates. In many cases, the presence of different modifications can be assumed on the basis of differences in double refraction, habit or rates of growth. Differences in crystal habit alone, however, are not sufficient since crystallization may occur on different faces. The transformation itself, which occurs at the boundaries between different modifications, is certain evidence of polymorphism; the determination of different melting points for adjacent crystal regions is also sufficient proof. In many cases study of the compound in a binary system may be helpful; for example, trinitrobenzene with the two unstable forms III (m.p. 109°C) and II (m.p. 110°C¹⁷⁹). The room temperature-unstable modification in an enantiotropic system usually crystallizes on rapid cooling to room temperature (I of Figure 28). However, the stable form may also be formed under these conditions. If, in this case, the tendency to transform is low, then enantiotropy might escape observation (e.g., resorcinol). If, however, a rapidly crystallized preparation is allowed to stand undisturbed at room temperature for several hours, then there usually appears spontaneously a region of form II which grows more or less rapidly as the stable form. The reversibility of the process can be determined by rewarming a preparation which has not completely transformed.

Often addition of a solvent will speed up transformation. For this purpose the coverslip is removed from a rapidly solidified preparation which is then covered with a new coverslip, and a drop of organic solvent is allowed to flow under while the crystals are carefully watched. If the first crystals to form are not stable at room temperature, there should appear in the crystal mass nuclei of form II (Figure 28). The first crystals to form often transform either instantaneously or more or less gradually under these conditions, for example, cholesteryl acetate, resorcinol, thiosinamine⁵⁶, betanaphthol³⁶ or veronal (see Exercises, page 158). In many cases it is sufficient to place a drop of solvent at the edge of an intact preparation, since the small amount of liquid which flows around and between the crystals is usually sufficient to bring about the transformation at room temperature (Lehmann³⁵).

Exact determination of the transition point depends, of course, on the rate of transformation. Quite often the process comes almost to a standstill near the transition point. In such cases the temperature interval in which the movement of the transition front can be observed should be narrowed gradually, by holding the preparation at various constant temperatures for long periods of time. An exact determination of the transition temperature is possible only through observation of the behavior in a two component system^{80,65} (page 90).

If the system is monotropic there are several possibilities: (1) on renewed heating the solidified melt may melt at a lower temperature than the original material. In this case the unstable modification crystallizes from the melt and then melts on renewed heating without transformation to the stable form. (2) Transformation may occur during reheating of the solidified preparation. The unstable form could transform either to the stable form, so that on further heating the melting point of the latter is observed, or to another unstable form possessing a higher degree of stability. The latter will then melt at its melting point or transform again (e.g., veronal). (3) Before the transformation is complete, the melting point of the unstable modification may be reached and this form may begin to melt. In this event, the melt will crystallize so that finally only the stable modification is present.

Transformations as observed under the microscope show certain dif-

ferences which, on the one hand, are characteristic of the substance and, on the other hand, are characteristic of the experimental conditions. The transformation may be progressive with a well-defined crystal-front, or large crystal areas may transform instantaneously (page 72), e.g., suberic acid or methylbutylketone-2,4-dinitrophenylhydrazones.

The change in appearance during transformation⁶⁸ (page 73) can, in spite of the effects of temperature, purity etc., be tabulated as follows:

(1) Diffusion-transformation. The transformation proceeds from numerous nuclei, e.g., 1,3-dinitrobenzene, alphanaphthol, beta-naphthol. The original crystals become, thereby, microcrystalline aggregates.

(2) Progressive transformation

(a) The crystal-front of the stable phase will grow from only a few nuclei and proceed with relatively slow rate as a well-formed crystal-front throughout the unstable region. There is, however, no relation between the orientations of the two phases.

(b) The crystal-front for the transformation is irregular although made up of straight lines corresponding to the crystal faces; there results from the transformation only a few irregular crystals, which replace the original crystal mass. The new crystals, however, are related directly to the crystals of the unstable phase, e.g., resorcinol, thiosinamine, novatophan, dibenzoylmethane, p-anisidine and many others.

When the transformation is instantaneous, a definite relationship always exists between the orientations of the structures in the secondary and primary phases; this relationship does not usually exist when the transformation is gradual. Although such a relationship between the orientations of the two modifications during the gradual transformation is not excluded it occurs, however, more rarely when the rate of transformation is high. The stable modification of p-nitrophenol is a paramorph¹⁸⁰ of the original form in spite of the fact that the transformation proceeds without apparent relation between the orientations. Frequently if the transformation progresses from a large number of nuclei it is difficult to determine whether a relation between the two orientations does exist. If, for example, form I of beta-naphthol³⁶ resulting from the melt transforms at room temperature to form II then the new modification is at least partly regularly oriented as a lamellar system. If, however, the transformation occurs at 100°C the resulting transformation starts from many nuclei and the resulting crystal mass is made up of individual single crystals apparently without relationship to the original structure.

Quite often in a microscopical preparation the transformation stops completely in one single region while it may progress quite rapidly in other regions. This is due in most cases to the presence of shrinkage cracks or other boundary structures, or to a concentration of impurities in that portion of the preparation.

There are examples in which the original spontaneously crystallizing unstable modification transforms before it can be observed, e.g., alpha-trinitrotoluene or stilbene. It is always possible to tell, however, that transformation has occurred and to recognize the transformation structure by shrinkage cracks and vapor bubbles appearing in the crystal mass. During crystallization from the melt these vapor bubbles form as a result partly of volume contraction and partly through insolubility of the vapor in the crystalline state. They arrange themselves in an orderly geometrical fashion along the boundaries between crystals. If the original structure has trans-

formed to a second modification, then the vapor bubbles which are present will not bear the proper relationship to the second crystalline phase. It would be possible, particularly by observing the preparation between crossed polars, to see that the vapor bubbles occur within a region of uniform crystallization. It is sometimes possible to stabilize one of these very unstable modifications by addition of a second component, for example, trional stabilizes the unstable form of stilbene¹⁰⁸.

Many crystalline preparations such as diphenyl and hexachlorobenzene, which are prepared by rapid crystallization following strong supercooling, will change on warming without appearing to go through a polymorphic transformation (e.g., diphenyl and hexachlorobenzene). The process is very similar to a polymorphic transformation but by careful observation may be distinguished from it in that the crystals which grow on one side of the preparation at the expense of others, are on the opposite side being "eaten up" themselves. Such changes are especially common with very volatile substances. (Translator's note: This phenomenon is designated as grain growth, recrystallization or boundary migration.)

A second means for the preparation of unstable forms is sublimation. The unstable polymorphic forms may often be prepared by sublimation at normal or reduced pressures. It has been estimated that about 80 percent of the organic compounds can be sublimed to give crystalline products^{134,181}. The crystalline phase obtained is dependent on the temperature, pressure, duration of sublimation, sublimation distance and the temperature differential between the source of material and the condensing surface (page 12). Because of the isolated position of the unstable crystals obtained by sublimation, they are not easily inoculated with stable nuclei and thus can be used much better for melting point determinations.

Sublimation can be carried out on the micro hot stage itself (page 14). Since the stable form in a polymorphic system has a lower vapor pressure, the stable form is formed at any given temperature at a lower pressure than the unstable form. The stable form will sublime at low pressures even after the unstable polymorph has completely vaporized. Even though an unstable form can be sublimed at higher pressures, diminished pressure is often an advantage since the temperatures required to obtain the unstable form are lower and more convenient. If diminished pressure is used, however, the time for sublimation should be kept as short as possible in order to lessen the possibility of transformation to the stable form. The most favorable temperature for sublimation of the unstable modification of a given compound can be determined only by experiment. General rules cannot be given to cover this situation and even the above ideas should be used only as rough guides.

Frequently the crystals obtained by sublimation are well-formed and the various crystallographic and crystal optical measurements can be determined directly. It is important to note the orientation of the crystals since different habits might be falsely interpreted as different polymorphic modifications. Differences are easily observed when the single crystals have a needlelike or platelike habit: For example, caffeine crystallizes in long needles, the cross-section of which is six-sided. If these needles lie perpendicular to the coverslip, they then appear as short hexagonal prisms¹⁸² which were considered by earlier investigators to be a different crystalline modification.

The crystals can frequently be rolled in a viscous liquid, e.g., corn syrup, Canada balsam, Aroclor etc., in order to determine the habit and the relationship of the different faces of the crystal. It is often possible

to measure the refractive indices on the sublimate using the immersion method¹⁸³.

The lattice structures of the different modifications of a given compound can show all degrees of differences; and usually the two forms will crystallize in different crystal systems. Recently the crystal geometric relationships between the various polymorphic modifications have been classified under the general classifications of "isotype, homotype and heterotype" (page 69). A few examples will illustrate the behavior of crystals of different modifications as formed through sublimation during the micro melting point determination. The melting point 209 and 213°C is given in the drug tables for atophane (2-phenylquinoline-4-carboxylic acid). During the micro melting point determination a small amount of sublimed atophane melts at a point several degrees before the main portion of the material. Better crystals of this sublimate can be collected at temperatures of about 120-150°C under diminished pressure of about 11 mm of mercury, a distance of 3 to 4 mm and a sublimation time of 1/2 to 3 hours.

Such a sublimate is reproduced in Figure 38. Each modification can readily be distinguished by its unique habit. The oblique-angled crystals are modification III (196°C) and the square-end crystals in the middle are modification II (Figure 34). On heating to 196°C the crystals of modification III melt, Figure 38b and c; form II has not melted and has, instead, grown (Figure 38d and e). The needle which in Figure 38a was very small is now elongated and thickened. Figure 38e corresponds to a temperature of 199°C and shows modification III completely melted. Figure 38e, taken at 205°C, shows form II growing rapidly at the expense of several nearby droplets. Even in Figure 38b the needlelike extension on the crystal of form II has grown toward a nearby droplet on the left. On contact with the drop the melt is absorbed and solidified by the crystal. In the same way the crystal has also absorbed the second drop in the upper left. The crystallization of these absorbed droplets is still not quite complete in Figure 38d; therefore, the large crystal is still surrounded by a small liquid boundary. The crystallization is complete, however, in Figure 38e. A similar, although not so definite process, has also occurred in the lower right portion of the field. The melting point of II is reached in Figure 38f.

The modification of atophane melting at 213°C was considered until recently to be the stable modification. A short time ago, however, the work of A. Kofler and Birner¹¹¹ disclosed an additional modification of atophane melting at 218°C. This form is, therefore, the stable modification (I) and forms melting at 213°C and 196°C are, respectively, modification II and III. Modification I appears only under special experimental conditions (page 80) and never during micro sublimation. In general, precipitation from solution gives the form most stable at that temperature. Frequently, however, the unstable forms may appear at the same time (nicotinamide¹⁸⁵). Folic acid¹⁸⁶ may be readily crystallized from a solvent as an unstable form.

An unstable modification can sometimes be obtained by seeding with an isomorphous substance. This can be done by direct isomorphous growth (page 85) or by use of less closely related lattices to increase the possibility of nucleus formation. For example, 1,3,5-trinitrobenzene (I) seeds the melt of picric acid, and as a result crystallizes a very unstable polymorph of picric acid melting at 75°C¹⁷⁹ (page 74). Styphnic acid also seeds picric acid isomorphously and forms another unstable modification melting at 101°C¹⁸⁷. Neither of these two unstable forms of picric acid have been prepared by any other means. In these cases there exists the highest possible relationship between the corresponding forms, a continuous series of solid

solutions over the entire composition range (page 124).

Nucleus formation can also occur with less relationship between the two forms; with limited solid solubility or even with immiscibility. The various cases are easily differentiated. Numerous experiments on nucleation have been reported by Weygand²⁸ involving very little relation between the forms concerned.

(b) Determination of the melting point of unstable modifications

Although the melting points of the unstable modifications of many organic compounds can be determined relatively easily either by micro or macro methods, there are a number of other unstable modifications whose melting points can be determined only with great difficulty, if at all¹⁸⁸.

There are, in general, two possibilities for the melting point determination of unstable modifications:

1. The Direct Determination:
 - (a) On crystals from the melt
 - (b) On sublimates
2. The Indirect Determination:
 - (a) From the melting point curve of the two component system
 - (b) With the help of eutectic temperatures with suitable test substances.

The procedure using crystals from the melt depends on whether one or several modifications (with or without the stable form) are present. With only one unstable form present the melting point can, in many cases, be determined directly without further effort, e.g., acetamide⁷⁹. If several modifications are present then there are two possible procedures. The transformation is not allowed to go to completion but the partially transformed preparation is immediately placed on an already preheated hot stage. With immediate further heating it is often possible to obtain the melting point of the single isolated crystals before they have transformed, e.g., picryl chloride¹⁸⁹ or nicotinamide¹⁸⁵. If, however, the transformation proceeds very rapidly, so that the preparation is completely transformed before placing on the already warmed hot stage, there is still a possibility of being able to determine the melting point of the unstable form before transformation is complete. In such cases, the following technique is used: The coverslip is removed from a preparation made up of the unstable and stable form in such a way that many small crystals of the unstable form remain attached to the coverslip. A portion of the preparation is then chosen where a large number of isolated crystals of the unstable form are present. The object slide is then covered with a new coverslip and placed on the prewarmed hot stage¹¹⁸. It is sometimes better with compounds of high volatility to use the coverslip which has been removed for the melting point determination and place it on a new object slide. The largest part of the unstable crystals may transform on further heating, but it is usually possible with patience to determine the melting point on isolated crystals of the unstable modification.

It is possible in this way to determine the melting point of the unstable modification II of chloro- or bromo-acetanilides, which always occur crystallized adjacent to crystals of the stable modification. The dimorphism

of these two compounds has been reported in the literature⁴⁶ without data on the melting points of the unstable forms. By these methods, the melting points of chloracetanilide II was found to be 157°C and for bromacetanilide II, 162°C. It is important in making these preparations to cool only to the point where crystallization occurs. If the preparation is cooled to a lower temperature, many shrinkage cracks result which may act as nucleation centers for the transformation to the stable modification.

In many cases the transformation nuclei are observed to arise at the edge of the coverslip. This nucleation can often be prevented if the preparation is ringed with a viscous material such as Aroclor, Canada balsam, Karo syrup, paraffin oil etc. It is, therefore, no coincidence that the unstable modification of pyrocatechol was first observed in a mixed fusion (page 93) with nicotinamide, the melt of which completely surrounded the pyrocatechol⁶⁵.

If it is not possible to determine the melting point of the unstable modification on crystals from the melt, the sublimate should then be tried¹³⁴ (page 11). Because of the higher vapor pressure of the unstable form, lower temperatures and, in general, normal pressures are used. There is a good possibility of determining the melting point of the isolated single crystals before transformation. In this way the melting point of the unstable modification of acetanilide (100°C) can be determined on the sublimate. This determination is impossible on crystals from the melt because of the transformation. There are data in the literature^{35,79,46} on the existence and crystallographic structure of the unstable modification of acetanilide, but no mention of the melting point.

The unstable modification may resublime before it can be brought to the melting point. In this case it may be possible to determine the melting point by placing the preparation on an already preheated hot stage.

There are, however, unstable modifications whose melting points cannot be determined by any of these direct methods. In these cases it may be possible by the use of indirect methods to determine quite accurately the position of the melting point. One method consists of the study of unstable modifications in mixtures with a second component which may or may not be isomorphous. Since the stability frequently increases through the addition of impurities, the melting point of the unstable form and a part of the melting point diagram can often be determined on mixtures. For example, the unstable modification of acetanilide is so stabilized in mixtures with phenacetin that a portion of the curve can easily be determined, the intersection of this partial curve with the ordinate shows a melting point for the pure substance of 100°C (Figure 39). This is the same melting point determined, as mentioned above, on the sublimate of unstable acetanilide.

In a similar way, modification II of 1,3-dinitrobenzene gives a melting point of 75°C according to Brandstätter¹⁹⁰ (Figure 36). This modification can be stabilized with 1,2,4-chlorodinitrobenzene; mixed crystals result on which the melting curve of the unstable form II can be determined up to the small content of 2 percent chlorodinitrobenzene. A value of 74°C is obtained as the melting point for the latter mixtures; this justifies the value of 75°C for the pure substance (Figure 40). This method of determining melting points was used for the unstable forms of methyl esters of mono carboxylic acids by Francis and Piper¹⁹¹ and by Phillips and Mumford¹⁹².

The second indirect method utilizes the eutectic temperature with a suitable test substance¹⁹³, provided that the system under examination is monotropic and that the two modifications possess approximately parallel

equilibrium curves (page 74) and, in addition, that neither modification forms mixed crystals with the test substance. If, then, these conditions are fulfilled, the melting point curves for the stable and the unstable forms in mixtures with the test substance will, in general, be parallel to each other. Figure 41 shows the behavior of the partial curves of three unstable forms of 1,2,4-bromodinitrobenzene with methyl acetanilide. Here the eutectic content of the stable form and the three eutectics of the unstable form of bromodinitrobenzene with methyl acetanilide were directly determined in mixed fusions. The eutectic of the stable form lies at 30 percent methyl acetanilide and 47°C. The eutectic temperatures of the unstable forms lie at 32, 29 and 21°C. The eutectic temperatures of the unstable forms determined directly are 51, 46 and 36°C. These values agree well with the values obtained by drawing a straight line from each eutectic point and parallel to the line connecting the melting point of I and its eutectic point. The behavior of the melting point curves in the system 1,2,4-bromodinitrobenzene-benzil (Figure 42) is entirely analogous.

B. Isomorphism

The crystal structure of a substance depends to some extent, naturally, on molecular structure. In general, highly symmetrical crystal structures are characteristic of simple molecules. Almost all elements, for example, crystallize in the cubic, tetragonal or hexagonal systems. All highly symmetrical organic compounds such as methane and its derivatives in which all four valences of carbon are joined to identical atoms or groups of atoms crystallize in the cubic system (e.g., CH_4 , CCl_4 , CBr_4 , and CI_4).

X-ray and electron diffraction have shown that these simple molecules possess a tetrahedral structure¹⁹⁴⁻¹⁹⁶ in agreement with classical valence ideas of Van't Hoff and LeBell. CBr_4 ¹⁹⁷, CI_4 ¹⁹⁸⁻²⁰⁰ as well as SiI_4 , TiBr_4 , TiI_4 , GeI_4 and SI_4 ²⁰¹ all crystallize in the cubic system. Other molecular symmetries may also be preserved in the crystal lattice; for example, GeN_3 crystallizes with trigonal symmetry as rhombohedra²⁰². The highly symmetrical molecule, urotropine (hexamethylenetetramine) crystallizes in a body-centered cubic lattice²⁰³. X-ray diffraction studies, however, have not shown a relationship between crystal symmetry and molecular symmetry, in general, as simple as indicated by the properties of the few materials mentioned above. Even with the above-named compounds noncubic polymorphic forms are also known in addition to the cubic modification. Benzene crystallizes as rhombic bipyramids, in spite of the fact that x-ray diffraction shows the molecule to possess regular hexagonal symmetry²⁰⁴. The loss of symmetry is accounted for by the arrangement of the benzene molecules; presumably the arrangement which expresses the symmetry of the molecule is not stable²⁰⁵. And, on the other hand, some materials with complicated molecules such as camphor or camphene crystallize in the cubic system. The arrangement of the molecules in the lattice may give a crystal of either greater or less symmetry than the molecule itself³¹⁻³³.

The relation between the crystal structure and chemical properties is apparent in that even small changes in molecular structure cause a definite change in the crystal structure. Furthermore, chemically related compounds are often similar crystallographically. For example, the crystal system and habit of the corresponding sulphates of potassium, rubidium and cesium are almost identical with only small and regular changes in the axial ratio so that the a axis becomes shorter with respect to the c axis through the

series from potassium to cesium²⁰⁶. Frequently other corresponding salts of similar constitution show similar variations.

Groth²⁰⁷ calls this phenomena "morphotropy"; however, the designation "isomorphism" is more common. Compounds closely related chemically tend to be isomorphous because the crystal structures of such compounds are almost identical.

The close resemblance between the crystal forms of chemically related materials and the importance of this phenomena was first recognized by Mitscherlich in 1821 with the salts potassium dihydrogen phosphate and potassium dihydrogen arsenate. Mitscherlich first proposed for this phenomena the term "isomorphism". He also recognized that the close relation between the crystal structures of isomorphous substances would permit them to form mixed crystals with each other.

While the first consideration of isomorphous substances was based principally on morphological details, investigations by Retgers²⁰⁸ have pointed out that all physical properties of isomorphous substances are a function of their composition. The degree of isomorphism is considered by Retgers and later by Nernst²⁰⁹ etc., to be measured by the extent of miscibility of the solid components.

The problem of isomorphism is even today usually studied in the field of mineralogy, where it is usually presented as very complicated. As a result a great deal has been written on the definition and description of isomorphism²¹⁰. The problem of isomorphism in inorganic materials has been studied by Goldschmidt²¹¹ and by many other authors. The corresponding study of organic compounds is still in the very early stages of development. Unfortunately, complete structure determinations are very difficult for organic compounds and most of the information so far available is a result of solid solution studies.

Mixed crystals have not only a geometrical meaning through goniometric or x-ray diffraction methods, but a mixed crystal is also a single thermodynamic phase^{92,206,213,214}. If two substances can be mixed in all proportions in the solid state, then crystals of definite and uniform composition are formed from the mixed melt (solution) with a composition which depends on pressure, temperature and composition of the melt. The physical properties of these mixed crystals are continuous though not necessarily linear functions of the percentage composition; they may even show a maximum or minimum²¹⁵. A crystal grows in the solution or melt of isomorphous materials just as it would from the melt or solution of either of the pure components.

There are several types of mixed crystals: (1) random substitution in which some molecules of one component are replaced by molecules of a second without regular arrangement but having a definite average composition (Figure 43); (2) ordered mixed crystals in which the lattice units of both components occupy definite, regular, repeating positions in the lattice "ordnungskonzentration", according to C. Wagner²¹⁶ (Figure 44); (3) interstitial solid solution in which the lattice units of the second component lie between the lattice points of the first without considerable displacement of the latter. (This is only possible with small lattice units of the second component and even then only to a limited degree (Figure 45)); (4) adsorption mixed crystals in which the foreign material is present in very small amounts and dispersed in molecular form through adsorption forces in the parent crystal. Since the various crystal faces show different surface arrangements of the lattice points, adsorption depends both on structure and direction. It is, therefore, selective²⁰⁶. Many dilute solutions of dyes will behave in

this manner. For example, phthalic acid crystals crystallized from a solution containing methyl red, methylene blue or fuchsin²¹⁷ are, depending upon the dye, colored more or less intensely red or blue. The color is homogeneous and the mixed crystals are pleochroic as a result of orientation of the adsorbed dye²¹⁸. Johnsen²¹⁹ describes these mixed phases as anomalous mixed crystals. Such mixed crystals were first examined in large numbers by O. Lehmann²²⁰ (about 300 cases). Further investigations were made by Rutgers²²¹, Marc and Wenk²²², Seifert²²³, and Neuhaus²²⁴ etc.

Only the first two of the above types of mixed crystals will be considered in the following discussion, i.e., random and ordered solid solutions. The essential difference between the systems showing mixed crystal formation and other systems lies in the degree of miscibility. With the interstitial or adsorption type of mixed crystals, the foreign material can be present in the lattice only in very small amounts. It has been proposed that the components of mixed crystals be designated as isomorphous only when at least 5 mole percent of the foreign component can be present in the lattice (Strunz²²⁵).

It should be noted that there are cases in which certain classification as mixed crystals or as another type of structure is not possible. Each particular case must be considered in light of the following discussion.

In the melting point diagram for a mixed crystal series of the substitution type, the melting points or solidification points lie on a continuous curve corresponding to thermodynamically single phases (page 98).

In order that two substances can form a continuous series of mixed crystals they must show certain chemical analogies and, in addition, similar crystal lattices. Similarity in lattice structure (isotype) is not alone sufficient, but there must also be present for mixed crystal formation corresponding similarity between active radii of the substituting units. Steinmetz²²⁶ mentions potassium and ammonium chlorides and sulfates as a system showing the highest order of continuous solid solubility. Continuous solid solubility is spoken of as isomorphism in the narrower sense (Machatschki⁶⁸, Nowacki²²⁷ etc.). Neuhaus²²⁸ requires strict iso-structural relationships for the two components forming continuous solid solutions. The term "isomorphous relationship" has recently been proposed to replace the expression "isomorphism"²²⁹. The same criteria are used, however — chemical structural similarities and miscibility. The terms isotype, homotype and heterotype are used to express the degree of similarity²²⁹.

The relationship between crystal structure and mixed crystal formation is formulated in the following way²³⁰. The formation of mixed crystals of two or more compounds has been considered as a certain indication of a chemical similarity between the molecules. Today, however, it is known that mixed crystal formation is not limited to isotypes but also to homotypes and even to heterotypes. It is necessary then to differentiate between these three types of miscibility.

The miscibility of nonisotype organic compounds may be easily explained by means of isodimorphism as suggested by Bruni and Groth. In these cases the two compounds are miscible, not in spite of lattice dissimilarity but because one component possesses an unstable modification which has the proper isotype relationship with the other component (page 100).

Complete miscibility may be impossible even with isotype structure if the cell dimensions are very different. In these cases (for example KCl:KI; size difference 22%²³¹) the result is usually limited to solid solubility (page 99).

A special degree of close crystal-chemical relationship which enables complete solubility is polymer isomorphism (Goldschmidt²³¹). In this case the elementary units of the two crystal types are not equivalent, but certain number multiples of the elementary units are related. Polymer isomorphism is found not only in minerals like the plagioclases (albite, $\text{NaAlSi}_3\text{O}_8$ and anorthite, $\text{CaAl}_2\text{Si}_2\text{O}_8$) where the Na and Si are replaced by Ca and Al. Additional types of substitution are the addition-, subtraction- and division-substitution²³³.

Investigations of the replaceability of definite atoms or atom groups with organic compounds has already been investigated by Groth, Bruni, Garelli etc. Bruni²¹³ calls such atom groups "isomorphogenes". The most important advance in this field is the expression of the replacement law of Grimm²³⁴.

IV	V	VI	VII	0	I
C	N	O	F	Ne	Na
	CH	NH	OH	FH	-
		CH ₂	NH ₂	OH ₂	FH ₂ ?
			CH ₃	NH ₃	OH ₃
				CH ₄	NH ₄
4	3	2	1	0	-1

Grimm's Replacement Series

The vertical columns in the table show the atoms and radicals which possess, according to Grimm, equivalent electron structures and are, therefore, capable of replacing each other isomorphously in crystals.

A series of investigations of organic compounds brings some agreement with but also some disagreement with Grimm's rule. One reason for this might be that many of the composition diagram determined by the macro-method are incomplete or inaccurate and because the relation between polymorphic forms in a given system have been almost completely neglected (page 76). Many of these disagreements could be resolved through use of micro thermal methods and especially through use of the mixed fusion (see Chapter VI, page 93).

C. Determination of Composition Diagrams

1. Microscopical Determination of Primary Point of Crystallization

The usual determination of the composition diagram of the organic substances is carried out through a series of experiments in which different mixtures of the two components are heated to determine the temperatures of primary crystallization and the corresponding breaks in the cooling curves. To complete the diagram the melting points of the pure components and of the eutectic are also determined^{92,214}.

The numerous difficulties arising in macro thermal analysis of organic compounds such as the tendency to supercool to form viscous liquids,

and the necessity for large amounts of material has led to the micro determination of melting points in the capillary tube (instead of the solidification point). In this way the melting point of the corresponding eutectic is also determined. The eutectic temperature should be constant, of course, for all possible mixtures. Stock²³⁵ calls this horizontal line the "sintering point curve", Rheinboldt²³¹ designates it as "the thaw point curve".

The observation of the eutectic temperature for different mixtures often permits a simple differentiation between systems forming a simple eutectic and those forming a molecular compound. Simple eutectic systems show a sinter point curve parallel to the eutectic line and rising rapidly only on both ends near the pure components. In a capillary tube the eutectic melt is apparent as very fine droplets on the glass wall. Since the amount of the resulting liquid phase (eutectic) depends on the eutectic temperature, the observation of the eutectic depends on the composition of the eutectic. The eutectic is always more difficult to observe the more closely the composition of the mixture corresponds to that of one of the pure components. With less than 10 to 15 percent of a foreign substance, the beginning of melting will not be visible in a capillary tube during macro determination at the eutectic temperature but only at a higher temperature.

The occurrence of a molecular compound is shown in the melting point diagram by a break in the sinter-point curve in the middle of the diagram. In this way a third substance, the molecular compound, is added to the system of the two starting materials (Figure 46). The melting point diagram now possesses two parts which are in contact with each other along the vertical line M-N, corresponding to the composition of the molecular compound. The molecular compound forms a eutectic with each pure substance and, as a rule, the melting points of these two eutectics are different. A mixture of composition M will give on crystallization a uniform crystal mass of the molecular compound which will melt sharply at the corresponding temperature, M. If the composition of the mixture lies either to the right or left of M, a second phase, A or B, will also crystallize. Mixtures between A and M begin to melt at E₂; those lying between M and B begin to melt at E₂. The sinter point curve which follows these eutectic lines rises sharply not only when the composition approaches that of the pure components but also toward the middle of the curve near the composition of the molecular compound (Figure 47). Through determination of the position of the peak of the sinter point curve the stoichiometric composition of the molecular compound can be determined. Other types of composition diagrams will be discussed in the next chapter on the fusion method.

Micro thermal analysis with the help of the microscope hot stage will give more information than the two macro methods described above. The attempts to introduce the microscope as a help in chemical investigations is very old. The earliest experiments with the microscope hot stage were made by O. Lehmann³⁵ in 1877, who in the following year published a large number of observations made with the help of his "crystallization microscope". Lehmann studied the identification of compounds and their polymorphism using molten droplets alone or with several droplets in contact with each other. This latter procedure he called "comparative crystal analysis". The chemist, however, paid little attention to this development of microscopical techniques²³⁷. Only very recently composition diagrams have been determined by microscopical methods. Grimm, Günther and Tittus²³⁸ have reported such determinations using the hot stage of Vorländer and Haberland; Lettré, Barnbeck and Lege²³⁹ with the melting point apparatus of Kofler and Hilbck²⁴⁰. The

same apparatus has been used by Lüttringhaus and Hauschild²⁴¹ and by Rheinbold and Mathias²⁴².

The microscopical investigations are made on a preparation between coverslip and slide using one of two techniques²⁴³. One consists of observation of the finely powdered mixture. This type of observation is directly comparable with the macroscopical determination in the capillary tube with the one difference that details of the melting process are much easier and more clearly visible with the microscopical magnification of 80 to 100 times. With this method the point of primary crystallization, i.e., the end of melting, of the particularly fine particles of the mixture must be ascertained. It is frequently an advantage to melt the mixture, crystallize it rapidly, and repowder the crystals in order to obtain as finely divided and uniformly mixed a preparation as possible, since the use of large particles would lead to high melting points. However, to determine only the eutectic temperature an especially fine powder is not necessary even with small amounts of foreign materials (under 2 percent). On the contrary, preparations can be too finely divided; this will be discussed in more detail later. Most of the above named authors used powdered mixtures (at times without a coverslip) for microscopical melting point determinations.

The second method of examination²⁴³ makes use of crystal films obtained by solidification of the melt between coverslip and slide. Although the first method is very useful for determination of eutectic temperatures, the second technique is of advantage in the determination of the point of primary crystallization or for the equilibrium melting point of the last residue of excess component.

The following method is usually used for the microscopical determination of the composition diagram. The eutectic temperature is first determined by finely grinding equal amounts of the two components between two object slides; placing a small amount of this powder on another object slide; covering it with a coverslip and warming it in the hot stage (page 39). At the eutectic temperature a part of the substance clearly begins to form liquid drops consisting of melt plus small unmelted crystals. The recognition of the eutectic temperature in a powdered preparation is clearly detectable even with only 1 percent of the minor component; even one-half or one-quarter percent can often be detected in the absence of solid solution formation²⁴⁴.

Enough material is used so that the entire space between the slide and coverslip will be filled. If the components of mixture are not too volatile the sample is melted without a coverslip. The melted drop is then covered with a preheated coverslip and crystallization is induced by placing the preparation on a cold metal block. If either component is volatile, then a coverslip must be placed over the crystals before melting and the melt should be mixed by movement of the coverslip before crystallization. In this way a comparatively fine-grained homogeneous preparation is obtained in which the excess component will then develop from a few centers pushing aside the residual melt so that local concentration differences result²⁴⁵. In such a preparation the resulting temperature would be too low in some portions of the preparation and too high in other portions of the preparation where the large crystals are formed. The preparation is then usually observed as close to the center of the coverslip as possible. The excess component will usually be visible above the eutectic temperature as a lattice-work or as individual crystals of approximately uniform crystal size. The preparation is heated further until only a small crystal residue remains; this temperature can be determined as an equilibrium melting point by heating and cooling the preparation over a narrow range to achieve slight growth and slight melting

of the last crystal residue.

The points of primary crystallization and the eutectic temperature are determined in this way on a number of mixtures of different composition, from about 10 to 90 percent. The composition diagram is then drawn through these points. The series of single determinations is best carried out with alternate A- and B-rich mixtures rather than with continuously increasing amounts of one component, since in this way more is learned about the morphology of the two components. If a morphological change of the crystal residue is found in a definite composition interval, for example between 30 and 40 percent, the further definition of the eutectic can be made by preparation of a mixture containing 35 percent and observing the type of crystal residue. It must be emphasized that with careful mixing and rapid solidification an accurate eutectic mixture can be obtained which will begin to melt at the correct eutectic temperature with the end of melting only 1 to 3°C higher. This range of 1 to 3°C results from the fact that even with rapid crystallization the crystal size is too coarse to allow the mixture to melt completely at the eutectic temperature. In the microscopical preparation of the eutectic mixture crystals of A and B can be seen lying adjacent to each other and dissolving only at temperatures a few degrees above the eutectic temperature because of local concentration. Sharp melting of a eutectic mixture is obtained only with very fine crystal size and exceptional mixing. Efforts to obtain as finely crystalline aggregates as possible through rapid crystallization make it necessary to remember that frequently unstable forms may be obtained in this way. Therefore, it is necessary to be familiar with the presence and behavior of possible polymorphs of each of the two components (page 68).

Binary systems having one highly volatile component or a molecular compound with a very much higher melting point than that of either component (e.g., picric acid plus α -naphthylamine) will show the same difficulties with the microscopical method as with the macro determination in an open capillary tube. In such cases the ordinary microscopical preparation in which the substance is placed between the coverslip and slide is useful only in a limited region on either side of the eutectic. At higher temperatures the loss of the volatile component results in a change in composition toward the less volatile component, so that high temperature values are obtained. One answer to this problem is the use of a second object slide in place of a coverslip. The melting of the sample as well as the crystallization must be carried out rapidly, and determination of the equilibrium melting point for the high melting mixtures in such preparations must be ignored since here a higher melting component becomes enriched very rapidly. Experience has shown here, also, that check determinations differ as a rule only from one-half to one degree.

An improved technique for handling such volatile components involves sealing the sample under the coverslip (page 17). The determination of the temperature of primary crystallization can then be determined easily and with the same accuracy as with nonvolatile materials. For particularly volatile substances the microbulb of Fischer (page 17) can be used or, still better, the micro molecular weight procedure described by Kofler and Brandstätter (page 58). For the use of the hot bar in simplifying these procedures see page 122.

2. Mixed Fusion

With the mixed fusion²⁴⁵ the zone of mixing between two crystal-

line compounds is observed microscopically during heating. Important conclusions can be drawn from these observations with respect to the behavior of both components.

The preparations necessary for this purpose are made up in the following way: A sample of the higher melting component is melted between coverslip and slide so that the melt occupies only one-half of the space beneath the coverslip (Figure 42, Part a). The melt is then allowed to solidify. The second component is then placed at the edge of the coverslip near the crystalline film already present. (Figure 42, Part b). The preparation is then heated so that the melt of the low melting component flows into the free space between the slide and coverslip. The introduction of the second component at b rather than at c (Figure 40) is an advantage, since this procedure avoids entrapment of air bubbles along the crystal-front of the first component. With similar or almost identical melting points introduction and melting of components A and B can be carried out at the same time.

The melt of component B dissolves a small amount of A to form the zone of mixing. This gives the two materials the opportunity either to mix to form solid solutions, a eutectic or a molecular compound. In the latter case, it is of advantage when making up the mixed fusion to remelt a large amount of component A after introduction of the second component. This gives a wider zone of mixing and thereby a better possibility for reaction. It is also possible, of course, to remelt and crystallize several times.

By rewarming the mixed fusion on the hot stage the appearance of the changes occurring in the zone of mixing can be definitely observed and used to indicate the type melting point diagram for the binary system involved.

(a) Systems in which solid solutions are not formed

When the two substances do not react with each other and are also not soluble in the solid state, then the eutectic must exist at a definite composition in the zone of mixing. On warming the preparation to the eutectic temperature this part of the zone of mixing will begin to melt first. It is helpful to observe the changes between crossed polars, since in this way the melt shows up clearly as a dark strip between the doubly refractive crystalline phases. Figure 49a shows a simple eutectic diagram; Figure 49b shows a schematic mixed fusion after heating to a temperature above the eutectic temperature. Figure 50 shows a mixed fusion of diphenyl and acetanilide photographed below (50a) and just above the eutectic temperature (50b).

Melting of the eutectic occurs with melting of the adjacent crystal region due to diffusion of the components. According to Tammann, heating of a mixture results first in reaching a temperature at which the lower melting component can migrate in the lattice and has a tendency to diffuse into the lattice of the second components. This results in decomposition of the second lattice which appears as melting.

As a rule the melting point of the eutectic is relatively lower²⁴⁷, the greater the melting point difference between the two components; at the same time the eutectic composition corresponds more closely to that of the lower melting component. The composition of the eutectic depends also on the slope of the two melting point curves; camphor, for example, has a very steep melting point curve. It appears in some cases as if the eutectic and the melting point of the lower melting component seems to coincide with each other (Figure 51)^{92,214}.

The formation of a molecular compound when two materials react with each other can frequently be recognized in the solidified preparation even macroscopically. The zone of mixing will show in this case a new phase appearing as a separate strip with a different type of crystal growth. To the two original components there is now added a third component, the molecular compound (Figure 52). On heating, such mixed fusions show melting of two eutectics. On one side of the contact zone is observed the eutectic between the lower melting component and the molecular compound (E_1) and on the other side the eutectic between the molecular compound and the higher melting component (E_2) (Figure 53).

Figure 54a shows a mixed fusion of 2,4-dinitrotoluene and naphthalene; these two components form an equimolecular. This molecular compound gives a eutectic E_1 melting at 52°C with dinitrotoluene (Figure 54b) and a eutectic E_2 melting at 55°C with naphthalene (Figure 54c).

If two molecular compounds occur with different percentage compositions these will also appear in the mixed fusion in the regions corresponding to the different compositions. Rewarming such preparations will show three different eutectics. Nicotinamide forms two molecular compounds with some dicarboxylic acids²⁴⁸. Figure 55 shows schematically the diagram for nicotinamide and decamethylene dicarboxylic acid. The photomicrographs (Figure 50) show a mixed fusion of these two components during heating. The eutectic E_1 melts at 117°C and the eutectic E_2 melts soon after at 119°C , while the separation of the molecular compounds through melting of E_3 occurs at 126°C . Two molecular compounds are also formed in the system hydroquinone-antipyrene²⁴⁹ (see Exercises). Nicotinamide exhibits three molecular compounds with pyrocatechol²⁵⁰.

Compounds which melt incongruently can also be recognized in the mixed fusion. In contrast, however, with congruently melting molecular compounds no second eutectic is formed here, i.e., no minimum melting point can be observed between the molecular compound and the higher melting component. The molecular compound does not melt to a homogeneous liquid but at a definite composition and crystals with a higher melting point^{92 214}. This case appears most often when the two components show a large difference in melting point. In Figure 58 three portions of the curve are shown corresponding to the three crystal types. The crystal phases in this mixture correspond to component A between a and E, to the molecular compound between Z and K, and to crystals of B between K and b. The highest point of the curve E-K, which corresponds in the usual diagram to the maximum melting point of congruently melting compound does not appear in this diagram; instead, this part of the melting point curve is obscured by the higher melting component (concealed maximum). Although the solidification process in the region between a and X proceeds in a manner typical of a simple eutectic, the mixtures containing larger amounts of component B show considerable differences. The vertical line M-M' correspond to the composition of the molecular compound. If a melt of this composition is cooled, component B will crystallize first; on reaching point M, however, the crystals of B already present decompose in the melt to give the compound AB (peritectic reaction). After completion of this reaction the only crystals remaining are those of the molecular compound AB.

If, instead, a mixture of composition N is observed, component B again separates first; on cooling to the straight line KP, component B again reacts through the melt to form AB. Since, however, the over-all concentration of the mixture is higher in component B than in the compound AB, some of the original crystals of B will remain. After complete crystallization there are, therefore, crystals of both B and AB present. With the mixture of com-

position R, then, B again crystallizes first and changes over at R' to AB; however, since B is completely absorbed in the A-rich melt, a melt with a percentage of A remains. On cooling further, AB crystallizes out until the concentration E is reached, at which temperature component A also crystallizes. The final crystal mass contains AB and A. Quite often, however, the reaction may not be complete because of rapid cooling and slow diffusion. The residue of the first component to crystallize may remain because it becomes surrounded on all sides by the reaction product AB. In this case the final product still contains B in addition to A and AB. The rate of diffusion is increased on heating the microscopical preparation to a temperature somewhat below the straight line K-P-H, and in this way the process may be forced to completion.

The hidden maximum, M, has a real significance; it represents the true melting point of the unstable addition compound, AB. This melting point may be observed in those cases where nucleation and crystallization of B fails to occur on heating above the transition temperature. Under these conditions the addition compounds melt homogeneously at the temperature M' (page 72).

A mixed fusion of a pair of substances with an incongruently melting molecular compound has the same appearance as one with a congruently melting molecular compound. The difference can be noticed only on warming; following melting of the lower eutectic the molecular compound gradually dissolves until finally at the temperature corresponding to the break in the melting point curve it completely disappears. In this way the changes described above corresponding to the concentrations between M' and K can frequently be followed. In Figure 58b assuming the temperature has reached point E, let the temperature rise now to the horizontal line K-P in the region where the curve for component B overlaps the curve for the molecular compound. Then a transformation will occur so that the molecular compound changes through the solution phase to component B at K (peritectic reaction). Confirmation of occurrence of a peritectic reaction is obtained through study of the concentrations in the region of the unstable maximum using the hot stage. These mixtures should melt incongruently with separation of crystals of B. The transition temperature is best determined by locating the three phase equilibrium temperature of melt AB and B. Examples of systems showing peritectic reactions are anthracene-picric acid, diphenylamine-picric acid, p-dinitrobenzene- α -naphthylamine, carbazole- α -trinitrotoluene (see Exercises) and most hydrates (page 20).

In the special case lying between the congruently and incongruently melting molecular compounds, the break in the melting point curve falls just at the maximum (Figure 59). The system, β -naphthol- β -naphthylamine is almost of this type²⁵². See also suberic acid-nicotinamide²⁴⁸.

In many cases which show molecular compounds the compound is not stable up to the eutectic temperature but starts to melt prematurely and incongruently. This type of molecular compound can be detected readily with thermal analysis under the microscope and especially with the fusion method. The system α -naphthol- β -naphthylamine forms an equimolecular addition compound which melts at 50°C with separation of crystals of β -naphthylamine, while the eutectic between β -naphthylamine and α -naphthol melts at 57°C. Observation of the point K is only possible by supercooling the α -naphthol-rich melt²⁵⁰ (page 135). In the system anthracene-2,4-dinitrophenol²⁵¹ long yellow needles of a molecular compound crystallize from a mixture containing 15 percent anthracene. The molecular compound is stable only in a melt containing excess anthracene and decomposes immediately in the presence of excess 2,4-dinitrophenol. The molecular compound melts incongruently precipitating

anthracene plates.

Some pairs of substances are distinguished from the above in that their liquid phases are not in all cases miscible with each other. The melting point diagram shown in Figure 60 illustrates this. Mixtures between the concentrations m and l melt to give two layers, the one a saturated solution of B in A, the other a saturated solution of A in B. The interval $m-l$ is called the "immiscibility region". With rising temperatures the mutual solubility increases a rule, thereby decreasing the restricted region between the two liquid phases until at the critical solution temperature (KLT) continuous miscibility again results. This behavior can be recognized in a single mixed fusion of, for example, resorcinol-triphenylmethane^{252,253} with no difficulty. On warming, the eutectic, of course, melts first. The resulting liquid phase (Figure 61) consists of a large amount of A (triphenylmethane) with a little B (resorcinol). On further heating more of B dissolves and B finally melts completely until at the temperature of the horizontal line $m-l$ the melt A is saturated with B; there now results a second liquid phase which consists of B saturated with A (Figure 61). These liquid phases are distinguished quite clearly without polarized light in the mixed fusion even in the presence of excess crystalline components A and B. If the stability of the melt permits, the critical solution temperature (KLT of Figure 61) can be reached at which the interface between the two liquid components finally disappears; for example, acetamide-benzil (Exercises page 178).

The investigation of mixed fusions makes it possible to determine the relation between unstable forms so that the melting point diagrams can, therefore, be made more complete in this respect. With the formation of metastable forms or through lack of knowledge of the existence of enantiotropy the melting point diagram determined by macro methods can be quite erroneous. For example, Kremann and coworkers²⁵⁵, reported a eutectic melting at 54°C for the binary system 2,6-dinitrotoluene-anthracene. This temperature, however, corresponds to the eutectic between anthracene and an unstable enantiotropic modification of 2,6-dinitrotoluene, while the eutectic with the stable form melting at 66°C melts, instead, at 62°C²⁵⁴.

If, in Figure 62, the unstable modification A_{II} crystallized instead of the stable form A_I then the eutectic with B_I will be found at the lower temperature E' rather than E . On the other hand, a mixed fusion between B_{II} and A_I will give a eutectic temperature E'' . Both unstable forms, A_{II} and B_{II} give with each other a eutectic E''' . All combinations except A_I-B_I involve unstable equilibria which are, however, not at all rare in the crystallization of organic mixtures. Unstable equilibria of this type often arise through slow crystallization. The large number of systems, however, transforms on warming to the stable phase. In Figure 63 a mixture N should begin to crystallize as molecular compound on cooling of the melt to the point a; instead, however, component B may crystallize at b rather than the molecular compound at a. In this case the composition of the melt changes with further crystallization of component B to E' , at which point the unstable eutectic crystallizes. In mixed fusions such unstable eutectics are frequently observed as a result of rapid cooling which seems to prevent nuclei formation of the molecular compound, e.g., diphenylamine-picric acid; azobenzene-picric acid²⁵¹; 1,3,5-trinitrobenzene-benzyl²⁵⁵; and veronal — pyramidon.

(b) Systems showing solid solutions (isomorphism)

Fusion methods have proved to be extraordinarily valuable for the study

of isomorphous behavior. The properties of isomorphous substances in the melt or saturated solutions of corresponding isomorphous substance are such that they grow as the same phase with each crystal growing across the zone of mixing with the same crystallographic orientation. In many cases, the differentiation of the system into the five Roozeboom types²¹³ can be made rapidly.

If mixed crystals between two substances are formed in all proportions the system shows continuous solid solubility^{92,214} and such substances are designated in the narrow sense as isomorphs (page 89). The melting point curves can be of three different types according to Roozeboom: Type I (Figure 64); Type II (Figure 65); and Type III (Figure 66). Only a single crystalline phase is obtained from any mixture in these systems; the melting point of each mixture lies on a continuous curve as a function of composition. For Type I the melting point of the mixed phase varies continuously from the melting point of the lower melting component to that of the higher melting component without maximum or minimum (Figure 64). The upper curve is the liquidus line, the lower curve is the solidus line. The region between the two curves is characterized by the fact that both melt and crystals are stable together. The solidification process starts with mixed crystals of composition *b* separating from a melt of composition *a*. The melt, as a result, becomes richer in A. From this melt, then, a mixed crystal must form which is still richer in A; the composition of the mixed crystals proceeds with decreasing temperature along the curve *ba'*, that of the melt along *aa'*. With sufficiently slow cooling the A-rich melt undergoes an exchange with the B-rich phase so that the end result is a crystal mass of the original composition *a*. On rapid cooling crystal layers result whose centers are richer in the higher melting component than the periphery.

Rapid cooling to a point below the solidus line will, as a rule, give mixed crystals of uniform composition. Since the above three types (Roozeboom) of solid solutions represent continuous solid solutions over the entire composition range, the appearance of crystallization of a mixed fusion will be the same for each. On nucleation the crystal-front of the higher melting component will grow with the same orientation through the zone of mixing and continue into the pure melt of the second component which, therefore, also crystallizes (b of Figures 64, 65 and 66). This crystallization process can, of course, be reversed by supercooling the melt. In this way growth of the lower melting component can be started; this then crystallizes across the zone of mixing to cause crystallization of the high melting component.

The classification of a system into one of the three above types can be very clearly determined by rewarming. With Type I the melting process begins in the pure low melting component (Figure 67 shows this behavior for the system propyl-3-methylbutyl barbituric acid (A) and n-butylpropyl barbituric acid (B) in the mixed fusion²⁵⁶. Figure 67a shows the crystallization front of B up to the melt of A. On lowering the temperature further B grows unhindered, though more slowly, through the zone of mixing (Figure 67b and c) into the melt of A. On rewarming the melting process begins in A (Figure 67d) and proceeds continuously towards B. Further examples are stilbene-azobenzene and naphthalene- β -naphthol.

Systems of Type II show melting of the low melting component first, followed by the second component leaving a strip of the high melting mixed crystals between (Figure 65). Optical isomers often show melting point diagrams of this type²⁵⁷.

With systems of Type III melting begins in the zone of mixing corresponding to a minimum in the composition diagram (Figure 66). Here the crystals on either side are apparently identical but with the middle melted away. During the melting process the temperature rise can be interrupted and the preparation can be slightly cooled, at which time crystallization proceeds again in the reverse direction. This experiment can be repeated again and again with the same preparation although each repetition will tend to broaden slightly the zone of mixing. An example of this is shown in Figure 68; dipropyl barbituric acid (m.p. 146°C), the higher melting component B is in contact with the melt of allylbutyl barbituric acid (m.p. 129°C; Figure 68a); in Figure 68b the crystallization front of B grows isomorphously into the mixed zone and has, in Figure 68c, completely solidified the entire preparation. Figure 68b and c shows the boundary of the material crystallizing on the right quite well as a result of difference in birefringence between the two components. The preparation is then rewarmed until the minimum mixture melts as shown in Figure 68d.

Roozeboom Types IV and V are differentiated from the first three types in that no continuous solid solutions exist between the two components. Instead of one type of crystal, two types are present in which each component can dissolve a definite amount of the other component. Those mixtures in the range KP' of Type IV, Figure 69, show with respect to their behavior on crystallization or melting great similarity to the process described above (Figure 58 between K and M) for the incongruently melting compound. If, in Figure 69, a mixture of concentration N crystallizes from the melt, the first phase to appear will be the mixed crystal O of composition near that of pure B. On further cooling a change occurs so that on reaching N' mixed crystals of composition S and a large amount of melt of composition X is present. At this temperature a change begins to occur in which both phases change into mixed crystals of composition P'. Since the melt is richer in B than the mixed crystals at P' (corresponding to the peritectic) after the new A-rich mixed crystals are formed, some of the original B-rich mixed crystals will still remain.

If the mixture R is allowed to crystallize in the melt, B-rich mixed crystals appear first which finally at R' again have the composition S. The liquid phase at this point is small in amount but has the composition K. Here again a change occurs, in this case with complete disappearance of the B-rich mixed crystals. If the preparation is cooled further to provide more opportunity for change to occur the preparation will ultimately solidify completely with only A-rich mixed crystals of composition R. If the miscibility decreases with decreasing temperature as is frequently the case, and if this change is similar to the line P'R'', then at R'' partial separation will occur with formation of B-rich mixed crystals. In Figure 69 the behavior of a solidified mixed fusion of Type IV on rewarming is shown. The beginning of melting occurs with the lower melting component A. On further increase in temperature the A-rich mixed crystals gradually melt. When the temperature reaches K there occurs a change of A-rich mixed crystals in the range KP throughout the region where B-rich mixed crystals have separated, in other words, the crystal-front of B-rich mixed crystals moves slightly to give a melt composition corresponding to K. Figure 70 represents a mixed fusion of dibenzyl-stilbene²⁵⁸. A peritectic reaction is also shown in Figure 71a; b, and c for mixtures containing 5-8% stilbene. On cooling the melt crystallizes to give dibenzyl type crystals; on heating to the peritectic reaction temperature (55°C) the mixed crystals begin to melt with simultaneous separation of crystals having the stilbene lattice structure (Figure

71a). Figure 71b shows the three phase equilibrium. On slight cooling below the peritectic temperature the transition reverses (Figure 71c).

Roozeboom Type V, Figure 72, behaves in a manner similar to a simple eutectic on melting or crystallization; however, the eutectic is made up not of the pure components but of mixed crystal phases. Puer²⁵⁹ has pointed out that there can be two reasons why a given system might show Roozeboom Types IV and V. First, the structures may be different enough so that each can tolerate only a small proportion of the second component. Second, the system may show isomorphism (see page 87).

In the first case, the two structures are isotypes but the lattice dimensions are so different that above a certain concentration continuous solid solubility is impossible, e.g., KCl-KI (size difference between Cl and I is 22%). Experience has shown that the upper limit for difference in ionic radii is 10-15%. Above this figure there will, in general, be only partial solid solubility. At higher temperatures continuous solid solubility may be possible; however, on cooling, separation into two phases occurs (Figure 73). If the region of non-solid solubility approaches the melting region then there will be two melting point curves corresponding to the two series A and B.

Such a system can sometimes be made to show continuous solid solubility at room temperature by addition of a third component. In the system Ag-Au-Cu²⁶⁰ there is continuous solid solubility; likewise in the two binary systems, Ag-Au and Au-Cu. Ag-Cu, however, shows only partial solid solubility even though all three are isotypes²⁶⁰. A similar situation exists in the pentachlorobenzene series²⁶¹. Separation of the solid solution into two phases at lower temperature may also be due to enantiotropic transformation²¹⁴.

(c) Isodimorphism and isopolymorphism

Roozeboom Types IV and V are related to isodimorphism which occurs when two dimorphous materials form two series of mixed crystals. If the two components form several series of mixed crystals corresponding to several polymorphic modifications, this is designated as isopolymorphism. Figure 74 shows a case in which two stable and two unstable modifications form continuous solid solutions with each other. Such behavior is characterized as parallel isodimorphism. According to Brandstätter²⁶² p-bromo- and p-chloroacetanilide form two series of mixed crystals analogous to Figure 74. The polymorphic forms of 2,4-dinitrobromobenzene and 2,4-dinitrochlorobenzene show four mixed crystal series of Type I between corresponding polymorphic forms (page 136)²⁶³.

Frequently, however, mixed crystal formation occurs not between two stable or two unstable modifications respectively but between a stable form and an unstable form. The melting point curves of both series of mixtures must, therefore, intersect; the position of the intersection determines the miscibility limits and the stability of the various forms. In Figures 75 and 76 the melting points of the stable forms are designated as A_I and B_I with A_{II} and B_{II} as the unstable forms. A_I forms with B_{II} and A_{II} forms with B_I , continuous series of mixed crystals. The two curves intersect in Figure 75 at point K; from the positions of the solidus lines the resulting gap in the mixture series can be constructed between m and l. With this type of intersection the melting point of A-rich mixed crystals rises with increasing content of B, with formation of a break in the curve at K (Type IV Roozeboom, e.g., dibenzyl-stilbene²⁵⁸). The two isodimorphous mixed crystals curves can,

however, intersect so that the point of intersection lies lower than the melting point of the lower melting component (Figure 76). There results in this case a eutectic consisting of the two types of mixed crystals (Type V Roozeboom, *e.g.*, benzalaniline-azobenzene²⁶⁴).

The mere fact of the appearance of isodimorphism is, in itself, not sufficient to prove the system is either Type IV or Type V. There is (Figure 71) a system illustrated in which Type III is combined with Type I (dibenzyl with azobenzene, or tolan²⁵⁸). In addition, more than two mixed crystal curves may intersect each other as shown in Figure 78.

Both series of mixed crystals can be obtained completely in many cases of isodimorphous mixed crystal formation although in other cases they may be only incompletely realized. Picric acid grows isomorphously with an unstable modification of 1,3,5-trinitrobenzene in the undercooled melt at about 105°C and forms the unstable modification III (melting point, 109°C). In a similar way, the stable modification of 1,3,5-trinitrobenzene grows isomorphously in the picric acid melt and forms an unstable modification of picric acid having a melting point of 75°C. These two modifications are completely miscible in all proportions according to Type I Roozeboom¹⁷⁹ (schematically shown in Figure 76). The mixed crystal series with crossed melting point curves between picric acid I and trinitrobenzene III (119°C) can be demonstrated experimentally¹⁷⁹ (page 136). It often occurs as a result of variations in stability that corresponding polymorphic forms of related compounds are not isotype but homotype or even heterotype. In such cases the formation of mixed crystals occurs not in spite of that but on the basis of isodimorphism. In the system picric acid-trinitrobenzene the stable forms are probably homotypes since picric acid is rhombic pyramidal and trinitrobenzene is rhombic bipyramidal.

In many cases only one of the two series of mixed crystals can be obtained throughout the entire composition range while the other series can be obtained only over a short range of its unstable existence. This is true, for example, in the system benzalaniline-azobenzene²⁶⁴ which forms continuous solid solutions having the azobenzene structure and in which the benzalaniline exists as an unstable modification. This series of solid solutions is obtained without difficulty on spontaneous crystallization of mixtures of any percentage composition. The transformation of the corresponding unstable mixture to the stable form of benzalaniline occurs usually only on warming. The mixed crystal series with the stable benzalaniline structure can be obtained at temperatures only a little above the stable part of the curve.

Azobenzene also forms continuous solid solutions with dibenzyl II²⁵⁸; this series can be obtained over the entire range with strong supercooling. A second mixed crystal series having the structure of the stable modification of dibenzyl, can be obtained only over a part of the composition range. Although many isodimorphous series cannot be obtained over the complete range, they are included under the classification isodimorphous when the mixed crystal formation can be obtained over a comparatively wide range.

The smaller the temperature difference between the melting points of the polymorphic forms of a given component in a system of mixed crystals showing intersection of the melting point curves, the nearer the intersection point, K or E in the diagram, approaches the higher concentration of that component. For example, in the system dibenzyl-stilbene²⁵⁸ (Type IV; shown schematically in Figure 69), point K lies at 3 percent stilbene. In the system p-dichlorobenzene-p-dibromobenzene (Type V) point E lies at 2 percent p-dibromobenzene. In both of the above cases isodimorphism is shown not by the two stable forms but by the stable form of one with the unstable form of the other component.

The very unstable form of dibenzyl melts at 50°C; the stable form at 53°C. In spite of the great instability of the unstable form this lattice structure becomes the stable one with only 3% of stilbene added to it. In the second case, p-dichloro- and p-dibromobenzene, the situation is due to enantiotropism. The unstable p-dichlorobenzene structure is stabilized by 2% p-dibromobenzene (page 136).

There are systems in which the addition of 1% of an isomorphous compound stabilizes an unstable lattice type. In the above-mentioned system, picric acid- α -trinitrobenzene¹⁷⁹ the unstable modification II (110°C) of TNB reverses its relative stability toward form III (109°C) with only 1% picric acid. Six percent of picryl chloride also stabilizes form III of TNB with respect to form II¹⁷⁹.

The terms "homotype miscibility" and "heterotype miscibility" may be used for organic systems in the same sense as they have already been used for metallic systems. For example, in the systems magnesium-cadmium and magnesium-lithium^{265,230} the latter shows two phases, hence only partial solid solubility. Magnesium-cadmium was investigated by Grube and Schiedt²⁶⁶ and by DeKlinger²⁶⁷ and found to show two phases with a peritectic reaction (see also Hume-Rothery and Rowell²⁶⁸).

New results by Hume-Rothery and Raynor show that Mg and Cd are hexagonal homotypes with continuous solid solubility²⁶⁹. This is a very interesting case in which the authors were able to measure the cell dimensions as a function of composition. Most systems described as hetero-²⁶⁰ homotypes show partial miscibility^{265,230} and may show intermediate phases²⁷⁰.

For aromatic hydrocarbons, according to Lüttringham²⁷⁰, fused ring and open form behave differently, so that the former comply with the Grimm's displacement principle²³⁴, while the latter do not. So, for example, the fused ring compounds, diphenylene oxide (dibenzofurane) and diphenylene sulfide (dibenzothiophene), are very similar chemically and, in addition, both have parallel arrangement of the benzene rings so that the observed continuous solid solubility is understandable. On the other hand, the valence angles for the two compounds, dianisyl oxide and dianisyl sulfide, are different from each other and, at the same time, the two benzene rings are free to rotate; as a result, macrothermal analysis of this system shows a single eutectic.

However, microthermal analysis shows that both pairs of substances, the open ring compounds as well as the fused ring compounds, behave similarly, and indeed both show isodimorphism. The fused ring oxide (O) shows continuous solid solubility with an unstable modification of the fused ring sulfide (S_{II}) while the lattice of the stable modification of the sulfide (S_I) can accommodate only a small amount of the oxide. The continuous series of solid solutions (O-S_{II}) formed by the fused ring compounds is Type III Roozeboom; the intersection with the curve for the stable form is a peritectic. Since the two curves overlap very closely they were not detected by the macrodetermination (Figure 79); for the open ring forms the solid solution (O-S_{II}) is of Type I Roozeboom (Figure 80), the partial curve for the stable modification cutting the melting point curve of the series of mixed crystals in a eutectic. All gradations of behavior are, in general, observed between these two general types of overlapping of the two curves (page 104).

Organic substances with linear molecules such as paraffins and their derivatives show in their isomorphous behavior less sensitivity to molecular size such as hexadecane, heptadecane, octadecane, hexadecanol, heptadecanol and octadecanol.

A special position is held by materials with approximately spherical

molecules. Very low lattice forces are usually associated with these crystals (e.g., carbon tetrabromide, hexachloroethane, camphor etc.). The spherical structure is enhanced with these molecules through rotation or other movements of the molecule. Differences in molecular structure are at least partially eliminated through rotation and the state of the molecule approaches that existing in the liquid stage. These compounds very frequently form, as a result, isotropic, i.e., cubic, crystal lattices. Rotation and other movement of these molecules in effect reduces the effect of chemical differences between the molecules and means, therefore, that not only the type of crystal lattice but also the miscibility (isomorphous behavior) depends less on the chemical structure than with other types of molecules.

Again with systems of this type there is lack of complete information (even in the composition diagram which considers polymorphism) from which the miscibility relationship can be clearly and unequivocally obtained except as deviations from Raoult's law. Pirch²⁷¹ has investigated many systems involving replacement of a chloro group with a bromo as well as many spherical type molecules.

(d) Stabilized intermediate phases

A special case of isodimorphism of organic substances is presented by the formation of the so-called stabilized intermediate phases²⁷¹ which may arise through the intersection of more than two mixed crystal series as mentioned above (Figure 78). This involves a mixed phase made up of the unstable modifications of both components which is, however, the stable phase in a definite region of the composition diagram. The stabilization of the intermediate phase can be explained on the basis that the transition temperature of one of the pure monotropic components is lowered below the melting point by the formation of solid solutions (Figure 29). (For details on the change of transition point during formation of mixed crystals see Roozeboom²⁷².) This effect is more noticeable in the case where the transition temperature (t of Figure 29) lies close to the vapor pressure curve of the liquid phase, f -f. Cinnamic acid shows a decreasing transformation rate of Form II with rising temperature, hence it can be assumed that the transition temperature lies close above the melting point. This assumption appears under the circumstances to be justified since cinnamic acid in solid solutions with hydrocinnamic acid becomes enantiotropic²⁷³.

The different positions of the curve showing stabilized intermediate phases are represented in Figures 81, 82, 83 and 84. In all four cases continuous solid solubility exists between the unstable modifications of both components, i.e., A_{II} and B_{II} . In Figure 81 these mixed crystal series intersect the curves of both stable forms A_I and B_I in two peritectic transition points K_1 and K_2 respectively (example: cinnamic acid-hydrocinnamic acid²⁷³). In Figure 82 there arises through intersection of the branches of A_I and B_I a eutectic E and transformation point K (example: benzalaniline-dibenzyl²⁶⁴ [see pages 133]). In Figure 83 the continuous solid solubility corresponds to Type III of Roozeboom. The intersection point with the stable branches forms two transition points (example: barbituric acid derivatives²⁵⁶). Figure 84 shows an unstable mixed crystal series of Type II, in which the stable branches intersect in two eutectic points (example: *d*- and *l*-bromocamphor²⁷⁴).

The case illustrated schematically in Figure 83 is represented thus far only in the system allylisopropyl barbituric acid (A) and ethylpropyl barbituric acid (B). This is shown in Figure 85²⁵⁶. Figure 86 shows schematical-

ly the resulting melting point diagram derived from the behavior of the mixed fusion (without the liquidus curves). The crystals of Figure 85 correspond to a solid solution, $A_{III}-B_{II}$ shown schematically in Figure 86 which corresponds to Type IV. With very little warming, B_{II} changes to the stable form B_I as can be seen in the right part of Figure 85b. The left portion of the figure shows first no change other than melting of the minimum mixture at the proper temperature (Figure 85c). If the preparation is heated slowly then A_{III} transforms slowly into the corresponding A_{II} whose curve $A_{II}-R$ is shown schematically in Figure 86. The crystals remaining in the center correspond to that part of the curve, RK; the minimum is melted away although on cooling this region grows back together again. In Figure 85d in addition to the mixed crystal series (corresponding to the curve PR) modification A_I begins to grow through A_{II} .

Both Figures 87 and 88 show intermediate phases in fatty acid systems. Palmitic acid and stearic acid (Figure 87) show a diagram corresponding to the schematic Figure 82. Stearic acid and pentadecanoic acid show two intermediate phases (Figure 88).

Various types of overlapping were also observed with other barbituric acid derivatives observed by M. Brandstätter²⁵⁶.

The melting point curves of many isomorphous organic two-component systems show maxima or minima. In the literature the appearance of a maximum, alone or with a minimum, has been interpreted as a molecular compound. The reason in some of these cases is that the observed maximum lies approximately at the position of a simple stoichiometric composition. The melting point curve with a maximum (Type II) has been observed in systems of optical isomers, for example, d- and l-carvoxime^{257,276}. H. Lettré²⁷⁷ has shown that epihydro- and dihydrolumisterol shows a melting point diagram of Type II Roozeboom, whose maximum corresponds to a molecular compound. The system β -naphthol- β -naphthylamine shows a maximum which has been attributed to a molecular compound²⁵⁰ on the basis of the reactivity between the phenolic hydroxyl and the amino group. Further examples of the appearance of maxima and minima are shown in the melting point diagrams of the systems; ethyl-(1-methylbutyl)-barbituric acid-ethylpropyl barbituric acid²⁵⁶; and methylbutylketone-2,4-dinitrophenylhydrazones⁶⁶ (page 114). Thermodynamically, however, the appearance of a maximum is obviously not sufficient proof of a molecular compound²⁷⁸. The influence of seeding can be readily investigated using mixed fusions. The problem of nucleation is quite complex since a large number of factors play a role. The influence of the phase boundaries are characterized by the melting point diagram for the materials in question^{279,280}. It has already been pointed out by Schaum²⁸¹ that inoculation of phases must be carried out very carefully because the small mechanical shock involved during seeding may cause inoculation of a new phase even though no structural relationship is present²⁸².

The recently introduced concept of "isomorphous nuclei" (Correns²⁸³) refers to the above described case of nucleation on the basis of definite lattice analogies. "Isomorphous nuclei" must not be confused with "related nuclei". The latter presumes a positive seeding effect without reference to or knowledge of the thermodynamic relationship. "Related nuclei" is, therefore, an entirely general concept; it includes not only the nucleation due to isomorphous growth but also nucleation by thermal shock²⁸⁴.

3. Melting and Crystallization of Mixed Crystals

Mixed crystals melt and crystallize over a small temperature interval⁹²

with compensating changes in melt and crystal composition. To obtain crystals of homogeneous composition from the melt it is necessary to: (1) crystallize under equilibrium conditions, or (2) supercool strongly below the solidus curve. In the first case more time is needed since diffusion in the solid state is very slow. Very often diffusion is not able to proceed fast enough to maintain equilibrium even in metals systems²⁸⁵. The result is crystals of varying composition.

This phenomenon is known in alloy systems as grain segregation²⁸⁵. With salt systems and especially when working with small quantities the system may be strongly supercooled so that it solidifies rapidly and homogeneously below the solidus curve.

A mixture of 80% NaNO_3 and 20% KNO_3 between a microscope slide and coverslip forms aggregates of spherulites when crystallized from the melt by quenching on a metal block (Figure 89). On reheating to the melting point these crystals change in composition. Heating experiments on such crystals have shown that the averaging of the composition of the mixed crystals occurs partly by diffusion and partly by a rhythmic process of melting and crystallization²⁸⁸. At 235°C on heating a rhythmical transformation of the crystals occurs.

The crystals which begin to separate at about 235°C show a peculiar rhythmic structure. The spherulitic growth shows concentric rings or spirals (Figure 90). On heating, growth of some rings occurs with disappearance of others. New nuclei may also appear. If the preparation is heated at a rate of 2-4°C per minute fewer transformations are observed up to the melting point. The composition of these crystals is different in different parts as shown by the behavior on melting. The lowest melting point, for example, of an 80% NaNO_3 -20% KNO_3 mixture is 235°C, whereas the final melting point is 276°C.

If this same mixture is cooled after complete melting crystal nuclei appear spontaneously at about 275°C. These crystals grow rapidly (8-10 seconds) as dendrites until they reach their neighbors (Figure 91). During further cooling the continued growth of these dendrites outward into the remaining melt continues slowly as a change begins to occur within the crystals themselves. The dendrites begin to show knob or ridgelike thickening and soon become individual, separate crystals as part of the structure dissolves and part crystallizes. This transformation is especially clear at the ends of the dendrites since as these dendrites continue to grow into the melt they are being dissolved by the newly initiated growth (Figure 92).

Crystallization in this system does not take place with complete equilibrium. The centers of the crystals are richer in NaNO_3 while the last portion to crystallize is richer in KNO_3 . The beginning of melting of such a mixture, therefore, often begins as low as 225°C.

The system NaNO_3 - KNO_3 has a minimum at 220°C. With the first appearance of melting the crystals show the characteristic spiral design (Figure 94). This design can usually be seen best with crossed nicols by the difference in interference colors. It can also be seen on further heating since the transformation occurs most readily in the low melting parts of the crystal pattern. At the melting point no part of the original structure remains; melting occurs in a rhythmic manner throughout the preparation (Figure 95).

During primary crystallization of these dendrites a peculiar rhythmic "recoil" of crystal growth can be observed especially if growth is slowed in one direction by cooling only from one side or by concentration variations in the preparation. This "recoil" is, according to Masing and Haase²⁸⁷⁻²⁸⁸, the basis for the segregation of one component during crystallization.

Such crystals called zone crystals occur with metallic alloys but in those systems can become homogeneous by heating for a long time at a high temperature²⁸⁵. Two experiments have been carried out in an attempt to homogenize NaNO_3 - KNO_3 zone crystals by annealing. In the first experiment no change was noted in the structure after 10 hours at 205-208°C. This temperature was chosen so that no melt would be present. In many experiments with both organic and inorganic systems even binary mixtures of very pure components show traces of liquid even 10°C below the eutectic melting point²⁸⁹.

The second experiment was performed at 224-226°C in order to determine the effect of a small amount of melt since the liquidus temperature is 235°C. After a short time a transformation is observed with many new nuclei forming throughout the preparation. After about 4 hours heating the crystal mass becomes quite homogeneous (Figure 96). The time required for homogenizing salt systems is ordinarily much longer than the time required for alloys. Other mixed salt crystals such as NaNO_3 - NaNO_2 show a similar behavior.

The appearance of rhythmic precipitation can also be observed in organic mixed crystal systems (e.g., phenanthrene:anthracene, Figures 97 and 98). Organic systems show some differences in appearance because of the general difference in crystal type but also because of the difference in solidus and liquidus temperatures for a given mixture. The greater this temperature difference and the greater the ease of crystallization the more clearly the zone crystals can be observed. The rate of temperature change also plays a role.

4. Three-Component Systems

The temperature of the ternary eutectic is observed in a manner analogous to that used with binary systems; a finely powdered mixture of the three components is observed during warming. The mixed fusion is used to determine the eutectic concentration²⁵⁵.

Different portions of the three-component composition diagram can be studied in the different concentration regions of the contact zone. The simplest procedure is to make up a mixed fusion of various mixtures with the pure substances. If, for example, in Figure 99 the intersection line I is to be studied then a mixture H is melted in a mixed fusion with component A. The contact zone will then contain all mixtures between A and M. On warming such a preparation the ternary eutectic E will melt, leaving phases A and B in the appropriate relationship to each other as shown in Figure 100. When the temperature first reaches the melting point of the binary eutectic m a strip of liquid will appear in the mixed fusion. If, however, on heating a preparation representing intersection line II which passes through the ternary eutectic point then on reaching the temperature E, as shown in Figure 101, a more completely melted strip appears in the contact zone. As a rule, the intersection line from A on which the ternary eutectic must lie can be determined with only a few experiments. In the same way components B and C are examined with corresponding mixtures of the other three components (intersection lines II and IV of Figure 99).

In these experiments concentrations were frequently found which correspond, for example, to intersection line 7 of Figure 99. It is obvious during heating of such a mixed fusion that the zone of mixing contains three branches of the curve corresponding to three types of crystals⁹². Component A melts in the concentration range Ar, component B between r and s and component C between s and N. Most of these intersection lines are characterized very sensitively by the mixed fusion so that they serve especially well for

control experiments even with quasibinary intersections, e.g., VI of Figure 99; these are valuable, for example, in the presence of a molecular compound.

Often the contact zone experiments with the intersection line method lead not to a single point but finally to a small triangle. The final determination is then made through successive approximation of the ternary eutectic composition with microscopic identification of the crystal type remaining after melting of the eutectic.

The following three-component systems have been investigated by the above methods:

1. Azobenzene-acenaphthene-benzil

Binary eutectics:

azobenzene-acenaphthene, 47°C and 34 percent acenaphthene,
azobenzene-benzil, 52°C and 38 percent benzil,
benzil-acenaphthene, 63°C and 43 percent acenaphthene,
26 percent acenaphthene, and 28 percent benzil.

2. 2,4-dinitrophenol-acetanilide-benzil (Figure 92)

Binary eutectics:

2,4-dinitrophenol-acetanilide, 79°C and 47 percent acetanilide,
benzil-acetanilide, 78°C and 32 percent acetanilide,
2,4-dinitrophenol-benzil, 69°C and 63 percent benzil

The ternary eutectic lies at 58°C and 42 percent benzil,
36 percent 2,4-dinitrophenol, 22 percent acetanilide (Figure 102)

These results deviate from the earlier investigations of Tammann and Botschwar²⁹⁰. These authors also reported an incorrect eutectic composition for the system 2,4-dinitrophenol-benzil (the correct value is 50 percent benzil rather than 63 percent).

3. 1,3,5-trinitrobenzene-fluorene-benzil (Figure 93).

The system contains two molecular compounds: one, a congruently melting equimolecular addition compound between trinitrobenzene and fluorene (M_1)¹⁶³, and an incongruently melting molecular compound between trinitrobenzene and benzil¹⁶³ ($M_2 = 2:1$).

Binary eutectics:

1,3,5-trinitrobenzene (T)-fluorene (Fl); $e_1 = 100^\circ\text{C}$ and 22 percent T, $M = 106^\circ\text{C}$, $e_2 = 88^\circ\text{C}$, and 64 percent T.
1,3,5-trinitrobenzene-benzil (B); $e_1 = 74^\circ\text{C}$ and 34 percent T, $u = 90^\circ\text{C}$ and 62 percent T.
benzil-fluorene; 69°C and 38 percent Fl.

The coordinates of the three ternary eutectics are:

E_1 (B, Fl, M_1) 60°C and 22 percent T, 33 percent Fl, 45 percent B
 E_2 (B, M_1 , M_2) 64°C and 40 percent T, 12 percent Fl, 48 percent B
 E_3 (T, M_1 , M_2) 78°C and 61 percent T, 13 percent Fl, 26 percent B.

5. Analysis of Mixtures

(a) Qualitative analysis

There is almost no other method which is as effective and rapid as the absorption method (see page 47) for the isolation and identification of both components of a binary mixture. The question as to which component of a

given mixture will be isolated by the absorption method is answered by determining which melting point curve of the composition diagram is intercepted by the line corresponding to that composition.

To isolate the other component of the mixture the composition of the mixture must be shifted to the other side of the eutectic composition. This can be done by solvent extraction, chromatography or etc. This is naturally much easier than complete purification of the substance since only a slight change in the composition of the mixture is necessary. The final purification is carried out by the absorption method.

Isolation of the second component is usually carried out in the following manner²⁹¹. The solubility of the already isolated component is found from the literature or by experiment and the solvents in which this component is most soluble are used to extract that component from the mixture thereby concentrating the desired second component. This operation is carried out by dropping the solvent drop by drop onto a small amount of the mixture placed at the center of a filter paper. Of course, either component may be dissolved and the desired component may be isolated as solid crystals on the top of the filter paper or by evaporation of the solution that soaks into the filter paper. The solution is isolated from the filter paper by washing with excess solvent. This solvent is easily evaporated on a microscope slide without spreading if the slide is placed on a hot metal plate having an opening in the center. The solution is placed drop-wise on the slide over the opening in the metal plate²⁹¹. The residue can then be immediately subjected to further purification by the absorption method without removing it from the slide.

It may sometimes be advantageous to start with the eutectic melt removed from the original mixture during isolation of the principal component by absorption. To do this the original sample, on a filter paper, is placed on a microscope slide covered with a second slide and heated to a temperature just slightly above the eutectic melting point. As soon as the filter paper is well soaked with eutectic melt it is removed and extracted with a solvent, preferably one in which the desired component will be most soluble. The desired component is then isolated from the solution by evaporation on a microscope slide and from the remaining second component by the absorption technique.

If these procedures do not give the desired result chromatography should be tried (see page 47). Here, too, the solution can be evaporated to dryness on a microscope slide as it drips from the adsorption column.

In some cases the second component may be concentrated by microsublimation. This is usually most readily accomplished when the two components differ greatly in melting point. Unfortunately, however, in this case the eutectic composition lies nearer a 50:50 composition. It is important to realize that the principal component is not always the easiest component to isolate. The composition of the mixture relative to the melting point curves of the composition diagram are the sole determinant as to which component will be isolated. If the high melting component (A) melts much higher than the low melting component (B) the eutectic composition may be very close to that of pure B and, in this case, pure A may still be isolated from mixtures containing only a low percentage of that component (see page 22). For a basis for judging volatility see page 11 and the data given in the tables under "Special Characteristics".

With mixtures containing three or more components the use of absorption methods for the isolation of one component is usually no more difficult than for two component mixtures. It is, of course, more difficult to isolate com-

ponents other than the principal one from such mixtures. The problem here again is to shift the composition of the mixture to another branch of the melting point diagram. When this is achieved, the absorption method may again be applied.

The problem is, of course, more complicated when the components of the mixture form a molecular compound. The phase obtained then depends on the composition of the mixture and the stability of the molecular compound. If the addition compound is formed it is often possible to shift the composition of the mixture through use of suitable solvents so that the absorption technique will give one of the two pure components. The occurrence of an addition compound in a given system is best determined using the fusion method (see page 93). If a stable addition compound exists in a given system it is then best to use chromatography in an effort to isolate one of the two pure components. Aluminum oxide is probably one of the best adsorbents for this purpose.

The absorption method cannot, in general, be applied to systems involving solid solution. If, however, the isomorphous materials are present as a mechanical mixture and not as mixed crystals the higher melting component can usually be concentrated by the absorption.

After the components have been identified in the mixture the question frequently arises as to whether other components are also present. The mixed melting point method can be used here provided there is a large enough difference between the eutectic temperature of the ternary system and the already identified binary system. This condition is, in almost every case, fulfilled.

The table shown below covers a few examples of this. The first column of numbers shows the melting points of the pure compounds and the next two columns show respectively eutectic temperatures of the binary and ternary mixtures. The mixtures for which melting points are given are characterized by preceding the melting point with the first letter of the name for that component of that mixture.

In the examples shown, the eutectic temperatures of the ternary mixtures lie as a rule far below the melting points of the binary mixtures. If two substances have been identified and determination of the eutectic temperature gives the correct result for these two substances, the presence of a third component can be excluded almost with certainty. Only in the last example in the table is the difference in the eutectic temperature of the two-component and the three-component mixture too small. With the help of the eutectic temperatures, the presence of phenacetin in a mixture of caffeine and pyramidon in a mixture of phenacetin and caffeine can be quite readily detected. However, the presence of caffeine cannot be determined with certainty in a mixture of pyramidon and phenacetin because the addition of caffeine lowers the melting point of the eutectic only from 82 to 81°C.

Fortunately, however, there are a few such exceptions. Application of the absorption method to mixtures containing caffeine usually results in the isolation of caffeine. This is because the eutectic point usually lies close to the side of the other components in the composition diagram since the melting point of caffeine is very high. Thus, even when it is present in small amounts, caffeine is usually isolated in small quantities. After the caffeine and both other substances have been identified the problem still remains whether further components are present. This question again can be easily answered if the eutectic temperatures have been determined.

The new procedure permits, among other things, a rapid and simple determination of the presence of isomers in a preparation. For example, commer-

Substance	Eutectic Temperature °C																		
	M.P. °C	Adipic acid amide	p-amino- acetanilide	Benzanilide	Acetyl-p- toluidine	p-Phenylene- diamine	Isobutyl- phenetidine	Phenacetin	Benzidine	Benzamide	Acetanilide	β-Naphthyl- amine	Acetyl-o- toluidine	o-Phenylene- diamine	Methylacet- anilide	m-Tolylene- diamine	Acetoacet- anilide	Acetamide	Lactamide
p-Anisidine	57	56	55	54	53	51	52	53,5	44	48,5	47,5	45	47	43	31	42	39	41	47
Formyldiphenylamine	73,5	65	68	63	64	63	61	65	48	61	53	45	57	40	40	40	44,5	60	61
Lactamide	78	73	70	74	73	72	73	72	71	62	67	71	64	58	57	60	60	softens	
Acetamide	80	78	71	77	73	67	75	73	67	54	62	70	63	57	60	57	50		
Acetoacetanilide	84	77	74	77	73	67	70	72	66	65	62	64	59	48	40	56			
m-Toluylenediamine	90	97	89	92	88	84	87	85	73	73	73	75	76	60	57				
Methylacetanilide	100	96	87	78	77	78	72	77	54	60	60	53	67	55					
o-Phenylenediamine	103	99	91	94	91	83	90	88	75	76	73	78	74						
Acetyl-o-toluidine	110	108	99	94	88	94	83	88	86	82	74	78							
β-Naphthylamine	112	106	102	98	95	93	88	91	83	88	82								
Acetanilide	115	112	101	97	99	93	86	90	88	82									
Benzamide	128	122	104	108	102	98	97	98	90										
Benzidine	129	124	109	110	105	93	101	98											
Phenacetin	135	130	117	113	109	109	104												
Isobutyl-phenetidin	135	131	119	111	105	111													
p-Phenylenediamine	141	136	114	122	116														
Acetyl-p-toluidine	148	147	120	121															
Benzanilide	163	159	134																
p-Aminoacetanilide	163	155																	
Adipic acid amide	227																		

cially available m-dinitrobenzene often shows premature melting of single particles below the known melting point. It was naturally assumed that the impurities were isomers although it could not be decided without further work whether the o- or p-compound, or both, were present. However, the above described method for use of the eutectic temperatures makes this identification quite easy. The melting point of m-dinitrobenzene is 91°C; the industrial preparation begins to melt at 64°C, which corresponds to the eutectic temperature of m- and o-dinitrobenzene. A small amount of o-dinitrobenzene is mixed with the commercial preparation, but it does not change the initial melting temperature from 64°C. On the other hand, addition of a small amount of p-dinitrobenzene lowers the beginning of melting to 60°C, the eutectic temperature of the ternary mixture. In this way it is determined without doubt that the industrial preparation of m-dinitrobenzene contains the o- but no p-isomer.

The presence of theobromine or theophylline or both in a caffeine preparation can be determined in a similar way. This procedure can also be used with advantage for other alkaloids.

In two-component mixtures and with a restricted number of possible components, the identification can be simplified still further. For this purpose the eutectic temperatures are determined and recorded for all possible two-component mixtures. In an actual analysis of an unknown two-component mixture, the eutectic temperature is determined first. One of the components is then isolated through absorption of the eutectic melt and identified by its melting point or when necessary by one of the other micromethods. From these data the possible other components showing identical or very similar eutectic temperatures are obtained. The two substances are positively identified by observing a contact preparation¹ between the mixture and the possible second components. It is not necessary in this case to isolate the second component.

This method is illustrated by the twenty anilides, and acid anides in the accompanying table which includes the eutectic temperatures for 190 possible binary mixtures. A mixture containing any two of these compounds can be identified rapidly and with certainty with the help of the table. Let us assume, for example, that a mixture with a eutectic temperature of 67°C is being analyzed, and that the absorption method enables us to isolate benzidine from this mixture. The table shows that acetamide is the only compound with a eutectic temperature of 67°C with benzidine. A small amount of acetamide is then mixed with the sample being analyzed and no lowering of the eutectic temperature is observed. In order to make even more certain since the table shows that acetanilide forms a eutectic with benzidine which melts at 66°C within the experimental error a preparation is made up containing the

¹Translators note: The German edition calls for identification of the two substances by a mixed melting point determination. A much simpler method, however, is the use of the "mixed fusion" as it has been described in the United States or the "contact preparation" as it is known in Europe (page 93). This can be carried out without the hot stage and very quickly by melting all of one component and a part of the second, then observing the zone of mixing during cooling. A discontinuity in rate of growth or type of crystal indicates with certainty that the two components are different. Absence of any discontinuity, on the other hand, proves the two to be identical.

TABLE 4

Substance	M.P. °C	Eutectic binary	Temperature °C ternary
m-Nitrophenol	96	m p 65	
p-Nitrophenol	114	m α 70	m p α 54
α-Dinitrophenol	114-115	p α 77	
m-Nitrophenol	96	m p 65	m p T 55
p-Nitrophenol	114	m T 73	
2,4,6-Trinitrophenol	122	p T 81	
p-Nitrophenol	114	α p 77	
α-Dinitrophenol	114-115	α T 79	p α T 61
2,4,6-Trinitrophenol	122	p T 81	
m-Dinitrobenzene	91	m o 64	
o-Dinitrobenzene	118	m p 77	m o p 60
p-Dinitrobenzene	174	o p 100	
Acenaphthene	94	B N 48	A B N 38
Benzil	95	A B 63	
α-Naphthol	96	A N 69	
Acetanilide	115	A M 60	
Benzanilide	163	B M 78	A B M 53
Methylacetanilide	100	A B 97	
Acetanilide	115	A B 95	
Bromural	150	A L 97	A B L 81
Luminal	174,5	B L 113	
Narkotine	174	C P 120	
Codein	155	N P 130	C N P 113
Papaverine	143-146	C N 132	
Codein	236	C Tp 199	
theophylline	272	C Tb 223	C Tb Tp 193
Theobromine	349	Tb Tp 252	
Pyramidon	108	Py Ph 82	
Phenacetin	135	Py C 103,5	Py Ph C 81
Caffeine	236	Ph C 125	

original mixture and a small amount of acetanilide. The eutectic temperature is found to be 41°C. Acetanilide is, therefore, not present and according to the table no other compound forms a binary eutectic with benzidine which melts near 67°C. The mixture is, therefore, definitely identified as benzidine and acetamide. Sometimes there are three possible second components indicated by the table. For Example, acetyl-p-toluidine shows a eutectic temperature of 73°C with three other substances in this table. In this instance it is necessary, therefore, to observe three mixed fusions.

The method is applicable to mixtures of any composition. The greater the excess of one component the more rapidly it is purified by the absorption method. The second component must be present in the melt in a sufficiently large quantity that the eutectic temperature will be clearly visible. Mixtures containing only 5 percent of one component are, however, no more difficult to analyze than mixtures containing higher percentages, and the time required is very short. The eutectic temperature of the mixture can be deter-

mined in a few minutes; the determination of the necessary eutectic temperatures for the table requires only a few days. Identification of any mixture of two compounds chosen at random from a table can be made on an average in about 40 minutes.

When only four substances are possible components of the mixture any of the 2-, 3-, or 4-component mixtures can be positively identified using the known eutectic temperatures and mixed melting point. To accomplish this the eutectic temperatures of all possible binary, ternary and quaternary mixtures are determined, involving in all eleven different mixtures. In Figure 104 the values for the system m-nitrophenol, p-nitrophenol, alpha-dinitrophenol and gamma-dinitrophenol are given²⁹⁰. Each substance is represented by the letter M, P, alpha, or gamma at the corners of the square. The eutectic temperatures of six possible two-component mixtures are shown on the sides and the diagonals of the square. The broken lines outside the square intersect to give the corresponding ternary eutectic temperatures. In the middle of the square is the eutectic temperature of the four-component mixture. If a eutectic temperature of 58°C is obtained during the analysis of the mixture only a mixture of m-nitrophenol, alpha-dinitrophenol and gamma-dinitrophenol is possible. However, if p-nitrophenol is added to this mixture the eutectic temperature characteristic of the four-component mixture is obtained, 46°C. Two ternary eutectic temperatures lie at 54°C. If during the investigation of a mixture a eutectic temperature of 54°C is obtained, the mixture must contain m- and p-nitrophenol with either alpha- or gamma-dinitrophenol. In order to distinguish between these two possibilities alpha-dinitrophenol and gamma-dinitrophenol are each mixed with the sample under investigation. The eutectic temperature will remain at 54°C for the former but will decrease to 46°C for the latter mixture if the original three-component mixture contained alpha-dinitrophenol and vice versa if the original three-component mixture contained gamma-dinitrophenol.

6. Quantitative Analysis of Two-Component Mixtures

(a) Refractive index

The use of refractive index of binary melts as an analytical method is based on the fact that refractive index is a linear function of composition in binary mixtures. The method is applied by measuring the temperature at which the melt has an index of refraction identical with that of a standard glass particle immersed therein. This method has been developed and used by Lindpaintner²⁹², Reimers²⁹³, and Dultz²⁹⁴.

The simplest examples are those in which the refractive indices of both substances can be determined with a single glass powder from the standard series. The two substances can be differentiated by the temperatures at which their refractive index becomes equal to that of the glass. The composition of the mixture is plotted as abscissa and the temperature of equality between the melt of that composition and a specific glass standard is plotted as ordinate. In Figure 95 there is a composition-refractive index diagram for cycloform (m.p. 65°C) and anestheline (m.p. 90.5°C). The latter shows at 120°C and the former at 66.5°C identity of refractive index of their melts with the glass 1.5403. The corresponding temperatures for all compositions of the mixture lie on a straight line connecting the values for the two pure components. The steeper the slope of this line the more accurate the quantitative analysis of those mixtures will be. In some cases, such as for the hexachlorocyclohexanes²⁹⁵ (Figure 106), the slope is very low. For the best

pair, the alpha- and gamma-isomers, the temperature difference is only 20°C, hence an analysis of mixtures of these two components can be determined to about ±3-5 percent.

Frequently it is necessary to use several glass powders in order to cover all compositions of the mixture. With the following two substances, uliron, 4-(4'-aminophenylsulfonamido)-phenylsulfonediethylamide (m.p. 196°C) and eubasin, p-aminophenylsulfonamide-phenylsulfonediethylamide (m.p. 196°C) (Figure 107), the glass standard 1.5898 can be used for mixtures containing up to only about 70 percent eubasin, since at temperatures above 210°C the mixtures decompose. For the eubasin-rich mixtures the glass 1.6010 is used. This glass cannot be used with mixtures containing less than 30 percent eubasin, since such mixtures solidify at the lower temperatures.

With mixtures of papaverin and atropine, it is desirable to use a third glass in the middle composition range (Figure 108). Pairs of substances having refractive indices with still greater differences require a larger number of glasses in order to reach all possible compositions, for example, novatophane and trional (Figure 109). The greater the difference in refractive index of the two melts the more accurate the determination since, then, a 1 percent change in composition causes a difference of many degrees in the temperature.

Mixtures of compounds having widely different refractive indices must be carefully mixed since otherwise different temperature values will be obtained in the same mixture and even in the same microscopic field of view. This has been emphasized in the work of Kofler and Baumeister²⁹⁶. It has been found, however, in the work with Baumeister that with careful mixing the error in analysis of a two-component mixture in which the substances have a large difference in refractive index is frequently less than 1 percent. Although the solid substance has been well mixed, even by the addition of a suitable solvent, it is often desirable to do additional mixing by lifting the coverslip from the molten microscopical preparations a few times. Mixing of the molten droplet is most effective when enough material has been used to fill at least one-half of the area under the coverslip. Figures 110-114 show the refractive index-composition relationships for several binary mixtures²⁹⁴. This technique has been applied by Brandstätter to the quantitative determination of 2,4-dinitrophenylhydrazones²⁹⁸ (Figure 115) as well as barbituric acid derivatives²⁹⁹. Fischer and Kocher³⁰⁰ analyze mixtures of vanillin and ethyl vanillin in a similar way.

The preparation of the required diagram is quite simple for the two-component system all of whose mixtures have refractive indices covered by a single glass. The two temperatures for the pure components need only be joined by a straight line. In order to be more certain, determinations on one or two mixtures (e.g., 50 percent and either 30 or 70 percent) are determined in order to show that these mixtures lie on a straight line. The rare case in which such deviation from linearity does occur will be considered later. In general, at least two points must be determined for each of the several glasses required in the analyses of mixtures. The determination of a diagram requiring several glasses will naturally take more time than when a single glass is sufficient. In some cases, only when it is necessary, the entire diagram is determined to make repeated determinations with the same pair of substances (see page 115).

The accuracy of the values depends naturally on the difference in refractive indices of the two components. In the system trional and novatophane (Figure 109) the error is less than 1 percent because of the large difference in refractive index of the two pure melts. In rare cases

the temperature values for the mixture do not lie on a straight line with respect to the concentration axis but may be either concave or convex²⁹⁶. In these cases a large number of mixtures must be examined in order to determine the diagram.

It would be expected in all other cases that the diagram could be calculated for a given system if the values were known for two glass powders and hence for the temperature coefficient (Lennartz³⁰¹). The difference in temperature corresponding to a refractive index difference of 0.0100 can then be calculated. With this value the temperature at which the melt will match in index any glass powder can be calculated whether it can or cannot be realized.

For example, if the middle line in Figure 108 is to be calculated the temperature value for glass powder 1.5301 must be determined for both substances.

Atropine according to the tables shows identity of refractive index with glass powder 1.5101 at 111.5°C and with glass powder 1.5000 at 135°C. In other words, 23.5°C corresponds to a refractive index difference of 0.0101 or a change of 23.27°C for an index difference of 0.0100. From these values the temperature difference between glass powder 1.5301 and 1.5101 can be calculated ($23.27^{\circ}\text{C} \times 2 = 46.54^{\circ}\text{C}$). Therefore, atropine melt will match the refractive index of glass powder 1.5301 at $111.5 - 46.5 = 65.0^{\circ}\text{C}$. In an analogous manner it can be shown that the refractive index of papaverin melt will match glass powder 1.5301 at a temperature of 247.5°C. If these two values for atropine and papaverin are plotted in Figure 108 and joined by a straight line the values obtained vary from the experimental curve and slightly more so at higher values than lower. For example, a 50 percent mixture would appear to be 45 percent papaverin using the calculated curve.

Lennartz³⁰¹ has reported the calculated and experimental relationship between refractive index and composition for a number of binary systems. He has found, however, that the calculated diagram agrees with the observed diagram only for certain systems and that in some cases variations of as much as 5 percent are observed. A definite explanation for this variation between the observed and calculated diagram is not yet known. Apparently the explanation lies in the fact that with certain substances the temperature coefficient of the refractive index is not constant throughout the required temperature range. (Translator — In all of this work the temperature coefficient of refractive index of the glass is ignored. This is of no consequence for any of these determinations since a given glass always has the same index at a given temperature. On the other hand, it could easily affect calculations based on the constancy of refractive index with temperature. This could be another contributing factor to the lack of agreement between the calculated and experimental curves.) As a result, the use of the calculated diagram is not justified for exact determinations. On the other hand, precalculation of the diagram has the advantage (especially with substances having widely different refractive indices) that a better choice can be made of the proper mixtures for the experimental determination of the diagram. This is true particularly in those cases where only the partial diagram is required.

If only a few quantitative determinations of the composition of a mixture of two substances are required and all within a limited composition range, a partial diagram is sufficient. The temperature values for both pure components are determined first either from the tables in Part II, or if they are not available there, by actual determination. The refractive indices of the mixtures under question are then determined. When the glass powders used for each of the two pure substances are not adjacent to each other in the

series, the glass lying midway between them is chosen for the mixture. Then it is necessary to determine the glass powder which has, at a suitable temperature, a refractive index equal to that of the melt, and the corresponding portion of the diagram is determined with this glass powder. Two points on the diagram must be determined with each of two suitable mixtures. An indication of the value for a suitable composition is obtained from comparison of the glass powders as, for example, in the diagram of trianol and novatophane, Figure 109, covering the range of thirteen glasses in the series (1.4880-1.6010). If the mixture in question corresponds to the middle of this series, then mixtures with 40 and 50 percent trianol are prepared. The next step is to determine the temperatures at which the refractive indices of the melts are identical with the index of this glass, in this case 1.5403. These values are then plotted in the diagram. After both points are connected with a straight line, the composition of the mixture in question can be determined. The percentage composition of other mixtures of these compounds is determined in a corresponding way.

By calculations based on the work of Lennartz³⁰¹, it is possible to dispense with preparation of one of the two known mixtures. The slope of the line for a particular glass is calculated for the analysis of the mixture in question, and the resulting partial diagram is used to determine the composition of the mixture in question. A mixture of this same composition is then made up to check the above result. If the two temperatures are in agreement the determination is complete. However, if there is a variation which it can be assumed will not exceed 5 percent, the calculated diagram can be corrected in the following way: The temperature value for the known mixture is plotted in the diagram and a line is drawn through this point parallel to the calculated curve. The composition of the unknown samples is determined from this corrected line. Even though the values obtained by this method involving a single mixture are not as exact over the entire range as those determined by the use of two different mixtures the accuracy for the points in question is still satisfactory. The work of Lennartz indicates a deviation not greater than 0.5°C with this procedure.

Decomposition on melting, high velocity, immiscibility of the liquid phases and too large a separation of the melting points of the two substances make the use of this method in the above described manner impossible. These difficulties are, however, eliminated by addition of a third component (Lennartz³⁰²). The two pure components, their known mixtures and finally mixtures with a suitable third component are studied. The ternary mixtures melt at lower temperatures than the pure components and their mixtures, hence the refractive index can be determined in a temperature range in which the substance will not decompose and in which the volatility is low. Enough of the third component is added so that the melting point is lowered sufficiently so that reproducible temperature values are obtainable for a single glass. As a rule from 25 to 60 percent of the third component is used. The amount used should, of course, be kept as small as possible since the difference in index will, therefore, remain larger and the accuracy of the determination will be greater.

In the preparation of the diagram for substances A and B, the refractive index of mixtures containing A with an equal amount of suitable third component C is measured. In a similar way the refractive index of B + C is determined. Likewise the necessary mixtures of A and B are always made with an equal amount of C. All of these points are plotted in the diagram in terms of the composition of A and B in the same manner as for the simple two-component system; it should be remembered, of course, when using the diagram that

the values obtained are not characteristic of pure A and B and their mixtures, but of their solutions in C. Thus, unknown mixtures of A and B must be mixed with C before their refractive indices are measured and before use of the diagram. If many analyses must be made with the same pair of substances, the complete diagram is prepared. Of the two substances in the system shown in Figure 116, codeine hydrochloride decomposes between 260°C and 270°C. Salophen (acetyl-p-aminosalol) proves to be a suitable third component for this system; with codeine hydrochloride it forms a eutectic melting at 146°C and with ephetonin (m.p. 189.5°C), a eutectic melting at 162°C. Mixtures containing equal parts of salophen have proved to be best since, with these mixtures, the refractive indices of the melt are measured at temperatures around 140°C, which is below the decomposition temperature of codeine hydrochloride and which even lies below the binary eutectic temperatures. These mixtures can also be easily supercooled so that measurements can be made below the actual freezing points. Finally, the difference in refractive indices of the basic mixture is great enough so that accurate results are obtained with this diagram.

Even in those cases where both components decompose on melting, the use of this procedure with a third component frequently gives good results. For example, with the system alpine hydrochloride and narcotine hydrochloride (Figure 117) analyses can be made when mixtures with equal amounts of salophen are prepared. Also pairs of substances whose melting points are too widely different (more than 80 to 100°C) for the simple procedure can be analyzed by adding a third component just as in the analysis of mixtures of decomposable substances. Cardiazol, for example, melts at 59°C and caffeine at 236°C, yet this pair of substances can be analyzed quantitatively by the addition of a third component. Optimum mixtures for this purpose are obtained by weighing out two melting point depressants, salophen and acetanilide, so that the mixture contains one of the pure compounds, cardiazol or caffeine (or a mixture of these two) plus salophen, and acetanilide in the ratio of 4:3:3. The diagram in Figure 118 shows the values obtained for such mixtures.

Substances whose liquid phases are not miscible can be brought into solution through the addition of a third component and can then be quantitatively analyzed by this technique of determining the refractive index of the melt. The third component must be a solvent for each of the two components. For example, the melts of thiosinamine and azobenzene are immiscible, but if phenylethylurea is added as a third component a homogeneous melt is formed over the entire composition range (Figure 119). An addition of 25 percent phenylethylurea is sufficient.

Lennartz³⁰² has analyzed systems of even three or more components by the refractive index method in combination with other physical and chemical methods of qualitative analysis.

(b) Melting point diagram

Another method for the quantitative analysis of mixtures is by thermal analysis³⁰³. This method is, in general, applied only to those substances which melt without decomposition and whose liquid phases are miscible. Extremely high volatility is also a disadvantage. The procedure is also complicated by polymorphism, molecular compounds, and by solid solutions. Since reproducibility by the glass powder method is, in general, better it is used if the refractive indices of the two components differ by a large enough amount.

The idea of analyzing two-component mixtures by means of the melting point diagram is not new. It has been used only infrequently, however, for example, for vanillin and its adulterants³⁰⁴, and for fatty acid mixtures³⁰⁵ because the procedure is troublesome and susceptible to many errors. Above all, in the earlier work it has frequently not been possible to determine which branch of the melting point curve the mixture represented. In the microprocedure described here these difficulties are avoided and the analysis is rapid.

With this procedure the two substances are first investigated by the fusion method (page 93) in order to determine their behavior in the presence of each other, and at the same time to determine the eutectic temperature.

After the mixed fusion has been used to determine qualitatively that the two-component system in question forms a simple eutectic, it is sufficient to determine the "end melting point" ("point of primary crystallization") of two or three mixtures in order to prepare the melting point diagram.

For this determination the different mixtures must be very finely powdered and intimately mixed in a small mortar. It is a good idea to grind the two components together in a few drops of an easily evaporated solvent (e.g., ether). Enough of this mixture is used for the microscopical preparation to fill the entire area beneath the coverslip. Melting must be very rapid and is best carried out by placing the preparation on the hot stage or the hot bar at a temperature 20°C above the upper melting point of the mixture. After the preparation is completely melted the coverslip is pressed lightly onto the object slide so that the preparation will be neither too thick nor too thin. Crystallization gives very finely divided uniform crystals in which the excess component is distributed evenly throughout the entire preparation. Slow solidification must be avoided since this will allow the excess component to crystallize from only a few nuclei. The large crystals thus formed would push aside the residual melt thus causing a local concentration of the principal component. In melting such a preparation a low value would be obtained in some spots and a high value in other spots in which the large crystals were formed.

In many cases it is recommended that during the rapid cooling process pressure be exerted on different sides of the coverslip in order to form as many crystal nuclei as possible. (Translator's note: In most cases, rapid crystallization is best affected by inverting the preparation and placing coverslip down on a cool surface.) To determine the end point of melting, the uniformly solidified preparation is heated and observed with polarized light between crossed polars. A point as near as possible to the center of the preparation should be observed, and the temperature should rise no more rapidly than about 2-3°C per minute in the region of the final melting point. After being heated above the eutectic temperature, the excess component remains in the melt as a lattice work or as a mass of small crystals of approximately the same particle size. On further heating, the temperature at which the last crystal residue disappears is recorded.

The reproducibility of the determination depends on the nature of the components and whether the composition of the mixture is near or far from the eutectic composition. Mixtures with compositions equal or close to the eutectic composition give, in general, less reproducible values (see page 39).

The diagram is drawn by recording the melting point of the pure components, A and B, the end melting point of the various mixture and the horizontal line corresponding to the eutectic temperature. Although the classical

methods of thermal analysis for the preparation of melting point diagrams require examination of ten to fifteen different mixtures, the use of three or occasionally even two mixtures is sufficient when micromethods are used. It is recommended that the 30 and 70 percent mixtures be determined first and their melting points plotted in the diagram. It is clear then whether an additional point is necessary and, if so, which composition would be most suitable. These few points are sufficient because molecular compounds and solid solutions are absent as shown by the fusion method; the melting points of the eutectic and the two pure components have been determined and because it is possible to determine very simply the position of the eutectic in relation to the previously determined points.

The latter determination is made with a mixed fusion, in which a pure substance is brought into contact with successive mixtures³⁰⁵. The mixture is melted between coverslip and slide, allowed to solidify, and the pure substance is added at the edge of the coverslip. Finally, the preparation is completely remelted and allowed to crystallize; the pure substance is then in contact with the mixture so that on heating the position of the eutectic with respect to that mixture and the pure substance can be determined. If the mixture and the pure component lie on the same side of the eutectic (e.g., Figure 120, lactophenin:phenacetin) no evidence of eutectic will appear in the contact zone on heating. If, however, the eutectic lies between the mixture and the pure component, the contact zone will melt as a eutectic and will become apparent as a dark strip between crossed polars. It is very easy to determine by this method whether the eutectic lies between 30 and 70 percent as in Figure 120, or whether it lies between one pure substance and one of these mixtures. If the lactophenin in Figure 120 is replaced by a lower melting substance the eutectic composition usually moves toward the left. It is possible to find systems of this type where the eutectic lies between the low melting component and the 30 percent mixture.

With the melting point of the pure substance, the end melting point of two or three mixtures, the position of the eutectic point in relation to these four points, and finally with the eutectic temperature it is possible to draw a sufficiently accurate melting point diagram. The diagram for lactophenin and phenacetin shown in Figure 120 is based on determinations of all mixtures from 10 to 90 percent. However, essentially the same diagram would have been obtained with the 30 and 70 percent mixtures alone.

In the determination of an unknown mixture in a given two-component system, the end melting point is determined in the manner described above. If it lies above the melting point of the lower melting component, the composition of that mixture can lie only on that branch of the curve closest to the higher melting component (for example, in Figure 120 at temperature above 118°C). It is possible to determine the percentage composition of such mixtures directly from the diagram. In all other cases it is necessary to determine first on which branch of the curve the mixture in question lies. There are many possible ways of doing this. For example, in many two-component systems the crystal form of the excess component can be recognized. A simple and certain method is the use of a mixed fusion between the mixture in question and one of the pure components. The branch of the diagram on which that particular mixture lies is clearly indicated by the appearance or absence of eutectic melt in the zone of mixing. The eutectic composition can be determined in this way to within ± 0.5 percent. Fusion methods are even more useful for the determination of composition diagrams involving addition compounds since the percentage composition of the addition compounds and the eutectics can be more accurately determined than

by the classical methods³⁰⁷.

The preparation of the diagram in the manner described above usually requires less than one hour. Investigation of a mixture when the appropriate diagram is available requires only a few minutes.

With many two-component mixtures, both the glass powder and the thermal methods are applicable for systems which, instead of a simple eutectic, give evidence of a molecular compound, mixed crystals or some other complication. In all these cases, however, one method or the other can be used since complications in the melting point diagram only very seldom affect the glass powder method and when the refractive indices are similar or nearly identical thermal analysis can be used. Isomers are often responsible for the latter difficulty and here thermal analysis can usually be applied without difficulty since molecular compounds between isomers are quite rare.

(c) Thermal analysis without weighing

In many systems it is possible with a combination of the glass powder method and the mixed fusion (see pages 29 and 93) to determine the concentration corresponding to molecular compounds, eutectics and maxima or minima of systems showing solid solution³⁰⁸. The method is as follows: A small quantity of the proper glass powder is placed in the center of a microscope slide and the mixed fusion is made up incorporating the glass powder throughout. On heating to the point of first melting one glass particle is observed using the red filter until it disappears. This temperature corresponds to the composition of the eutectic and can be determined from the composition-refractive index relationship for that system. For example, in the system phenacetin-benzamide (Figure 121) the eutectic lies at 99°C and 51 percent benzamide as determined by the fusion method alone. A glass particle of index 1.5301 at the exact spot in the zone of mixing corresponding to the first material to melt disappears at a temperature of 122°C. This corresponds to 51 percent benzamide, the eutectic composition.

The composition of a molecular compound can be determined in a similar manner. As soon as the last portion of the addition compound melts a glass particle in that exact region is observed until it disappears on further heating. In the system *a*-trinitrotoluene-fluorene (Figure 122) glass powder 1.5794 disappears at 95°C corresponding to 42 weight percent fluorene. The calculated weight percentage of the equimolar compound is 42.2 percent.

It is obviously of greatest importance in using this method to be certain that the glass particle under observation lies precisely in the lowest melting eutectic region or on the point at which the last crystals of addition compound disappeared depending on the composition to be determined. It is a good idea to orient the preparation so that the center of the cross-hairs in the ocular falls within the desired region and preferably within a properly placed glass particle. Movement of the preparation is carried out with the slide manipulator from outside the stage in order to avoid the necessity of removing the glass bridge and hot stage cover plate.

In most cases the refractive index of the melt for the binary system involved requires more than a single refractive index standard, for example, phenacetin-benzamide. In this case the data have to be calculated in terms of a single glass powder, i.e., 1.5301 (Figure 121). This calculation is simple enough using the temperature coefficient which is, in turn, calculated from any two temperature-index values for that substance. The temperature coefficient gives the refractive index decrease for each 1° increase in temperature; the range for this coefficient for nearly all organic compounds

lies between 0.0002 and 0.0009. Another way to express the coefficient is to calculate the temperature change corresponding to a refractive index change of 1.0000 (see page 115)³⁰⁹. Phenacetin shows in an index of 1.5101 at 134.5°C and 1.5000 at 156.5°C, hence a change of 22°C corresponds to a change in index of 0.0101. The temperature change corresponding to an index difference of 0.0100 can easily be calculated at 21.78°C.

The calculation of the temperature value corresponding to glass 1.5301 for phenacetin is therefore: $134.5^{\circ}\text{C} - 21.78 (1.5301 - 1.5101 / 0.0100) = 91^{\circ}\text{C}$. This value is therefore plotted in a diagram for glass 1.5301 with a corresponding temperature of 152.5°C for pure benzamide. The value of 91°C for phenacetin is imaginary, of course, since this compound cannot be supercooled to this temperature. In fact, this glass powder cannot be used for phenacetin-benzamide mixtures containing more than about 70 percent phenacetin. This powder is, however, the most suitable for the determination of the eutectic composition in this system since the eutectic contains less than 70 percent phenacetin.

The accuracy of this procedure for determination of eutectic composition is greater the less the preparation has to be superheated since diffusion within and mechanical movement of the melt increases as the temperature rises. The lowest possible glass powder is therefore chosen. The best value for the eutectic composition is obtained when the two pure components still remain unmelted, thus enclosing the eutectic melt between two solid phases. Also, the larger the temperature coefficient the more accurately the composition can be determined. In the system benzamide-phenacetin where the entire composition range covers only 61°C each degree corresponds to 1.6 percent. In the system dibenzyl-azobenzol (Figure 123) each degree corresponds to only 0.5 percent, hence the eutectic composition may be determined with much greater accuracy.

The eutectic or addition compound composition determined in this manner is usually checked by weighing out a mixture corresponding to that composition and using a mixed fusion to check or correct the percentage.

The glass powder method is especially useful for the determination of the composition of maxima and minima since these cannot be determined so accurately by the fusion method alone. In dibenzyl-azobenzene, for example, the position of the minimum is very difficult to determine because it is so flat (Figure 123). The glass powder method, however, shows this minimum to be at 32 percent whereas earlier work by Kofler and Brandstätter²⁵⁸ using the fusion method showed 30 percent. A temperature of 51°C was found for this minimum composition for glass 1.5794.

This technique can also be used to determine the solubility relationships in systems showing partial immiscibility of the liquid phases. In the system acetamide-acenaphthene the melt exists as two immiscible liquids at 93°C. The compositions of these liquids are easier to determine using the glass powder method than by thermal analysis (Figure 124). In the mixed fusion the two solutions are seen separated by a dark line (meniscus). Acetamide, which can be easily supercooled, shows an index of 1.4339 at 58°C whereas acenaphthene shows an index of 1.5898 at 121.5°C. Because these two indices are so different the temperature coefficient of composition is very great (Figure 124). In a mixed fusion the acetamide melt near the meniscus shows an index of 1.4339 at 102°C instead of 58°C and on the acenaphthene side the melt shows an index of 1.5898 at 113°C instead of 121.5°C. Using Figure 114 these data can be seen to indicate that acetamide melt dissolves 13 percent acenaphthene and acenaphthene dissolves only 2 percent acetamide. These data determined without weighing agree very well with the

values determined by thermal analysis. The method is most accurate when the mixture has a high critical solution temperature since only in this case is the composition relatively unchanged with increasing temperature.

(d) Determination of composition diagrams using the hot bar

Composition diagrams can be very quickly and simply determined using the hot bar^{303, 304}. If a mixture is placed on the hot bar in a long strip of scattered crystals it is possible to clearly recognize three different zones. Figure 115a shows a simple eutectic diagram and at the side schematic illustrations of the appearance of corresponding mixtures on the hot bar. In the lowest temperature region corresponding to composition M the crystals of the mixture remain dry and unmelted. In the middle region which lies above the eutectic melting point there are cloudy drops of partially melted mixture. In the high temperature region which is sharply separated from the middle region the droplets are completely clear (Figure 115b). In mixture E, corresponding to the eutectic composition, the middle zone is completely missing (Figure 115c) and in Mixture N (Figure 115d) this middle zone is longer than for mixture M (Figure 115b).

The point of primary crystallization corresponds to the temperature at the point between the completely clear and the slightly cloudy droplets. The powdered and carefully mixed sample is best spread on the hot bar with a small sieve. The two temperatures are then read after a few seconds when the demarcation lines between the zones no longer move. The melting temperatures are best observed using a hand magnifier of about 6X magnification.

For systems showing a simple eutectic the mixture is weighed out and ground in a small mortar with a few drops of volatile solvent. In general, it is better, wherever possible, to mix the two components by melting. The mixing is better in this case and the border between the completely melted and the cloudy droplets is much sharper. The cloudy droplets obtained from a mixture prepared by melting and then grinding are much easier to differentiate from clear droplets than when mixing by grinding alone has been used because the crystal size is smaller and more light is scattered.

The complete melting of a mixture for mixing purposes is easily done on the hot bar. The sample is placed on a thin plate of copper or aluminum (0.4 to 0.8 mm thick by about 12 x 17 mm) or on a coverslip. The preparation is placed on the hot bar about 20°C above the temperature of primary crystallization. After melting the preparation is removed from the hot bar and cooled rapidly by placing it on a cool surface. The melt is seeded, if necessary, to induce crystallization. The crystalline mass is then transferred to a microscope half-slide and pulverized using a second half-slide or a metal spatula. The sample is next spread on the hot bar using a sieve or in some manner giving good distribution so that the individual droplets are not larger than the head of a pin.

The reading of the lowest temperature at which a clear melt is present is usually easier than in a capillary tube. Even colored substances generally cause no difficulties and the method is very rapid. About three minutes are sufficient to melt the mixture, crystallize the melt, pulverize the sample, spread it on the hot bar and read the temperatures.

Very strongly volatile compounds cannot be studied in this way. However, a given component has to be very volatile indeed to sublime so rapidly that the melting points cannot be determined before the composition of the mixture changes appreciably. Mixtures containing salicylic acid, benzoic acid and veronal will give good melting point data in spite of their strong

tendency to sublime.

The eutectic melting point can also, of course, be determined on a given mixture by noting the temperature between the dry crystals and the cloudy droplets. This boundary is easier to see the closer the composition of the mixture lies to the eutectic composition. This technique is far superior to the capillary method for determination of the eutectic temperature. In the system pyramidon-phenacetin the eutectic lies at 80°C and 32 percent phenacetin and the eutectic melting point may be easily and quickly determined in all mixtures from 5 to 90 percent phenacetin.

The eutectic melting point determined with the hot bar does not always agree with the value determined using the hot stage because of the much greater sensitivity of the latter. The eutectic melting point can be accurately determined microscopically in mixtures containing only 1 percent of one component.

This technique can be used for the analysis of binary mixtures³⁰⁷. For proof of identity of two mixtures it is usually sufficient to determine the point of primary crystallization (or end melting point) for each sample and for a mixture of the two samples. This technique may be especially useful in checking whether a mixture of several drugs matches a doctor's prescription or matches the contents as printed on the label. In this case a known mixture is made up for comparison with the unknown sample as outlined above. In this same manner other compositions, such as explosives, preservatives or annealing salts, may be checked.

In systems involving addition compound formation, mixing of the mixture by grinding alone is insufficient. If the various eutectic melting points as well as that of the addition compound are to be clearly defined the sample must be melted before pulverizing. In some cases the addition compound may be formed by grinding the two components together or on heating together on the hot bar. However, to make certain only one eutectic melting point is observed the mixture must be thoroughly mixed by melting.

In order to avoid errors of interpretation of the melting point data all pairs of substances studied by the hot bar technique should be examined first by the mixed fusion (see page 93) to determine the general type of composition diagram.