

Pharmaceutical Industry Practices on Genotoxic Impurities

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CRC Press

Taylor & Francis Group

Boca Raton London New York

CRC Press is an imprint of the
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CRC Press

Taylor & Francis Group

6000 Broken Sound Parkway NW, Suite 300

Boca Raton, FL 33487-2742

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Printed on acid-free paper

Version Date: 20140618

International Standard Book Number-13: 978-1-4398-7420-2 (Hardback)

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14 Salt Formation of Pharmaceutical Compounds and Associated Genotoxic Risks

Soojin Kim

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14.1 INTRODUCTION

The issue of genotoxic impurities in drug substances or products has received widely publicized attention in the pharmaceutical industry in recent years.¹ A few cases have raised particular concerns because genotoxic impurities were inadvertently generated

during the syntheses of drug substances using common solvent systems and reagents, which were otherwise considered safe and harmless. Many published cases of genotoxic impurities in drug substances involved the process of salt formation during their manufacture.²⁻⁶ Salt formation in general is vitally important in drug substance synthesis as well as overall pharmaceutical development and manufacture. This chapter provides an overview of solid forms and salt formation and their significance in drug development and manufacture.

Salts are defined as acid–base pairs in the solid state, involving proton transfer or neutralization and the ionic interaction between the acid and base species. Salt formation is controlled by factors influencing the acid–base reactions. In principle, every compound possessing acid or base functional groups can participate in the acid–base reaction. Salt formation is determined by the relative strength of the acidity and basicity of the chemical species involved, which also determines the stability of the resulting salts. Salts in the solid state can be either crystalline or amorphous. When a salt form is mentioned with respect to pharmaceutical development, it is typically a reference to the solid state, particularly a crystalline state of the salt.

Salt formation is widely used and often a critical process step for drug substance synthesis and manufacture. In addition, salt forms of drug substances play a critical role in solid dosage form development for drug products. Typically, any reference to “salt formation” of a compound is essentially directed to crystallizing a salt form of the compound, as almost all the reasons and advantages of forming salts are related to the necessity or feasibility of crystallizing a compound. The crystalline properties of salts should be one of the deciding factors in using the salt form as a drug substance or as a process step in the synthesis.

This chapter examines some fundamental concepts including the more obvious reasons for salt formation from drug substances and the salt form selection process for drug dosage form development. Also, the general relevance of salt formation in the synthetic processes for drug substance manufacture is reviewed. Finally, some of the associated genotoxic risks in salt formation based on specific cases and possible measures for preventing or minimizing the risk of generating genotoxic impurities in salt formation are covered.

14.2 DRUG SUBSTANCE SALT FORMS

The vast majority of drug substances are in the solid state. Not only the drug substances used in solid dosage forms but also those used in parenteral, inhalation, intranasal, or solubilized drug products are almost always in the solid state, not the liquid state. Furthermore, most drug substances are in a crystalline state of the solid for many reasons. Hence, crystallization is a critical final step of the synthesis of drug substances.

The crystalline state provides essential stability and consistency for drug substances. Compared to materials of liquid form or solid amorphous form, crystalline substances are typically more chemically and physically stable. In addition, they have consistent chemical and stoichiometric compositions as well as consistent physicochemical properties such as density, solubility, and dissolution. Many of the physicochemical properties of drug substances have direct effects on bioavailability

and other biopharmaceutical properties. Crystalline materials can be manufactured more consistently batch to batch into products with consistent properties and performance compared to amorphous solids. Consistency in the physicochemical properties as well as the biopharmaceutical properties and the physical/chemical stability of drug substances are of paramount importance in drug product development and manufacture, as they ultimately ensure consistent performance and stability of dosage forms and finished drug products.

Drug compounds often possess acidic or basic functional groups. The majority of drug substances contain amine or carboxylic acid functional groups, which are ionizable and capable of forming salts. It is reported that an estimated half of all the drug molecules used in medicinal therapy are administered as salts.⁷ Therefore, salt formation of a drug substance may be one of the crucial operations in drug development.

What are the primary purposes of having salt forms as the final form of drug substances? Salt formation is a well-known and very effective technique to modify and optimize the physicochemical properties of a drug substance without changing the molecule.^{8,9} The crystal structure of a salt form is usually completely different from that of the free base or acid of the molecule, and it also differs from one salt to another. As a result, physicochemical properties of a given substance such as melting point, density, hygroscopicity, solubility, and dissolution rate can be easily modified by producing salt forms. The major reasons for using salt forms as drug substances may be categorized as discussed in Sections 14.2.1 through 14.2.4.

14.2.1 SOLUBILITY/DISSOLUTION/BIOAVAILABILITY ENHANCEMENT

Improving the solubility and dissolution rate of poorly soluble drugs is one of the primary reasons to prepare salt forms for drug substances.^{10,11} Formation of a salt is an effective way to change aqueous solubility and dissolution rate, which have direct effects on pharmacokinetics and bioavailability. It is a consensus in the pharmaceutical industry that drug candidates in development during the past few decades have been increasingly more complex in chemical structure and more lipophilic in general, and as a result, typically have become much less soluble in aqueous media.¹² Despite the availability of many formulation techniques and particle engineering technologies to increase the bioavailability of poorly soluble drugs, salt formation is the simplest and most common approach to increase solubility and dissolution rate and thus to improve bioavailability.

Sufficiently high aqueous solubility is a critical requirement in developing various pharmaceutical dosage forms, not only for the most common solid oral dosage forms such as immediate release tablets and hard-gelatin capsules but also for several other dosage forms including parenteral injections, oral syrups, nasal drops, eyedrops, and so on. Whereas for oral dosage forms a certain minimum solubility and an adequate dissolution rate in the pH range of the gastrointestinal tract may be required, for liquid dosage forms, particularly injectable solutions, a considerably higher aqueous solubility is typically an indispensable requirement.

For this reason, many examples of salt forms exist for marketed drug products. Coumadin® (warfarin sodium), a well-known anticoagulant, is poorly soluble as the

free acid and hence is administered as the sodium salt in both the solid oral and the injectable dosage forms.¹³ Reyataz® (atazanavir sulfate, an HIV protease inhibitor) is another example. The free base has very low aqueous solubility and inadequate bioavailability, but its sulfate salt has a reasonable solubility profile and can be formulated into successful solid oral dosage forms with acceptable bioavailability.¹⁴

14.2.2 DISSOLUTION/SOLUBILITY CONTROL

Salt forms are also used to control drug dissolution for various purposes.^{8,15} For example, salts are prepared to decrease the solubility of drug substances used in liquid suspension formulations, typically developed for children, the elderly, and patients who have difficulty swallowing solid dosage forms. Limiting the solubility of drug substances used in liquid suspensions can help maintain the stability of the suspension, as well as ensure consistent solid-state properties such as particle size and polymorph, which may significantly impact the absorption and safety of the drug.

In addition, limiting the solubility of highly soluble drugs is often used for taste masking, particularly for liquid suspensions, by reducing the solution concentration of drug substances that have unacceptable taste. Taste improvement is often achieved by forming salts or complexes with long-chain fatty acids such as pamoic acid, stearic acid, and palmitic acid or by forming typically less soluble salts with calcium or with saccharin, which can also serve as a sweetener.

Many cases exist of marketed products using less soluble salt forms for liquid suspensions and/or taste masking; one of them is Terramycin® (oxytetracyclin), an antibiotic compound for which a calcium salt was developed for the suspension formulation.¹⁶ The less soluble calcium salt provided improved chemical stability in suspension and palatability over the more soluble HCl salt, which was used in conventional tablet forms but had a serious stability problem (hydrolysis in aqueous solution) as well as a bitter taste. In the case of the antibacterial drug Tequin® (gatifloxacin), pediatric formulations were developed by forming crystalline complexes with stearic acid and palmitic acid that effectively masked the bitter taste.¹⁷

Another important application of salt forms of lower solubility is in the development of controlled-release dosage forms, which are designed to release the drug at a controlled rate over an extended period. Drug release can be slowed down by the selection of a salt having suitable solubility and dissolution profiles. The drug substance metoprolol in Lopressor® and Toprol XL®, marketed for hypertension treatment, is employed as metoprolol succinate for an extended-release formulation, whereas metoprolol tartrate is used for an immediate-release formulation.¹⁸ Tofranil® (imipramine), a drug for the treatment of depression, used a hydrochloride salt for conventional tablets but was later developed as a pamoate salt with lower solubility for the controlled-release formulation.¹⁹

Salts with lower solubility may also provide advantages in the formulation process. For example, in wet granulation processes, which are typically carried out to prevent the segregation of powder constituents and improve the flow and compaction properties of powder blends, when water is used as the granulation liquid salts with high aqueous solubility may experience undesirable effects.²⁰ Excessive surface

dissolution of the particles may occur or partial dissociation of the salt into the free form, resulting in uncontrolled recrystallization or partial form change during post-granulation drying. This may result in adverse consequences for the bioavailability of the formulated drug.

14.2.3 CHEMICAL/PHYSICAL STABILITY

The majority of pharmaceutical drug products are in solid dosage forms, such as capsules or tablets. The solid form of a drug substance must have suitable properties for large-scale manufacturing as well as adequate stability to ensure reliable product manufacture and long shelf life. Crystalline materials usually have superior chemical and physical stability compared to noncrystalline materials. For example, they are generally less hygroscopic and thermally more stable and possess high and well-defined melting points. They are also more stable to oxidation or hydrolysis than liquid or amorphous materials.

Salt formation provides opportunities for the formation of crystalline materials when the drug substance does not crystallize on its own. For ionizable compounds, forming an ionic pair with a counterion may provide chances to induce ordered phase formation where the free base or free acid molecules do not readily assemble into an ordered crystalline state. Furthermore, even when crystalline phases of the free compound are available, crystalline salt forms can often provide more enhanced physical characteristics compared to the crystalline form of the free compound alone, in terms of nonhygroscopicity, higher melting point, greater density, and superior chemical/physical stability. Examples include the drug substances for Plavix® (clopidogrel bisulfate), an antiplatelet agent,²¹ and Glucophage® (metformin hydrochloride), an antidiabetic drug.²² For both drugs, the free bases were not isolable as stable solid forms and exhibited unstable characteristics as oils or amorphous solids, but the salt forms provided acceptable stability and were successfully developed into the solid dosage forms. During the course of the life cycle of these drugs, several other salt forms have been produced by various parties for different dosage forms and product launches.

14.2.4 PROPRIETARY SOLID FORMS

It is sometimes advantageous to look for alternative salt forms of a drug substance for additional patent protection, especially in the United States. Finding a new salt form and developing its drug product may offer new opportunities for improved physicochemical properties, which can lead to developing a new dosage form, a new route of administration, and possible extension of the product line and drug patent life. There are many examples of successful drug life cycle management for well-established drug substances. After being successful in the market with one dosage form (e.g., conventional immediate-release tablet), additional dosage forms such as an injectable product, a controlled-release product, an inhalation dosage form, a topical application form, or a transdermal dosage form can be introduced with different salt forms providing different physical properties.^{15,23}

An example of this case is Voltaren® (diclofenac), a widely used nonsteroidal anti-inflammatory drug. Since the early success of the conventional tablet (diclofenac

sodium salt), various products from new salts have been introduced into the market, including a sustained-release tablet (diclofenac resinate), an injectable form (diclofenac sodium), a topical gel (diclofenac diethylamine), and additional tablet products (diclofenac potassium and diclofenac free acid) with or without new indications.²⁴ Other examples include dihydralazine for hypertension treatment for which a sulfate salt was used for the conventional tablet form (Apresol®) and, later, a mesylate salt was used for the injectable formulation (Nepresol®).¹⁵ In addition to these examples, there are numerous cases in the pharmaceutical industry where different salt forms of a drug have been used either to extend the exclusivity of a product line or to circumvent infringement on another party's patent for a specific solid form of a drug.

14.3 SALT FORM SELECTION

Form selection for a new drug substance or active pharmaceutical compound (API), whether a salt form or a free form, is a vital part of drug development. The search for a suitable salt form and the selection of a salt form usually take place during early development and involve comprehensive evaluation of physicochemical and biopharmaceutical properties for drug development including melting point, solubility, stability, bioavailability, and processability as well as manufacturing conditions and scalability.

14.3.1 ACTIVE PHARMACEUTICAL INGREDIENT FORM SELECTION PROCESS

Up until the 1990s, prior to the adoption of the more systematic API form screening and selection processes now widely practiced in many pharmaceutical companies, salt forms were often chosen in more empirical ways by medicinal chemists or pharmaceutical scientists. Often, a particular salt form was chosen based on the first attempt of synthesis and the ease of crystallization or based on previous experience or familiarity with the counterion. Nonoptimal choice of the form may lead to later problems such as difficulties in dosage form development or issues of manufacturability or stability, associated with increased costs of development and production down the road. Therefore, a rational form selection based on the evaluation of relevant properties for obtainable solid forms including salts would be essential to avoid the costly and inefficient process of switching the form later during development.

Over the past 10–20 years, many pharmaceutical companies have adopted more systematic screening processes as well as a rational approach to form evaluation and selection.²⁵ These activities are typically handled by an interdisciplinary form selection team or committee, comprising scientists representing different functions in the company. The team usually goes through a process of screening, evaluation, and form selection through some type of decision tree or form selection strategy, considering key criteria for drug substance solid forms. Many aspects of drug development are also taken into account, including process development and scalability as well as the expected dose and required solubility/bioavailability for the dosage form design. Figure 14.1 shows an example of such a process showing a common framework of the form selection decision tree. The details may differ, but the general criteria are similar for the form selection of new drug substances across the pharmaceutical industry.^{26–31}

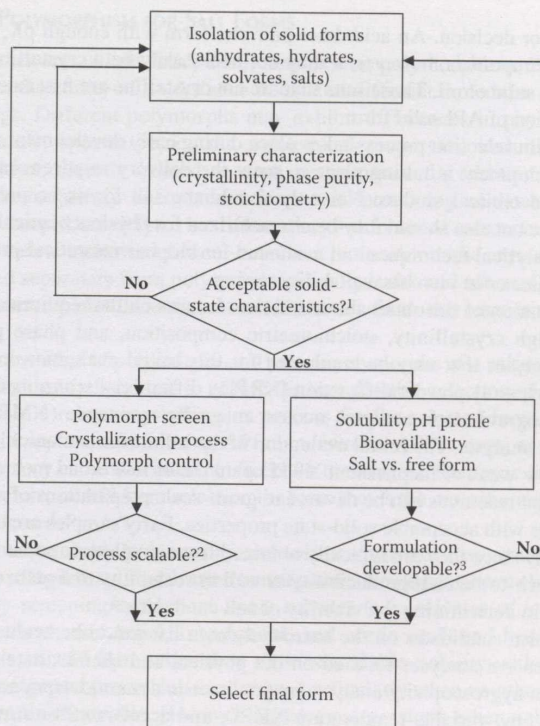


FIGURE 14.1 Active pharmaceutical ingredient solid form selection process.

¹ Highly crystalline, single phase, consistent stoichiometry, thermodynamically stable form, high enough melting point, nonhygroscopic, no volatile content, and physically and chemically stable (in elevated temperature, humidity, and light).

² Can be consistently crystallized on scale with robust polymorph control.

³ Can be developed into a robust drug product with acceptable bioavailability, excipient compatibility, and stability in formulation process and dosage form.

14.3.2 SALT FORM SELECTION CRITERIA

Most often, a salt form screen is called for due to inadequate bioavailability of a poorly soluble compound in its free form. When a salt is considered for a drug substance form, typically there is a clear limitation of the free acid or free base, whether it is undesired physical characteristics, weak crystallinity, less than optimal stability, or poor solubility, and thus a salt form would provide advantages over the free form.

The main selection criteria for API solid forms including salt forms mainly consist of crystallinity, stability, bioavailability, and manufacturability. Consideration of a salt for a suitable drug substance form involves first the question of whether it is feasible to be crystallized. Crystallinity of a salt form is the first step in the

consideration or decision. An acid–base pair can form with enough pK_a difference between the compounds; however, it may not necessarily be a crystalline form but an amorphous solid or oil. Those salts that are not crystalline are first ruled out from the consideration of API solid form.

The salt form selection process takes place during early development when rapid synthesis development is taking place to meet the delivery requirements for early toxicology and clinical studies. Not only should the salt forms be prepared in a speedy manner but also should they be characterized for physicochemical properties by various analytical techniques and evaluated for biopharmaceutical properties by solubility studies or in vivo bioavailability testing.

Early evaluation of the obtainable salt forms focuses on the requirement for consistency of high crystallinity, stoichiometric composition, and phase purity. The solid-state analyses that may be conducted for this initial evaluation include optical microscopy, x-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), thermogravimetric analysis, nuclear magnetic resonance (NMR), titration, and elemental analysis. The initial evaluation of the salt forms can quickly eliminate those that show weak or inconsistent XRPD patterns or low/broad melt endotherms by DSC, so that resources can be devoted to gram-scale preparations of a few viable salt candidates with acceptable solid-state properties. Early samples are usually limited in quantity since they are typically obtained from small-scale screening experiments, but batch-to-batch reproducibility as well as scalability to a gram scale would be important in determining the viability of salt candidates.

The salt form candidates on the narrowed-down list would be evaluated further via more extensive analyses focused on the physical and chemical stability of the forms, such as hygroscopicity analysis by gravimetric dynamic vapor sorption, hot-stage microscopy, variable-temperature XRPD, and accelerated or stressed stability tests. Stability is a very important aspect when considering the API solid form; physical and chemical stability in the solid state as well as in the slurry state (in both aqueous and organic media) is monitored for any changes or degradation.

The viable salt forms are compared among themselves for their physicochemical characteristics, as well as against the free base or free acid form of the compound. In addition, relevant biopharmaceutical measurements such as pH-solubility profile, intrinsic dissolution, and/or in vivo bioavailability studies in animals are carried out for those that show acceptable stability characteristics. The comparison of bioavailability often determines the preference of one salt over another or the nonsalt form.

The salt form selection process described here is based on rapidly assessing the essential properties and eliminating nondevelopable forms. Compared to the extensive evaluation of full list of relevant properties for all available salt forms, this process saves time and resources during early development when form selection needs to be made within a short time with limited material. It is similar to the salt selection strategy outlined by Morris and others³¹ in which expeditious evaluation of viable forms and selection were accomplished through a multitier approach: conducting the least time-consuming experiments first to give a go/no go decision followed by progressively more time-consuming and labor-intensive experiments. Such a form selection practice is commonly used nowadays in many pharmaceutical companies to enable rapid progress of drug development.^{25,28,30}

14.3.3 POLYMORPHISM FOR SALT FORMS

Like any other crystalline solid, a salt may exhibit polymorphism. Polymorphism is defined as the ability of a substance to exist in different molecular arrangements and packings. Different polymorphs may exhibit differences in physicochemical, thermodynamic, spectroscopic, and mechanical properties, including solubility and dissolution, directly related to drug performance. In addition, pseudopolymorphism is frequently encountered for a salt, producing solvates or hydrates. Pseudopolymorphs are defined as different crystal forms of a compound involving the stoichiometric inclusion of water or solvent molecules in the crystal lattice; they are classified separately from polymorphs since hydrates and solvates are in a strict sense considered to be different chemical entities from the respective anhydrous compound.

Sound knowledge of polymorphs and pseudopolymorphs of a salt form candidate and their relationship is critical to ensure the best possible selection of the API form and to develop a robust crystallization process for the selected form. Any uncontrolled form transformation during API manufacture, API storage, or drug product manufacture may cause detrimental effects on the drug performance and regulatory issues. Therefore, in API form selection the form that is the most thermodynamically stable is preferred.

Many of the unstable polymorphs and solvates/hydrates displaying low melting points or low dehydration/desolvation temperatures would have been eliminated during the early screening/evaluation. Some solvates (e.g., solvates of toxicologically safe solvents such as ethanol, isopropanol, and acetic acid) and hydrates may be acceptable as an API form when they show reasonable stability within acceptable ranges of storage and processing conditions, particularly when no other nonsolvated or stable form is obtainable. However, in most cases, where other stable forms are available, they would be a less desirable option due to concerns of stability, both physical and chemical. In addition to generally poor thermal stability, hydrates and solvates may also have poor chemical stability demonstrated by the chemical degradation caused by water or solvent under stressed conditions. Some hydrates show variable levels of hydration depending on relative humidity and would pose an issue regarding the inconsistency of API batches with different API compositions resulting in variable potency of the drug. For the same reason, there are usually processing concerns related to drying hydrates or solvates in API manufacture. It is easy to inadvertently overdry the hydrates or solvates during the API drying process, to a partially or fully dehydrated/desolvated state, which may cause a reversible or irreversible change in the crystal structure. In general, hydrates have lower solubility than the corresponding anhydrous form, which may be another consideration in whether a hydrate is acceptable or not as the API form.

Anhydrous forms are typically preferred over hydrates or solvates because they are generally expected to have higher melting points (e.g., $>200^{\circ}\text{C}$) and thus superior thermal stability and nonhygroscopic behavior compared to hydrates or solvates. However, some anhydrous forms can also convert to hydrates on contact with water or in high-humidity conditions or can show hygroscopic nature which may cause them to have physical or chemical stability only within limited humidity ranges.

For salt form selection, the thermodynamic stability of the polymorphic/pseudopolymorphic forms of the salt should be investigated to sort out the most stable form among them. Polymorph screening may be performed on a salt form candidate via high-throughput screening or parallel manual screening experiments, the details of which are described in Section 14.7. Once the thermodynamically most stable form is identified, a process to consistently crystallize the desired form should be developed. Robust polymorph control in API manufacture is essential as the solid form's purity is an important quality specification for API batches, because it not only affects the API's consistency and stability but also can impact the drug product's performance including absorption/efficacy, safety, and stability. Sometimes, the propensity of a salt to form polymorphs or pseudopolymorphs and the ease or difficulty of the manufacturing process to robustly control the desired polymorph may be a factor for preferring one salt over another.

14.3.4 SALT FORM VERSUS FREE FORM

Salt formation is an extra step in the synthesis of a drug substance. It requires additional time and resources for development and manufacture including added cost for the counterion material and solvents, processing time, as well as the expected loss of a portion of the API in the crystallization step. In addition, the salt formation adds molecular weight to the drug substance, potentially resulting in a weight burden to the drug product, especially when the dose requirement is high. A high pill burden (tablet count) is typically a critical disadvantage in marketing a new drug and patient compliance and may even be a reason to halt the development of a compound altogether. Therefore, the salt form to be selected should provide a clear advantage over the free form of the compound to be considered a necessary option. If a crystalline form of the free acid or base exists and it has acceptable physicochemical properties, stability, manufacturability, or bioavailability compared to its salt forms, the free form of the compound is preferred for development, unless there are other reasons to choose a salt form such as a special dosage form design, intellectual property protection, or drug life cycle management.

14.4 COUNTERIONS FOR DRUG SUBSTANCE SALT FORMS

In the salt formation of drug substances, an important aspect to consider first in selecting counterions is their safety and toxicity. Usually, a list of counterions is drawn for a salt form screen from those from the drug salt products already on the market or from the list "generally recognized as safe" (GRAS), the Food and Drug Administration (FDA)-approved substances that can be added to food.³² Additionally, acceptable daily intake values are assigned to some commonly used counterions by the Joint FAO/WHO Expert Committee on Food Additives.³³ Even for biologically inert counterions, there may be upper limits of intake that need to be considered for safety in making a salt form selection. Accordingly, the selection of counterions may also be influenced by the route of administration, projected dose, and treatment length of the drug.

For a given drug substance, the choice of counterions that are feasible for salt formation depends on the basicity or acidity of the ionizable groups in the compound. When the pK_a values of the ionizable functional groups in the molecule are known, the potential salt-forming counterions can be proposed based on the pK_a difference between the acid and base groups. It is commonly known that a minimum difference of about 2 to 3 units between the pK_a values of the ionizable group and the counterion is required for the formation of a stable salt, especially with drug substances that are weak acids or weak bases.^{25,28} In cases of insufficient pK_a difference between the ion pairs, even if a complex is formed it may undergo disproportionation more easily back to the components in solution (e.g., gastrointestinal tract), resulting in the precipitation of the free acid or free base.

Multiple counterions may be viable for salt formation based on the pK_a value, but not all would produce a stable crystalline salt form. Typically, a variety of salts would be prepared for a new drug substance candidate by either high-throughput screening or manual preparation from a list of potential counterions. Only those that are highly crystalline and physically and chemically stable would be scaled up for a more comprehensive assessment of their properties and their suitability for the intended dosage form to be developed.

On the other hand, cocrystal formation is possible between a drug substance and one or more inert materials, including both neutral and ionizable compounds, whose salt formation is not feasible based on the pK_a values. Cocrystals, defined as homogeneous crystalline materials composed of multiple components with defined stoichiometry, are usually distinguished from salts based on the nonionic interaction between the components such as hydrogen bonding or van der Waals interaction. Similarly to salts, cocrystals offer the possibility of altering physical properties of the compound such as enhanced solubility, dissolution rate, and stability characteristics. For some compounds that cannot form a physically stable crystalline material, cocrystals may offer a potential to form a stable crystal as a complex where the crystallinity is aided by the presence of another molecule. Many cases of cocrystals having desirable physicochemical properties, stability characteristics, and improved solubility/bioavailability compared to the free form have been developed as APIs and are reported in the literature.^{34–36}

Stable cocrystals are often identified from standard salt form screens or from specially designed cocrystal screens. For a given drug substance, there are unlimited possibilities for countermaterials to be used for screening or crystal engineering for cocrystal formation, among those that are pharmaceutically acceptable and GRAS for drug product use, which may include any combination of organic or inorganic materials. Some of the most common ones found in the literature are glutaric acid, benzoic acid, succinic acid, fumaric acid, adipic acid, tartaric acid, maleic acid, salicylic acid, saccharin, caffeine, and so on.³⁷

In standard salt form screens with many different counterions of different ionization potentials, one may find a salt or cocrystal of the ionizable compound depending on the degree of ionization or proton transfer. Advanced analytical techniques, such as single crystal x-ray diffraction and solid-state NMR, which have become widely available in recent years, allow for a distinction between salts and cocrystals. However, in the absence of such detailed characterization one may often rely

on pK_a information to estimate the acid–base pair’s likelihood of forming a salt or not. In the early development of a drug substance, when the pK_a information is not clear or accurate, especially for drug substances with low aqueous solubility, there is usually no clear picture on the nature of the acid–base interaction or the extent of proton transfer. Therefore, for a complex formation between ionizable compounds, especially for weakly acidic or basic compounds that are most often encountered as drug substance candidates, the distinction between a cocrystal and a salt may not be obvious until more extensive analysis into the crystal structure and molecular interaction is conducted.

14.4.1 COUNTERIONS FOR BASIC DRUG SUBSTANCES

The majority of drug substances are weakly basic compounds.¹¹ Weakly acidic compounds are less common, and a few are zwitterionic compounds or neutral compounds. Acids that are used to supply counterions for salt formation with basic drug substances include inorganic acids (e.g., hydrochloric acid, hydrobromic acid, sulfuric acid, and phosphoric acid), sulfonic acids (e.g., methanesulfonic acid [MSA], ethanesulfonic acid, benzenesulfonic acid [BSA], and isethionic acid), carboxylic acids (e.g., acetic acid, maleic acid, fumaric acid, succinic acid, and benzoic acid), hydroxy acids (e.g., citric acid, tartaric acid, malic acid, and mandelic acid), and possibly amino acids (e.g., glutamic acid and aspartic acid).²⁸ Table 14.1 lists all the acidic counterion sources used for salt formation with basic compounds in FDA-approved drug products.³⁸

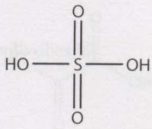
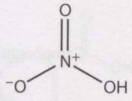
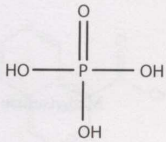
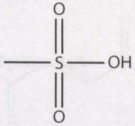
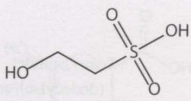
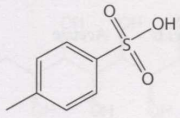
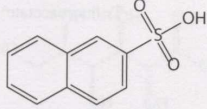
Hydrochloric acid (HCl) has been the most commonly used acid for making salts of basic drugs. According to several reports^{8,10,38} that list all the pharmaceutical salts that were commercially marketed or approved by the FDA over the past several decades, the frequency of hydrochloride salts historically is shown to be 40%–60% of the total number of salt forms of basic compounds. With its very low pK_a , hydrochloric acid can nearly always form a salt with a wide range of basic compounds, including weakly basic ones with low pK_a , although crystallization of the salt form is not guaranteed. HCl is commonly available and used as an aqueous solution (36% w/w). For water-sensitive salt formation or processes requiring anhydrous conditions, a dilute HCl solution of anhydrous organic solvents or anhydrous HCl gas is occasionally used.

Despite its popularity, there are potential disadvantages related to the general physical properties of hydrochloride salts as well as undesirable aspects of hydrochloric acid as a counterion. Hydrochloride salts often provide unacceptably high acidity in liquid formulations (e.g., parenteral products), and as solids they frequently show a hygroscopic nature and lower melting points and/or decomposition temperatures than other salts due to the volatility of HCl. The corrosive nature of hydrochloric acid can give rise to a concern related to the corrosion or discoloration of process equipment during the salt synthesis; likewise, some hydrochloride salts can be corrosive to tableting tools in drug product manufacture. Both cases can cause risks related to metal contamination of batches from the processing equipment.

Hydrochloride salts usually encounter the common-ion effect, the phenomenon of reduced solubility of a salt due to the presence of chloride ions in the gastric

TABLE 14.1

Acidic Counterions Used for Salt Formation with Basic Compounds in FDA-Approved Drug Products (up to 2007)

Salt Formers	Name	Structure
Inorganic acids	Hydrochloride	$\text{H}-\text{Cl}$
	Hydrobromide	$\text{H}-\text{Br}$
	Hydroiodide	$\text{H}-\text{I}$
	Sulfate	
	Nitrate	
	Phosphate	
Sulfonic acids	Mesylate (MSA)	
	Isethionate (2-hydroxyethanesulfonic acid)	
	Tosylate (4-methylbenzenesulfonic acid)	
	Napsylate (naphthalene-2-sulfonic acid)	

(Continued)

TABLE 14.1 (Continued)
Acidic Counterions Used for Salt Formation with Basic Compounds in
FDA-Approved Drug Products (up to 2007)

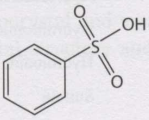
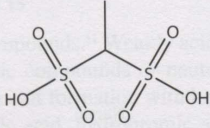
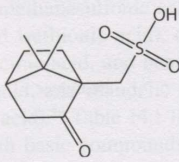
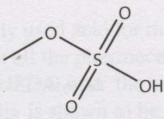
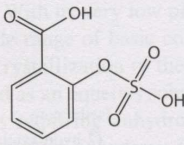
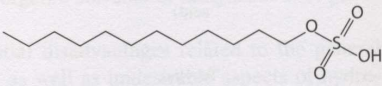
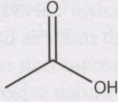
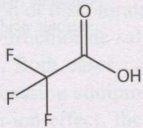
Salt Formers	Name	Structure
	Besylate (BSA)	
	Ethandisulfonate ^a	
	Camphorsulfonate ^a	
	Methylsulfate	
	Sulfosalicylate ^a	
	Laurylsulfate ^a (dodecylsulfuric acid)	
Carboxylic acids	Acetate	
	Trifluoroacetate ^a	

TABLE 14.1

Acidic Counterions Used for Salt Formation with Basic Compounds in FDA-Approved Drug Products (up to 2007)

Salt Formers	Name	Structure
	Maleate	
	Oxalate ^a	
	Benzoate ^a	
	Naphthoate ^a	
	Fumarate	
	Malate	
	Glucelate ^a (glucoheptonic acid)	
	D-gluconate	

(Continued)

TABLE 14.1 (Continued)

Acidic Counterions Used for Salt Formation with Basic Compounds in FDA-Approved Drug Products (up to 2007)

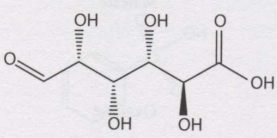
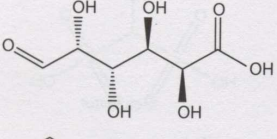
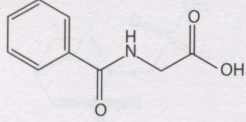
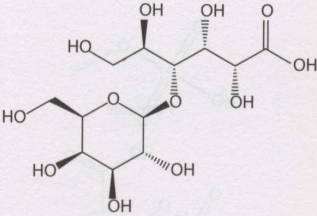
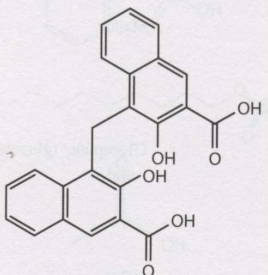
Salt Formers	Name	Structure
	D-glucuronate ^a	
	Galacturonate ^a	
	Hippurate ^a [2-(benzoylamino) acetic acid]	
	Lactobionate ^a (4-O-β-D-galactopyranosyl-D-gluconic acid)	
	Pamoate	

TABLE 14.1
Acidic Counterions Used for Salt Formation with Basic Compounds in
FDA-Approved Drug Products (up to 2007)

Salt Formers	Name	Structure
Hydroxy acids	Chlorotheophyllinate ^a	
	Citrate	
	Lactate	
	Succinate	
	Tartrate	
Fatty acids	Oleate ^a	
	Octadecanoate ^a (stearic acid)	

Note: A total of 38 different acids were reported. ^a16 acids were used only once.

fluid, causing a negative effect on the bioavailability.^{9,25} The common-ion effect is reported by numerous cases in the literature, where hydrochloride salts exhibited lower solubility and/or dissolution rate compared to other salts when administered orally or in the presence of HCl or NaCl in solution. For example, in the case of terfenadine (Seldane®, an antihistamine), the aqueous solubility of its hydrochloride salt was reduced 10-fold when dilute NaCl was added, due to the common-ion effect.³⁹ Likewise, a hydrochloride salt of doxycycline (Vibramycin®, an antibiotic), although more soluble in water than the free base form, showed a much slower dissolution rate than the free acid in the presence of dilute HCl solution.⁴⁰

Sulfuric acid is another strong acid frequently used for salt formation with a wide range of basic compounds. It is available as a liquid form. Since it is a dibasic acid, it can potentially form 1:1 or 2:1 (i.e., 2 mol of basic compound to 1 mol of sulfuric acid) salts. Typically only one form of the salt would be chosen, and the process is controlled to consistently produce the salt crystals of desired stoichiometry in a single phase.

Hydrobromic acid (HBr) is also a strong acid, similar to HCl, and HBr is also volatile and corrosive to metals. It is often used as an aqueous solution (48% w/w) or as the anhydrous gas. HBr has been used relatively often in the past for API salt formation. However, in recent years concerns related to the chronic toxicity of bromide ions, causing neurological symptoms, have been reported^{41,42} and therefore diminished the usage of HBr in drug substances.

Phosphoric acid is a weaker acid but preferred in API salt forms due to its relatively favorable safety profile compared to other counterions and often produces physically and chemically stable crystalline forms. It is mainly used as an aqueous solution (85% w/w) as the anhydrous form is either a highly hygroscopic solid or a viscous liquid.

Methanesulfonic acid (MSA) is another strong acid that has found prevalent use in APIs. It is reported that mesylate salt usage has increased significantly during the past few decades to second (to HCl) in the order of frequency among the salts with anionic counterions, now roughly comprising 10%–15% of the salts of basic compounds.¹⁰ However, in recent years concerns regarding the liability of forming genotoxic impurities with hydroxylic solvents or even some excipients have decreased its popularity in API uses.^{43,44} In general, MSA has a good tendency to form stable crystalline salts with a wide range of bases. It often produces highly crystalline, nonhygroscopic salt forms. The acid is used as the neat anhydrous liquid, which is convenient in large-scale operations.

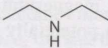
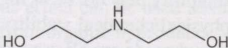
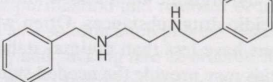
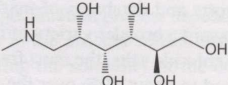
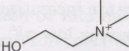
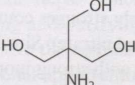
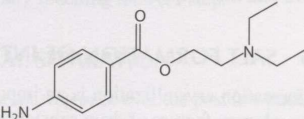
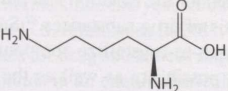
Other commonly used acids for APIs include tartaric, citric, fumaric, succinic, maleic, acetic, and malic acids. A total of 38 different anions have been used as counterions in the drug products approved in the United States that are listed in the Orange Book Database,³⁸ which has been published by the FDA since 1981.

14.4.2 COUNTERIONS FOR ACIDIC DRUG SUBSTANCES

For acidic drug substances, inorganic bases (e.g., sodium, potassium, or calcium), organic amines (triethylamine and ethanolamine), or basic amino acids (arginine, lysine, meglumine, and choline) can be used as counterions. Table 14.2 lists all the bases used as counterions (a total of 15) for the drug substances that were approved by the FDA up to 2007.³⁸

TABLE 14.2

Basic Counterions Used for Salt Formation with Acidic Compounds in FDA-Approved Drug Products (up to 2007)

Salt Formers	Name	Structure
Inorganic cations	Sodium	Na
	Potassium	K
	Calcium	Ca
	Magnesium	Mg
	Silver ^b	Ag
	Zinc	Zn
Organic amines	Diethylamine ^b	
	Diethanolamine ^b	
	Benzathine ^b	
	(N,N'-dibenzylethylenediamine)	
	Meglumine	
	Piperazine ^b	
	Choline ^b	
	Tromethamine [tris(hydroxymethyl) aminomethane]	
	Procaine ^b	
Cationic amino acids	Lysine ^b	

Note: A total of 15 different bases were reported. ^b8 bases were used only once.

Among the basic counterions, sodium ion is most frequently used for salt formation with acidic drug substances. Historically, sodium salts make up 60%–90% of all the salts of acidic compounds.^{8,38} Sodium ions from sodium hydroxide or sodium bicarbonate/carbonate can potentially form salts with a wide range of acids, and the resulting salts usually have high aqueous solubility; however, it is well known that sodium salts are often hygroscopic. Sodium salts are popular due to the safety of the sodium ion, which is naturally abundant in physiological fluids in the gastrointestinal tract; however, sodium salts are also subject to the common-ion effect and may suffer from the adverse effects of reduced solubility or dissolution *in vivo*, and thus lower bioavailability.⁴⁵

Potassium, calcium, and magnesium are the other commonly used inorganic basic counterions. They can potentially form salts of high crystallinity. Although their aqueous solubility may not be as high as that of the sodium salt, they often provide superior physical/chemical stability. Potassium hydroxide, calcium hydroxide, and magnesium hydroxide are all used conveniently for scale-up operations.⁴⁶

Lysine and arginine are the basic amino acids extensively used to form crystalline salts of acidic drug substances. Often when the salts formed from inorganic basic counterions have less than optimal stability or bioavailability, the salts from these amino acids may provide the needed stability or bioavailability. Due to the chirality of these compounds, special care is needed to ensure the chiral purity of the material used and the resulting product.

Several reports and databases of marketed salt forms^{10,38} indicate that there is a strong trend toward a broader variety of counterions in recent decades. For example, the fraction of chloride salts, the most frequently encountered salts in FDA-approved drugs, decreased to about 40% over the past decade from 53%–64% in 1981–1996, whereas the fractions of several other counterions increased during the same period.³⁸ This trend was explained as a stronger need to improve physicochemical properties of new drug substances that are becoming ever more complex in structure. This is related to the fact that there are increasingly more compounds with low aqueous solubility and weak basicity having low pK_a , which require stronger acids to form salts. It is noteworthy that 77% of the salts of the basic drugs listed in the Orange Book are prepared with relatively stronger counterions (hydrochloride, hydrobromide/bromide, sulfate/bisulfate, and nitrate). Similarly, 74% (14 out of 19) of salts of the acidic drugs listed are prepared with strong inorganic bases such as NaOH and KOH. Many believe that this general trend is also expected to continue in the future.^{10,38}

14.5 SALT FORMATION OF INTERMEDIATE COMPOUNDS

Salt formation crystallization is an important means of processing for the development and manufacture of drug substances. Crystallization is one of the most widely used techniques for the isolation and purification of organic compounds particularly in the synthesis of drug substances.⁴⁷ Salt formation often offers the opportunity to isolate a difficult-to-crystallize compound into a highly crystalline state, providing a purification possibility as well as the possibility of isolating easy-to-handle and stable solid products in a scalable manner.

In the synthesis of drug substances, key intermediates often need to be isolated. The isolation of key intermediates provides the opportunity to eliminate process

impurities and byproducts generated during processing and to store the bulk intermediate compound in a stable solid form until it is ready for further processing or transfer to other locations. In addition, key intermediates are characterized and registered as part of important manufacturing control in regulatory filings.

Crystallization is an essential means for the isolation of synthetic substances. Some compounds can naturally crystallize as a free form from a given reaction mixture and solvent system. Others are induced to crystallize from solutions. Along a synthetic route, salt formation and crystallization may be the only economical and scalable means to separate an intermediate compound from its side products. Often, when an organic compound forms a salt with an inorganic counterion its solubility is dramatically changed. The salt typically becomes more soluble in aqueous media but highly insoluble in organic solvents; hence, the salt tends to precipitate from the organic solution of the reaction mixture. This effect can be used advantageously to isolate difficult-to-purify reaction products from organic byproducts, which in most cases remain in the organic solution.

Crystallinity in a salt affords a means of purification and removal of unwanted impurities. Crystallization can be described as a solidification process from the liquid state as a result of molecular self-assembly and packing into an ordered structure, which enables the purging of foreign molecules (impurities) that do not fit into the ordered structure. For a highly effective purification process, there should be a high degree of molecular selectivity of crystal growth when it takes place in a controlled manner. Furthermore, the effectiveness of a purification process depends on the relative solubility of the individual components in the process solvent system. Salt formation in many cases assists in differentiating the solubility of the components in organic process solvents.

Salt formation of intermediate compounds is not different from that for APIs in terms of counterion screening and selection. However, more emphasis is put on the manufacturability and purification potential rather than the physicochemical properties or stability of salts. For the salt formation of intermediates, there is generally more freedom in the choice of counterions and solvents because of the lesser degree of toxicological concern at the earlier synthetic steps. Typically after the salt formation is carried out for the purpose of isolating and purifying an intermediate, there would be subsequent salt-break steps to remove the counterion, followed by further reactions and workup steps, providing additional opportunities to clean up any undesired residual reagents or solvents before finally reaching the API step.

14.6 SALT FORMATION FOR CHIRAL SEPARATION

The number of chiral drugs has increased at a soaring pace over the past few decades. They are now estimated to represent about 40% of all drug sales worldwide⁴⁸ and also constitute over 50% of new drug applications since the year 2000.⁴⁹ Moreover, among the top 10 pharmaceutical products by U.S. retail sales in 2011, 7 have chiral APIs.⁵⁰ The development of synthetic processes for drug substances requires efficient methods for producing various chiral compounds in an enantiomerically pure form. Despite the available alternative techniques, chiral resolution via diastereomeric salt formation remains the most widely used method for preparing pure enantiomers.^{7,51}

The resolution method via the formation of a salt between a racemic acid and an optically active base was discovered by Pasteur in 1853.⁵¹ He found that the chiral basic compounds quinine and cinchonine form diastereomeric salts with racemic tartaric acid and used the difference in the crystalline forms and solubility between the diastereomeric salts to separate L-(+)-tartaric acid and D-(-)-tartaric acid.

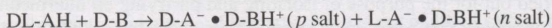
The separation of enantiomers is achieved by first forming crystalline diastereomeric compounds, salts, or dissociable complexes. Salt formation is a preferred method for the resolution of acids or bases compared to the formation of covalent diastereomers due to the simplicity of the salt formation and the ease of cleavage to the original constituents.

An optically active acid or base is used as the resolving agent to form crystalline diastereomeric salts with a racemic base or acid. The desired diastereomeric salt is then separated from the mixture of diastereomeric salts by fractional crystallization from a select solvent system based on the salts' difference in solubility and/or ability to crystallize.

A suitable resolving agent and solvent system may be searched in a systematic way through high-throughput automated or manual screening, similarly to the API salt form screening process. The screen can provide conditions to form crystalline salts and the solvent system in which the diastereomeric salts have a sufficient difference in solubility. Typically, polar solvents such as alcohols work more effectively than other solvents by providing a greater difference in solubility between the diastereomeric salts. Although any optically active acid or base can be used in principle, its use as a resolving agent is sometimes limited by its availability and cost as well as toxicity.

For effective resolution, high crystallinity of at least one of the diastereomeric salts and difference in their solubility are essential. In addition, the diastereomeric salts must not cocrystallize or form a complex with each other. After the preferential crystallization and isolation of the desired diastereomeric salt from the mixture, the desired enantiomer is separated as a pure form by the salt break from the resolving agent, which can be recovered for reuse.

The process may be summarized by Scheme 14.1,⁵¹ which corresponds to the treatment of a racemic acid, DL-AH, with an optically active base, D-B, to form salts, *p* (positive) and *n* (negative) or the inverse.^a



SCHEME 14.1 Formation of diastereomeric salts, *p* and *n*, from the reaction between a racemic acid DL-AH and a resolving agent, chiral base D-B

One of the salts, *p* or *n*, which has a lower solubility in the particular solvent system would crystallize out, whereas the other would mostly remain in the mother liquor. The effectiveness of the resolution would depend on the degree of difference between the solubilities of the two diastereomeric salts. The desired enantiomer can then be recovered by the isolation of the crystals from the mother liquor, followed by the salt break to separate the enantiomer from the resolving agent. The flow diagram representing a typical resolution is shown in Figure 14.2.⁵³

^aBy convention, a diastereomeric complex in which both the components have the same sign of optical rotation, (+ +) or (− −), is called a *p* (positive) isomer, whereas a diastereomer in which the components have opposite signs of rotation, (+ −) or (− +), is called an *n* (negative) isomer for convenience.⁵²

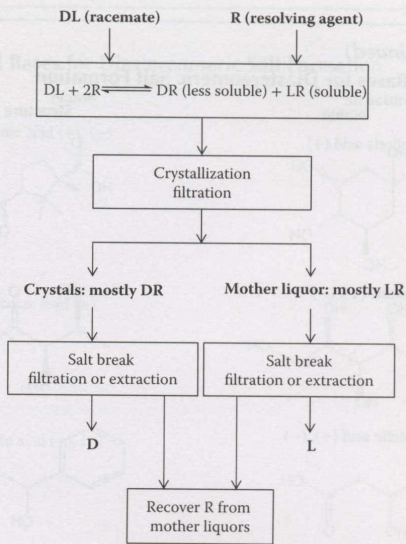


FIGURE 14.2 Flow diagram of chiral resolution by diastereomeric salt formation.

TABLE 14.3
Chiral Acids and Bases for Diastereomeric Salt Formation

Salt Formers	Name	Structure
Acids	Tartaric acid (+), (−)	
	<i>O,O'</i> -dibenzoyltartaric acid (+), (−)	
	<i>O,O'</i> -di- <i>p</i> -toluoyltartaric acid (+), (−)	

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TABLE 14.3 (Continued)
Chiral Acids and Bases for Diastereomeric Salt Formation

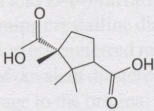
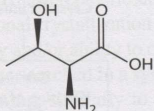
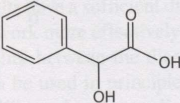
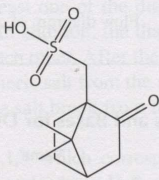
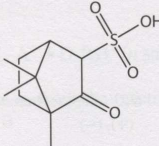
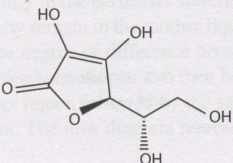
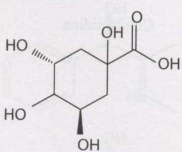
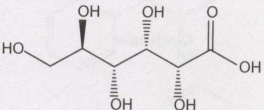
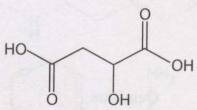
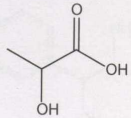
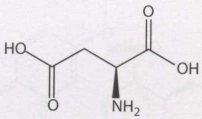
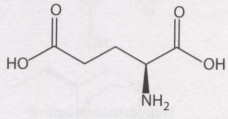
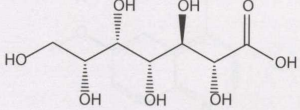
Salt Formers	Name	Structure
	Camphoric acid (+)	
	Threonine (+), (-)	
	Mandelic acid (+), (-)	
	Camphor-10-sulfonic acid (+)	
	Camphor-3-sulfonic acid (+)	
	Ascorbic acid (+)	

TABLE 14.3

Chiral Acids and Bases for Diastereomeric Salt Formation

Salt Formers	Name	Structure
	Quinic acid (+), (–)	
	Gluconic acid (+)	
	Malic acid (+), (–)	
	Lactic acid (+), (–)	
	Aspartic acid (–)	
	Glutamic acid (–)	
	Glucosheptonic acid (+)	

(Continued)

TABLE 14.3 (Continued)
Chiral Acids and Bases for Diastereomeric Salt Formation

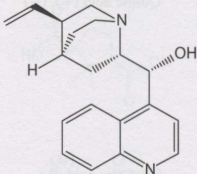
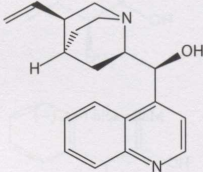
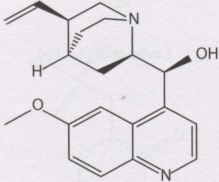
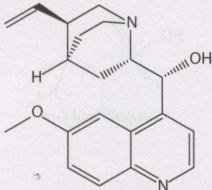
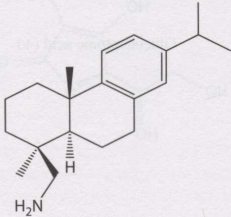
Salt Formers	Name	Structure
Bases	Cinchonidine	
	Cinchonine	
	Quinidine	
	Quinine	
	Dehydroabietylamine	

TABLE 14.3
Chiral Acids and Bases for Diastereomeric Salt Formation

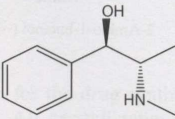
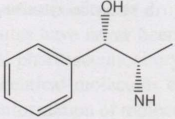
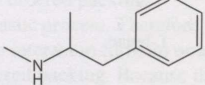
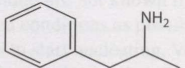
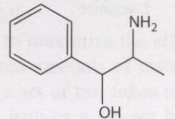
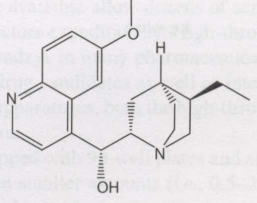
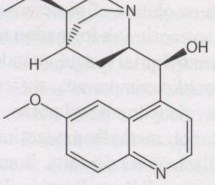
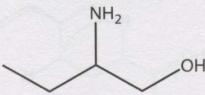
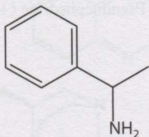
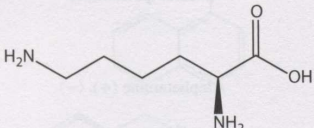
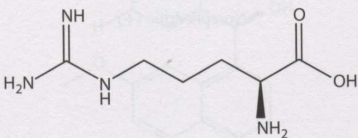
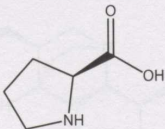
Salt Formers	Name	Structure
	Ephedrine (+), (–)	
	Pseudoephedrine (+), (–)	
	Deoxyephedrine (+), (–) (methamphetamine)	
	Amphetamine (+), (–)	
	Norephedrine (+)	
	Hydroquinine	
	Hydroquinidine	
(Continued)		

TABLE 14.3 (Continued)

Chiral Acids and Bases for Diastereomeric Salt Formation

Salt Formers	Name	Structure
	2-Amino-1-butanol (–)	
	α-Methylbenzylamine (+), (–)	
	L-lysine	
	L-arginine	
	L-proline	

For the resolution of basic compounds, some of the frequently used chiral acids for diastereomeric salt formation are camphorsulfonic acid, tartaric acid, camphoric acid, dibenzoyltartaric acid, mandelic acid, malic acid, and lactic acid. For the resolution of acidic compounds, optically pure bases such as cinchonidine, cinchonine, quinidine, dehydroabietylamine, ephedrine, pseudoephedrine, deoxyephedrine, aminobutanol, methylbenzylamine, and amphetamine may be used as counterions to form diastereomeric salts. Some of the common chiral acids and bases used as resolving agents^{51,53} are listed in Table 14.3.

It should be noted that many of the chiral resolving agents frequently used in the early years are genotoxic. Prior to the 1980s, alkaloids such as brucine, strychnine,

and quinine were some of the most commonly used resolving agents for the resolution of chiral acids.⁵¹ However, some of them, particularly brucine and strychnine, are known to be poisonous, Ames positive, or genotoxic; thus, these toxic agents are seldom used for pharmaceutical manufacture in modern times.

14.7 SALT SCREEN

The development and application of salt formation for the drug synthesis of both APIs and intermediates originate from screening. All crystallization starts from nucleation. Often, a new synthetic compound, in a synthesis of a new drug substance, has never been crystallized. This means the molecules have never been configured to assemble into an ordered solid state. Virtually all pharmaceutical crystallization takes place in the solution state. Complex pharmaceutical molecules can often be aided by solvent molecules to configure a favored conformation or molecular interaction amenable for molecular self-assembly into an ordered packing.

Initial nucleation of a new compound is a stochastic process. Therefore, it is desirable to maximize the chances for the molecules to interact to form an ordered packing or a certain conformation to facilitate an ordered packing. Because the specific conditions to trigger the nucleation of a new compound are not known initially, it is beneficial to subject the compound to as many varied conditions as possible to maximize the chances for self-assembly of the molecules to start nucleating. Variations in solvents, temperature, concentration, and composition can be applied. Screening is a way to provide all the variations and conditions rapidly.

Screening is done on a small scale (1–100 mg) to maximize the efficiency and number of experiments in a short time using limited amounts of material. In the simplest way, salt screens can be conducted using a set of test tubes or vials containing mini stir bars in a temperature-controlled bath or a reactor block. Many parallel reactor systems that are commercially available allow dozens of screening experiments to be carried out in miniature reactors expeditiously. High-throughput screening methods⁵⁴ are routinely applied nowadays in many pharmaceutical companies to prepare potential salt forms of new drug candidates as well as intermediates. Figure 14.3 shows examples of screening apparatuses, both the high-throughput automated system and the manual screen system.

A high-throughput screening system is equipped with 96-well plates and an automated liquid-handling robot system to use even smaller amounts (i.e., 0.5–2 mg of a given compound in each well) to provide hundreds of experiments. A typical salt screen is performed by dispensing minute quantities of the given compound and stoichiometric amounts of counterions as solutions in different solvents to the wells and subjecting the plates to different conditions such as changing temperature, solvent evaporation, changing the solvent composition by adding cosolvents/antisolvents, or any combination of conditions. Then the contents of the wells are observed for any salt crystal formation and/or analyzed by XRPD or Raman spectroscopy.

Crystallization of salts involves a two-step process: first, the formation of the salt compound by the acid–base reaction, and then, the nucleation of the salt molecules. Both steps must occur in order to have a desired hit in a salt screen. Solids observed

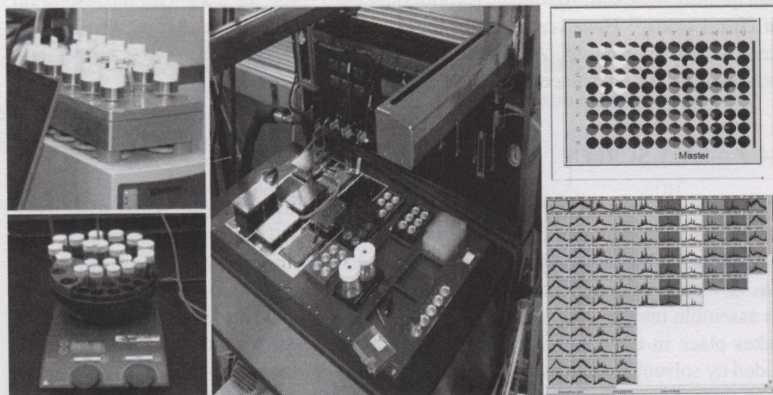


FIGURE 14.3 Examples of salt-screening apparatuses: manual screen tools (left) and high-throughput automated screen system and x-ray powder diffraction analysis (center and right).

in many of the wells may not be crystalline; in addition, crystals observed in some wells may not be salts but rather just the free compound or the counterions. For this reason, any positive hits are normally verified by preparing relatively larger quantities (a few grams) of the salts and by subjecting them to a variety of analyses to identify them as viable salt candidates for further assessment of developability.

Finding hits through the salt-screening process provides ideas for the conditions to crystallize the salt and also the first nuclei or seeds that can be used for subsequent scale-up experiments. Once a crystal is spontaneously nucleated, the compound can be crystallized easily by directed crystal growth onto the seed crystals like layering on a template. In many cases, subsequent experiments find the compound to crystallize on its own through molecular self-assembly without seed crystals, even those compounds that were very evasive and difficult to nucleate previously.

The precise original conditions from the screen where a particular salt crystal form was obtained may no longer be necessary to be repeated to further grow the crystals. For example, seed crystals of a salt formed in one solvent system can often be propagated in other solvent systems. For this reason, the screening is usually conducted using all kinds of solvents, including those toxicologically less desirable. Once the seed crystals of a viable salt form are obtained during the screen even from a less desirable solvent system, it is often possible to develop a scalable crystallization process using friendlier and safer solvent systems such as alcohols, acetates, and acetone, especially for APIs.

14.8 SALT CRYSTALLIZATION PROCESS

Salt crystallization processes typically start from solutions or dissolution of a free base or acid in suitable organic solvents. The solvent must provide adequate solubility to solubilize the compound in a reasonable volume. If not, the volume efficiency will be low and the process cannot be scaled up to be an economical manufacturing

process with sufficient productivity and throughput. The acid or base counterion should be miscible with the free base or free acid solution to form a homogeneous solution to allow uniform acid–base reaction to generate the ionic pair species.

The process must be amenable to the generated salt crystallizing from the solution. If the generated salt has a low solubility in the original solvent system in which the free base/acid is dissolved, the salt would naturally crystallize out of the solution promptly as it forms. Otherwise, the salt would remain dissolved in the solution. Subsequently, the dissolved salt can be crystallized by means of lowering the solubility, such as changing the temperature or solvent composition via antisolvent addition.

In developing a salt crystallization process, several things must be considered. First is the accurate control of the stoichiometric ratio of the acid and base species. This is especially important for the salt formation of APIs for which the crystal phase purity and the product pH are critical for ensuring consistent API properties and thus the performance of the drug products. The stoichiometry of the salt product may be affected by the accuracy of quantitation of the free acid/base amount in solution and calculation of the proper amount of the counterion to be added. Overcharging or undercharging counterions often results in the precipitation of one of the components and causes an undesired mixture of solid phases. The addition mode of the counterion can also have an impact on the purity and quality of the product. Too fast an addition or inadequate mixing during the addition of the counterion may easily lead to poor crystallinity or polymorph control of the salt or even new impurities being generated from extreme local pH.

Other considerations include proper choice of the solvent or antisolvent for the desired purification and yield. Suitable solvents and antisolvents are selected through the solvent screen and solubility measurements for the necessary polymorphic form control (for APIs) and the balance between the required purification and the desired yield. The toxicological safety of the solvents and the potential impact of the residual solvent should also be considered in selecting the process solvent. For API salt crystallization, the solvents used are preferably class 3 solvents listed in the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use guideline for residual solvents.⁵⁵ Alcohols and acetone are usually the most commonly used solvents for the final API step crystallization due to their excellent solubilizing properties, toxicological and environmental safety, low cost, low boiling points for ease of drying, and water miscibility which makes them a convenient choice as cosolvents with aqueous systems.

In a salt crystallization process, a particular concern is potentially rapid precipitation due to the abrupt change of solubility upon formation of the salt. This can lead to poor crystallinity and possible entrapment of solvents and impurities in the salt crystals and adversely affect the product quality. Therefore, proper controls and procedures are essential to avoid uncontrolled precipitation of the salt product. The controls may involve suitable management of solution concentrations, crystallization initiation by seeding, dilution and controlled addition of the counterions, and monitoring and controlling pH. Development of controlled crystal growth processes for salt formation is an essential part of process development for the synthesis of drug substances to achieve optimal and consistent quality.⁵⁶

14.9 GENOTOXIC RISKS ASSOCIATED WITH SALT FORMATION

In a synthetic process for a drug substance, a genotoxic impurity may be introduced as a starting material, reagent, intermediate, or catalyst and may also emerge as a byproduct, isomer, or degradation product. Typically, the genotoxic impurity issue arises when a particular compound used in the synthesis or produced as a byproduct is found to be genotoxic, obviously requiring tight controls in the downstream steps, yet remains in the product undesirably. In most cases, the impurities from preceding steps or starting materials should be removed by crystallization or salt isolation to meet the required specification, as discussed in the Sections 14.5 and 14.8.

Issues of genotoxic impurities related to the salt formation itself are typically caused by counterions and/or solvents. For obvious reasons, the direct use of any known genotoxic compounds should be entirely avoided in the final API step. In earlier intermediate steps, potential or known genotoxic compounds may be used occasionally as reagents, counterions, or solvents if unavoidable for reasons of selectivity or efficiency of a specific reaction, separation, purification, or key isolation purpose, essential for the overall drug synthesis. In such cases, the necessary process and analytical controls would duly be in place for the subsequent steps to remove the genotoxic impurity upstream of the final step.

In other scenarios, genotoxic risks are associated with inadvertent reactions between safe counterions and solvents or inadvertent degradation or byproduct formation. Most of these unintended instances are unlikely under normal circumstances; however, some of these inadvertent instances, especially related to the API final step, are well known to have caused serious consequences including failure of batches and, worse yet, product recall from the market.

When genotoxic impurities arise as a result of unexpected occurrences in otherwise ordinary situations, usually no genotoxicity-related analytical methods or controls are in place, and the contamination of the genotoxic impurity in clinical or commercial batches may go unknown until much later, posing a serious threat to the safety of patients. The prominent case of Viracept® in 2007 raised the awareness of genotoxic risks especially for such unexpected incidents and the need for vigilance even for seemingly routine processes.²

The Viracept (nelfinavir mesylate) product recall due to genotoxic impurity contamination originated from the inadvertent reaction between the counterion MSA (used in the API salt formation) and the solvent ethanol (used to clean the storage tank where the acid was kept). Residual ethanol left in the storage tank reacted with the acid over a long period of storage time to form high levels of ethyl methane sulfonate (EMS), a genotoxin, which was carried into the API salt formation and remained in the product. The EMS presence went unnoticed and undetected throughout both API and drug product manufacture until the tablets were consumed by some patients and adverse effects were noticed. It was a good manufacturing practice (GMP) oversight that caused the unfortunate incident, with a devastating outcome.

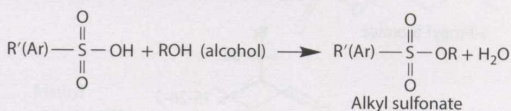
Alkyl sulfonates such as EMS can be generated from the reaction between sulfonic acids and short-chain alcohols such as methanol, ethanol, *n*-propanol, and isopropyl alcohol, as shown in Scheme 14.2.⁴³ MSA, BSA, and *p*-toluenesulfonic acid (tosic acid) are some of the commonly used counterions to form API salts, that is,

mesylate, besylate, and tosylate, respectively. Formation of these sulfonic acid salts in the presence of an alkyl alcohol presents the risk of generating genotoxic alkyl sulfonates, such as methanesulfonates, benzenesulfonates, and toluenesulfonates.

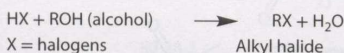
Alkyl halides are also well-known genotoxins that can be potentially generated during salt formation when hydrogen halides (e.g., HCl and HBr) used as counterions react with an alcohol used as solvent, as shown in Scheme 14.3. The genotoxins methyl chloride, ethyl chloride, and isopropyl chloride were reported to have been observed during the preparation of hydrochloride salts from methanol, ethanol, or isopropyl alcohol, respectively.^{57,58}

Esters of sulfuric acid, such as dimethyl sulfate and diethyl sulfate, are another type of genotoxins^{59,60} that may potentially occur during salt formation employing sulfuric acid. They are produced from the esterification of sulfuric acid with an alcohol, as shown in Scheme 14.4. All of these genotoxins—halogenated alkanes, alkyl esters of sulfuric acids, and alkyl esters of alkane- and aryl-sulfonic acids—known to be alkylating agents form via the interactions between strong acids and alcohols during the process of salt formation. These alkylating genotoxins are summarized in Table 14.4.⁶¹

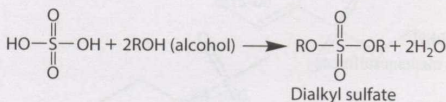
Acetamide (melting point = 80°C) is another genotoxin^{62,63} reported in the literature that is formed through the hydrolysis of acetonitrile catalyzed by a strong acid, as shown in Scheme 14.5. Acetonitrile is sometimes used as a solvent for salt formation and is susceptible to hydrolysis when excess strong acid is present in the salt formation process to produce acetamide.



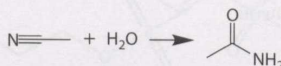
SCHEME 14.2 Formation of alkyl sulfonates by the reaction between sulfonic acids and alcohols



SCHEME 14.3 Formation of alkyl halides by the reaction between hydrogen halides and alcohols



SCHEME 14.4 Formation of dialkyl sulfate by the reaction between sulfuric acid and alcohols



SCHEME 14.5 Formation of acetamide by the hydrolysis of acetonitrile

TABLE 14.4
Alkylating Genotoxins

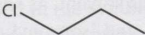
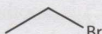
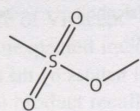
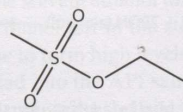
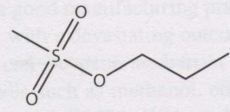
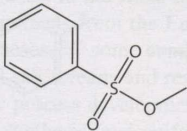
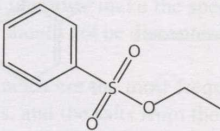
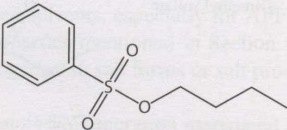
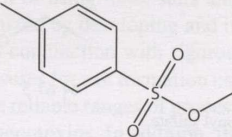
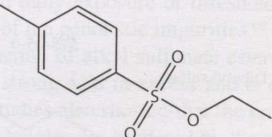
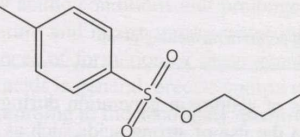
Type	Compound Name	Structure/CAS #	Properties
Alkyl halides	<i>n</i> -Propyl chloride	 540-54-5	Liquid, b.p. 46°C
	<i>i</i> -Propyl chloride	 75-29-6	Liquid, b.p. 35°C
	<i>n</i> -Butyl chloride	 109-69-3	Liquid, b.p. 77°C
	Ethyl bromide	 74-96-4	Liquid, b.p. 37°C
	<i>n</i> -Propyl bromide	 106-94-5	Liquid, b.p. 71°C
	<i>i</i> -Propyl bromide	 75-26-3	Liquid, b.p. 59°C
	<i>n</i> -Butyl bromide	 109-65-9	Liquid, b.p. 100°C
Esters of alkyl sulfonic acids	Methyl methanesulfonate	 66-27-3	Liquid, b.p. > 200°C
	Ethyl methanesulfonate	 62-50-0	Liquid, b.p. > 200°C
	<i>n</i> -Propyl methanesulfonate	 1912-31-8	Liquid, b.p. > 200°C

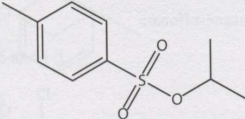
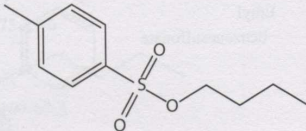
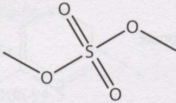
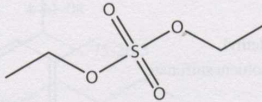
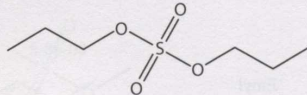
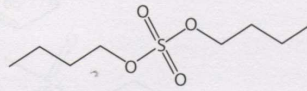
TABLE 14.4
Alkylating Genotoxins

Type	Compound Name	Structure/CAS #	Properties
Esters of aryl sulfonic acids	Methyl benzenesulfonate	 80-18-2	Liquid, b.p. > 200°C
	Ethyl benzenesulfonate	 515-46-8	Liquid, b.p. > 200°C
	<i>n</i> -Butyl benzenesulfonate	 80-44-4	Liquid, b.p. > 200°C
	Methyl toluenesulfonate	 80-48-8	Solid, m.p. 25°C
	Ethyl toluenesulfonate	 80-40-0	Solid, m.p. 30°C
	<i>n</i> -Propyl toluenesulfonate	 599-91-7	Liquid, b.p. > 200°C

(Continued)

TABLE 14.4 (Continued)

Alkylating Genotoxins

Type	Compound Name	Structure/CAS #	Properties
	<i>i</i> -Propyl toluenesulfonate	 2307-69-9	Liquid, b.p. > 200°C
	<i>n</i> -Butyl toluenesulfonate	 778-28-9	Liquid, b.p. > 200°C
Esters of sulfuric acid	Dimethyl sulfate	 77-78-1	Liquid, b.p. > 200°C
	Diethyl sulfate	 64-67-5	Liquid, b.p. > 200°C
	Dipropyl sulfate	 598-05-0	Liquid, b.p. > 200°C
	Dibutyl sulfate	 625-22-9	Liquid, b.p. > 200°C

Note: b.p., boiling point; m.p., melting point.

Many cases of genotoxin generation during salt formation, including those listed earlier, involve the use of strong acids such as MSA, HCl, HBr, and sulfuric acid in the presence of alcoholic solvents. What would be the best strategy to reduce the risk associated with these genotoxins and the salts? One might think that a surefire way to eliminate the risk is to completely abolish the use of these acids and alcohols from the

synthetic processes of drug substances. However, such extreme measures would have a significant bearing on API form screening/selection, crystallization development, purification, isolation of key intermediates, and decisions on synthetic routes and may hamper the development of efficient, economical, and scalable process for drug synthesis.

Particularly concerning sulfonic acids, in the wake of the Viracept incident and with the additional regulatory requirements from the European agencies related to the use of sulfonic acids in API syntheses,^{64,65} some companies have taken the steps of removing sulfonic acids from API salt screens and restraining their use as counterions for salt formation in synthetic process development. However, sulfonic acids have many uses in all steps of the synthesis, and sulfonic acid salts can provide many beneficial properties as API solid forms or as isolable solid forms for synthetic intermediates. Several articles in the literature make the specific point that sulfonic acid salts have many advantages and should not be discounted, especially during the assessment of API salt forms.⁴⁴

All of the aforementioned strong acids are the most frequently used counterions for API salt formation for basic drugs, and the salts from these strong acids account for a large majority of the API salts approved and marketed during recent decades,³⁸ as discussed earlier. Alcohols (ethanol, isopropanol, *n*-propanol, and methanol) are also some of the most commonly used solvents, especially for API salt crystallization, due to their many favorable properties mentioned in Section 14.8. Therefore, avoiding these acids and alcohols altogether in salt forms or salt processes would be difficult, if not impossible.

On the other hand, the risk of genotoxin generation associated with the use of strong acids and alcohols in salt formation can be managed after weighing the available options and balancing the benefit of using these salts and alcohols against the potential risk. The risk can be minimized by developing and implementing the processes with robust process control in conjunction with rigorous analytical monitoring. Appropriate process control measures for salt formation can be put in place after systematic investigations to define the reliable ranges of process conditions that avert undesirable reactions producing the genotoxins. In addition, implementation of suitable control points in the manufacturing process along with prudently set in-process specifications based on the permitted daily exposure or threshold of toxicological concern would ensure robust control of the genotoxic impurities.⁶⁶

Studies have shown that the formation of alkyl sulfonate esters in alcohols, for example, requires the presence of a strong acid in excess and is entirely inhibited by a slight excess of a base.^{67,68} The studies also showed that the undesired reactions producing alkyl sulfonates and alkyl halides are hindered by the presence of water in the solution and weaker acids such as phosphoric acid do not catalyze the reactions.^{43,67} Therefore, avoiding strongly acidic conditions and prolonged exposure of acids to an alcohol at a high temperature and incorporating water into the process when possible would reduce the chances of formation of these genotoxins. In salt crystallization processes using strong acids in general, precise control of the stoichiometric ratio of the acid to the base (according to the accurately quantified amount of the base in solution) would avoid strongly acidic conditions. This can be achieved by stringent in-process pH control during the salt formation process, controlled addition of the acid, and adequate mixing in the reactor during the acid addition.

Even in those atypical cases where a process experiences an excursion of extreme pH or high temperature, since most of these alkylating genotoxins exist as liquids at ambient conditions (Table 14.4), these genotoxins should remain in the mother liquor, given a well-controlled crystallization process for product isolation. Efficient filtration and subsequent washing should ensure their removal from the crystallized product. For this reason, in many cases where genotoxins may form in the solution their actual content in the product may be well below the specification limit. For alkyl halides, which are mostly volatile liquids with low boiling points, it should be even easier since they may be evaporated when drying the isolated product. The issue of genotoxin contamination would surface if a genotoxic impurity gets entrapped in the product due to occlusion in the crystals via a poorly executed crystallization. Aside from the alkyl halides, most of these alkylating genotoxins have high boiling points, and once they are formed and entrapped in the crystals they would be difficult to be removed. Therefore, as described in Sections 14.5 and 14.7, conscientious control of properly designed crystallization processes that encourage crystal growth rather than precipitation would prevent the entrapment of impurities and ensure their removal from products. Aspects of a properly designed crystallization include appropriate rates of temperature ramping, antisolvent addition, and/or counterion addition along with a well-planned seeding point at the right concentration/composition.

Poorly controlled processes pose risks even when genotoxic impurities are not formed in the actual process. Entrapped residual solvents, particularly alcohols, from uncontrolled precipitation can further react with strong acids in subsequent synthetic steps or during drying to create an undesired genotoxic compound. Even small amounts of residual alcohol in API can, in principle, interact with a strong acid used in the downstream formulation process to produce trace amounts of a genotoxin in the drug product. Therefore, prudent attention to any potential combination of residual alcohols in isolated compounds or APIs and use of strong acids in the subsequent steps is important to decrease the risk of formation of genotoxic impurities.

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