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REVIEW ARTICLE

Submicron Emulsions as Colloidal Drug Carriers for Intravenous Administration: Comprehensive Physicochemical Characterization

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Abstract □ Submicron injectable emulsions have been gaining more and more attention in the last few years, mainly as a vehicle for the intravenous administration of lipophilic drugs rather than as an improved delivery system for drug targeting. Submicron emulsions are available, clinically well accepted, and successfully marketed. Novel original emulsion formulations are being extensively investigated and already exhibit improved pharmacological activity; this fact underlines the promising therapeutic properties of these colloidal drug carriers as vehicles for potent lipophilic drugs. It is therefore essential to carry out a comprehensive physicochemical characterization of these submicron emulsions. It is the objective of the present review to emphasize the need for physicochemical studies in the design of new submicron emulsion drug delivery systems and to describe the different approaches available for carrying out such a complete examination of the emulsion colloidal drug carrier system.

Emulsions are heterogeneous systems in which one immiscible liquid is dispersed as droplets in another liquid. Such a system is thermodynamically unstable and is kinetically stabilized by the addition of one further component or mixture of components that exhibit emulsifying properties. Depending on the nature of the diverse components and of the emulsifying agents, various types of emulsions can result from the mixture of immiscible liquids. Invariably, one of the two immiscible liquids is water, and the second is an oily substance, often a long-chain triglyceride. Whether the aqueous or oil phase becomes the dispersed phase depends primarily on the emulsifying agent used and the relative amounts of the two liquid phases. Hence, an emulsion in which the oil is dispersed as droplets throughout the aqueous phase is termed an oil-in-water (O/W) emulsion. When water is the dispersed phase and oil is the dispersion medium, the emulsion is termed a water-in-oil (W/O) type. All pharmaceutical emulsions designed for parenteral administration are of the O/W type.

There has been renewed interest in emulsions as a vehicle for delivering drugs to the human body, especially into the bloodstream through parenteral administration. Extensive research has been published during the last decade and well reviewed by numerous authors.¹⁻⁷ As previously reported by Pranker and

Stella,⁶ the reasons for using parenteral emulsions as a drug administration vehicle include solubilization of poorly water-soluble drugs, stabilization of hydrolytically susceptible compounds, prevention of drug uptake by infusion sets, reduction of irritation from or toxicity of intravenously (iv) administered drugs, potential for sustained-release dosage forms, and possible directed delivery of drugs to various organs.

Emulsion Design

A comprehensive study of a novel drug submicron emulsion should involve various stages (Figure 1).

Requirements for a Parenteral Emulsion—iv emulsions, like all parenteral products, are required to meet pharmacopeial requirements.⁸ The emulsions must be sterile, isotonic, nonpyrogenic, nontoxic, biodegradable, and stable, both physically and chemically. Furthermore, the particle size of the droplets must be $<1\ \mu\text{m}$ and generally ranges from 100–500 nm. With larger particle sizes, a potential oil embolism may occur.⁹

Submicron emulsions intended for parenteral administration are designed for the incorporation of lipophilic and hydrophobic drugs, which exhibit poor aqueous solubility. Inclusion of hydrophobic drugs in the innermost oil phase presents special problems related to the solubilization of the drugs.^{10,11} However, these problems generally can be overcome by techniques such as the elevation of temperatures and the use of additives to increase the oil solubility of hydrophobic drugs.¹² It should be emphasized that the incorporation of physostigmine in an emulsion formulation with phospholipids as the sole emulsifier caused phase separation.¹³ The addition of other drugs to emulsions for iv application also resulted in reduced stability or cracking.^{1,6} In a previous study,¹³ it was demonstrated that the inclusion of a nonionic emulsifier, poloxamer, was required to prolong the stability of the physostigmine emulsion. The enhanced stability was then attributed to the probable formation of a complex interfacial film between poloxamer and the phospholipid molecules at the oil-water interface. This suggestion was supported by numerous experimental results presented in the cited study. The combination of phospholipids and poloxamer as an "emulsifying complex"

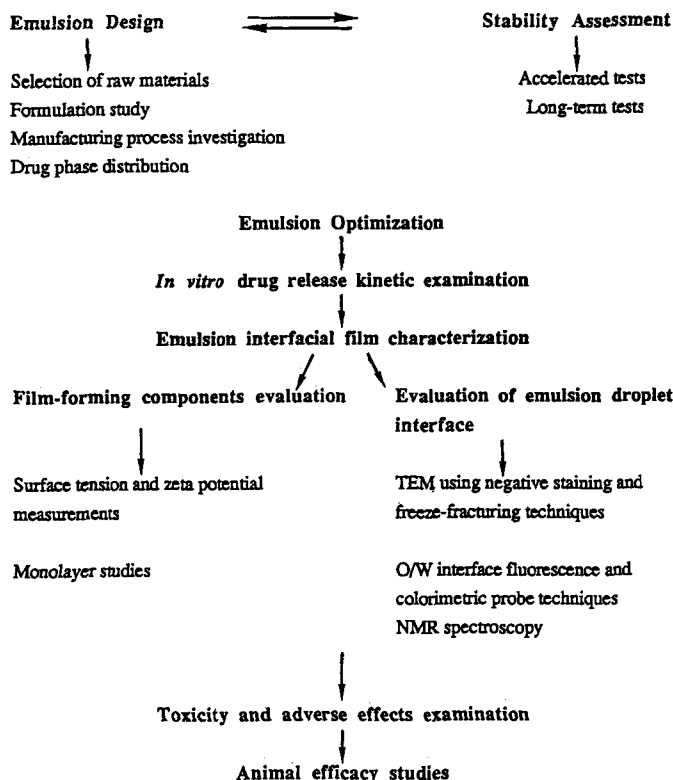


Figure 1—Various stages involved in a comprehensive study of a novel colloidal drug carrier system based on a submicron emulsion.

is most commonly used in the stabilization of medicated parenteral O/W emulsions.^{12,14–20} It should be emphasized that such a combination of emulsifiers already has been used in iv fat emulsions and has been found to be free from toxic effects.²¹ In view of the results reported, optimal experimental conditions for stable emulsion formation should occur when a drug is incorporated into the inner phase of an emulsion. Consequently, it is essential to optimize the emulsion preparation manufacturing process and to make appropriate choices of a mixture of excipients. Generally, stabilizers that are cosurfactants in nature should be added to the medicated submicron emulsion formulation.

Excipient Selection—For complying with the requirements for parenteral emulsions, careful selection of excipients needs to be performed. Special attention should be given to two major excipients in the emulsion formulation—the oil and the emulsifier(s). A detailed description of the excipient specifications for parenteral emulsions was presented by Hansrani et al.¹ Only the major aspects of the physicochemical properties of the excipients that should be considered are outlined below.

Oil—In previous studies, the oil phase of the emulsion was based mainly on long-chain triglycerides (LCT) from vegetable sources (soybean, safflower, and cottonseed oils).^{2,5} The oils need to be purified and winterized to allow the removal of precipitated wax materials after prolonged storage at 4 °C. Known contaminants (hydrogenated oils and saturated fatty materials) should be minimized.

The use of medium-chain triglycerides (MCT) in fat emulsion formulations increased extensively during the 1970s.^{22–24} MCT are obtained from the hydrolysis of coconut oil and fractionation into free fatty acids that contain between 6 and 12 carbon atoms. MCT are esterified with glycerol and are 100 times more soluble in water than are LCT.²⁵ MCT have been used mostly in fat emulsion formulations in combination with LCT.^{26–30} MCT recently were used in

medicated emulsions because of their increased ability to dissolve high concentrations of liposoluble drugs.³¹

Emulsifier(s)—Most of the known synthetic and efficient emulsifiers are toxic upon parenteral administration because of hemolysis. The emulsifiers most frequently used in parenteral emulsion formulations are phospholipids (generally from egg yolk sources), block copolymers of polyoxyethylene-polyoxypropylene (poloxamer) and, to a lesser extent, acetylated monoglycerides.^{32–35} Other emulsifiers, such as fatty acid esters of sorbitans (various types of Spans; ICI Americas) and polyoxyethylene sorbitans (various types of Tweens; ICI, UK), are already approved by the various pharmacopeias for parenteral administration and can therefore be considered for emulsion formulation design. However, it should be kept in mind that heat exposure after steam sterilization can alter the emulsifying ability by reducing the aqueous solubility and result in final phase separation.

Additives—Additives are needed to adjust the emulsion to physiological pH and tonicity. Glycerol is usually recommended as an isotonic agent and can be found in almost every parenteral emulsion.^{36–42} The pH is adjusted to the desired value with an aqueous solution of NaOH or HCl, depending on the value that should be reached. The pH of the emulsion is generally adjusted to 7–8 to allow physiological compatibility and maintain emulsion physical integrity by minimizing fatty acid ester hydrolysis of MCT–LCT and phospholipids.⁴³ Furthermore, emulsion stabilizers are often needed to protect emulsions from oxidation or phase separation. Although the first step is easy to accomplish by the addition of antioxidants or reducing agents, such as tocopherols, deferroxamine mesylate, and ascorbic acid, the second one is much more difficult to accomplish because it is related to interfacial degradation. The properties of the emulsifying interfacial film are altered by the incorporation of liposoluble drugs, so a stabilizing agent capable of localizing in the interfacial film should be added. Such molecules are generally amphipathic and are poor surfactants but can stabilize the film by enhancing molecular interactions and increasing the electrostatic surface charge of droplets. A well-known stabilizer is oleic acid or its sodium salt.^{43–45} Cholic acid, deoxycholic acid, and their respective salts also have been shown to markedly improve drug-incorporated emulsion stability.³¹

Manufacturing Process—The mean droplet size of iv emulsions must be smaller than the finest capillaries likely to be encountered in the vascular system; otherwise, an oil embolism can occur. Emulsions prepared by use of conventional apparatus, e.g., electric mixers and mechanical stirrers, etc., show not only large droplet sizes but also a wide particle size distribution and are often unstable.

A submicron emulsion can be prepared by use of a two-stage pressure homogenizer in which the crude dispersion is forced, under high pressure, through the annular space between a spring-loaded valve and the valve seat. The second stage occurs in tandem with the first, so that the emulsion is subjected to two very rapid dispersion processes.^{1,46,47} The influence of various mixers and homogenizers is depicted in Figure 2. As can be seen, a coarse dispersion is characteristic of emulsions prepared with a magnetic stirrer or with a simple homogenizer of the stator-rotor type. The use of high-shear mixers decreases the mean droplet size to the range of 1.1–0.65 μm . A monodispersed submicron emulsion can be prepared efficiently with a high-pressure homogenizer. High-shear mixers should be used for premixing before the homogenization process is conducted by repeatedly passing the coarse dispersion through the high-pressure homogenizer.

The emulsion preparation process involves the following steps, which are schematically illustrated in Figure 3.

(1) Three different approaches can be used to incorporate the drug and/or the emulsifiers in the aqueous or oil phase.

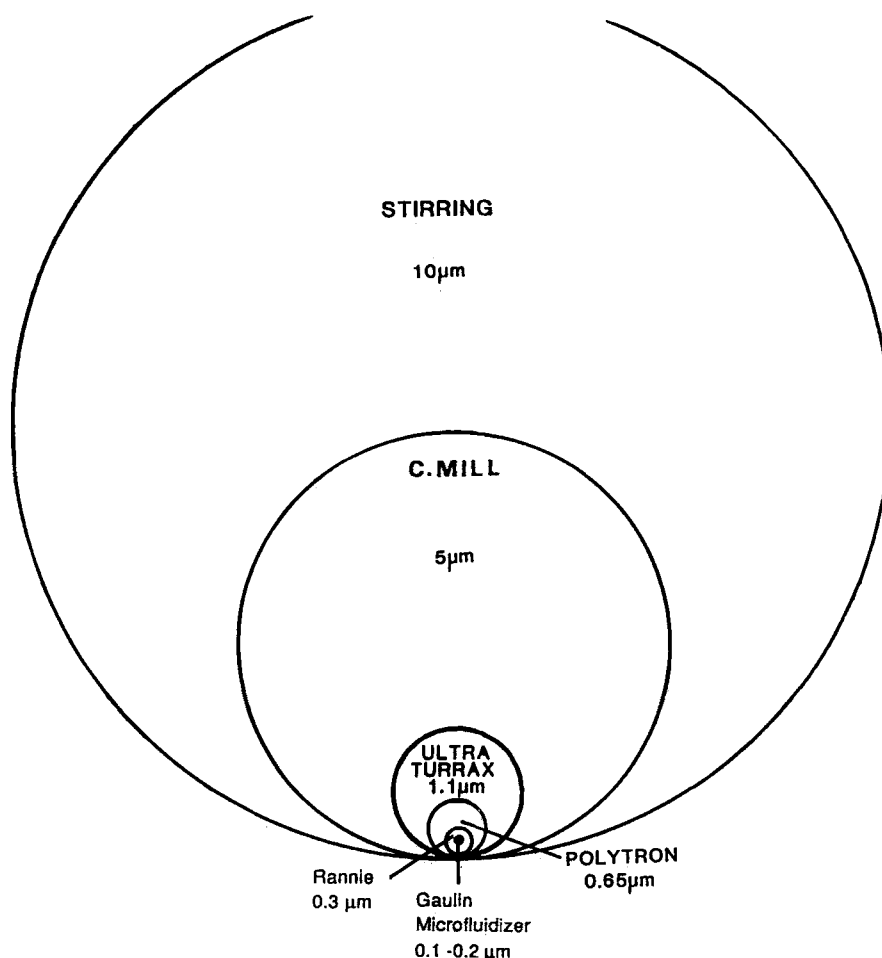


Figure 2—Effect of emulsification equipment on the mean droplet size of a submicron emulsion. C. MILL, Brogli Co., Appschwyl, Switzerland; ULTRA TURRAX (Janke Kunkel GmbH, Staufen, Germany); POLYTRON (Kinematica Ltd., Luzern, Switzerland)

The most common approach is to dissolve the water-soluble ingredients in the aqueous phase and the oil-soluble ingredients in the oil phase. The second approach, which was used in fat emulsion preparations,¹ involves the dissolution of an aqueous-insoluble emulsifier in alcohol and then the dispersion of the alcohol solution in water, evaporation, and total removal of the alcohol until a fine dispersion of the emulsifier in the aqueous phase is reached. The third approach, which was mainly used for amphotericin B incorporation into an emulsion, involves the preparation of a liposome-like dispersion.^{4,15} The drug and phospholipids are first dissolved in methanol, dichloromethane, or chloroform or a combination of these organic solvents and then filtered into a round-bottom flask. The drug-phospholipid complex is deposited into a thin film by evaporation of the organic solvent under reduced pressure. After sonication with the aqueous phase, a liposome-like dispersion is formed in the aqueous phase. The filtered oil phase and the aqueous phase are heated separately to 70 °C and then combined by magnetic stirring.

(2) The oil and aqueous phases are then emulsified with a high-shear mixer at 70–80 °C.

(3) The resulting coarse emulsion (1–5 µm) is then rapidly cooled and homogenized into a fine monodispersed emulsion (0.1–0.5 µm) with a two-stage pressure homogenizer.

(4) Finally, the pH content of the emulsion is adjusted to the desired level, and the emulsion is filtered to discard coarse droplets and debris generated during the emulsification and homogenization processes.

Usually, the whole preparation process is conducted in a laminar flow hood under a nitrogen atmosphere in case excipients and drugs sensitive to oxidation are used. Sterilization is normally achieved either by the use of a standard steam procedure (autoclaving) or by the maintenance of aseptic conditions during the entire preparation process, depending on the sensitivity of the active ingredients to elevated temperatures.

High-pressure two-stage homogenizers of the Gaulin or Rannie type (APV Gaulin, Hilversum, The Netherlands, or APV Rannie, Inc., Albertsland, Denmark, respectively) or microfluidizers (Microfluidics Corp., Newtown, MA) based on an interaction chamber mechanism not disclosed by the manufacturer can be used to decrease the droplet size of the emulsion to the submicron size range.¹²

Emulsion Characterization—Droplet Size—Particle size distribution is one of the most important characteristics of an emulsion. For example, sedimentation and creaming tendencies during long-term and accelerated stability tests of an emulsion can be conventionally monitored by measuring the changes in the droplet size distribution. A wide range of particle sizes are found in emulsion systems, as evidenced by iv fat emulsions that should contain particles in the range of 50 nm–1 µm and emulsions that are used as contrast media in computerized tomography and that contain particles 1–3 µm in size. Particles >5 µm in size are clinically unacceptable because they cause the formation of pulmonary emboli.³⁹ Such particles are sometimes present because of inefficient homogenization or instability of the emulsion. Hence, it is necessary to determine their sizes even if they are

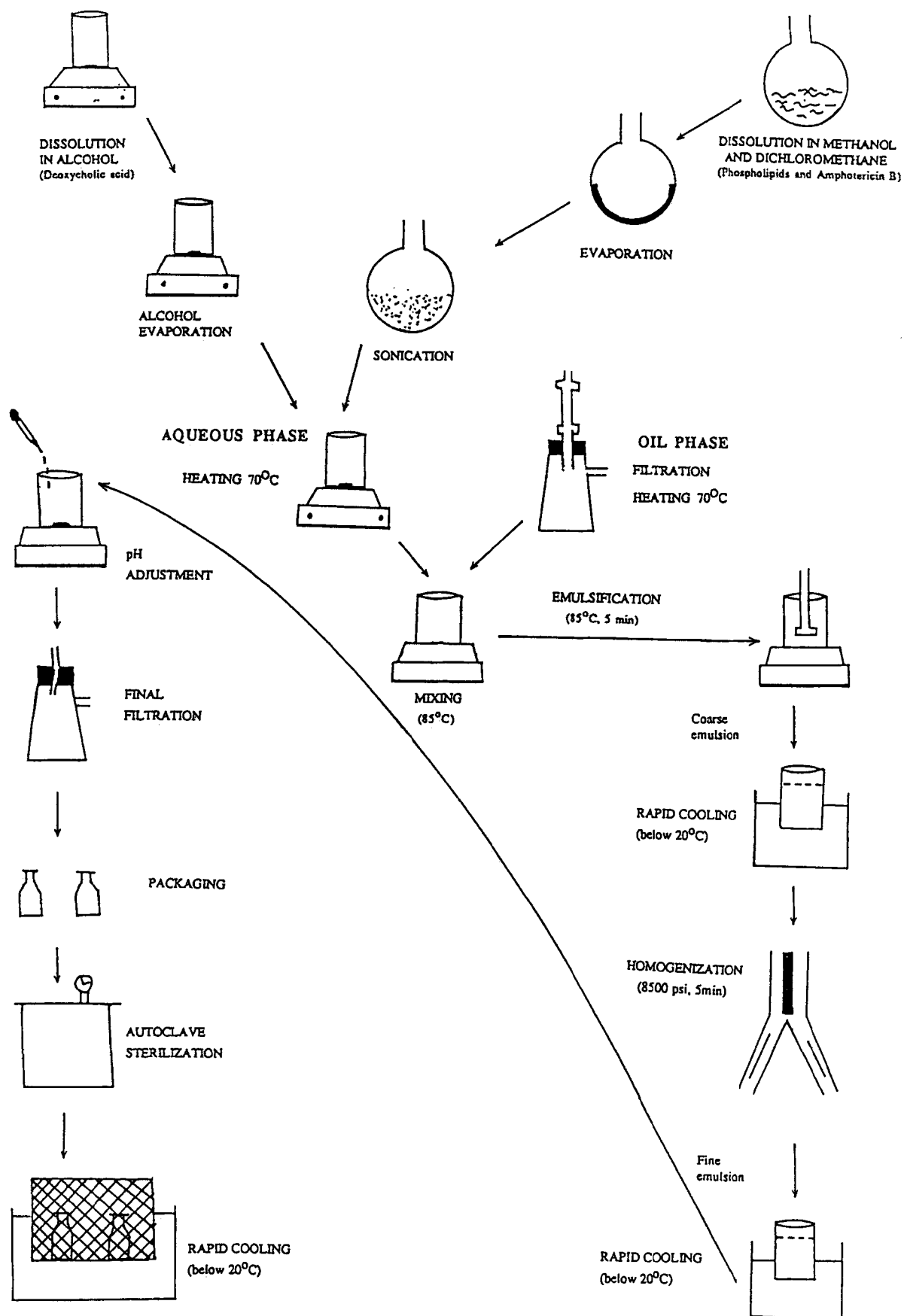


Figure 3—Schematic description of the submicron emulsion manufacturing process.

present in small numbers. Therefore, two complementary particle size analysis methods, namely, the photon correlation spectroscopy (PCS) method,⁴⁸⁻⁵¹ which is considered the most appropriate for studying droplets <1 μm in size, and the computerized laser inspection system,⁵² which can measure droplet sizes >0.6 μm , are needed to effectively cover the measured size range of 50 nm–10 μm . The advantage of the laser inspection systems, e.g., Galai Cis 1 (Galai Co., Migdal Haemek, Israel) over the widely used Coulter Counter system is that there is no need for an electrolyte solution, which can affect the stability of the emulsion.

Droplet Surface Charge—The electrical charge on emulsion droplets is measured by use of either a Zetasizer (Malvern Instruments, Malvern, England) or the moving-boundary electrophoresis technique, which has been shown to yield accurate electrophoretic mobility data.⁵³ The shape of an electrophoresis cell and the method used to convert the electrophoretic mobility to the zeta potential have been clearly reviewed.^{13,37}

Emulsifiers can stabilize emulsion droplets, not just through the formation of a mechanical barrier but also through the production of an electrical (electrostatic) barrier or surface charge. The electrical surface charge of droplets is produced by the ionization of interfacial film-forming components. The surface potential (zeta potential) of an emulsion droplet is dependent upon the extent of ionization of the emulsifying agent. The extent of ionization of some phospholipids present in lecithin is markedly pH dependent.⁵⁴⁻⁵⁶

Commercial lecithins are in fact a mixture of phospholipids that vary in composition. They may comprise phosphatidylcholine, which is zwitterionic in form and neutral over a wide pH range, as the major component, together with negatively charged phospholipids, such as phosphatidylethanolamine. In addition, other components, such as cholesterol, are present and may affect the interfacial film charge.

Rydhag^{57,58} suggested that emulsion stability may be optimized by the selection of commercial lecithins that contain appropriate amounts of negatively charged and uncharged phospholipids, which would result in the formation of an interfacial lamellar liquid crystalline phase. Attempts were made to enrich a mixture of purified phospholipids with negatively charged phospholipids after extraction and removal of neutral components, such as cholesterol, from a crude commercial lecithin product⁵⁹ by the addition to the mixture of phospholipids of a small quantity of negatively charged phospholipids.^{38,60} It is believed that emulsions prepared from such highly negatively charged phospholipids will exhibit high zeta potentials and will be less sensitive to the addition of small amounts of monovalent and divalent electrolytes.^{61,62}

High zeta potentials (> -30 mV) should be achieved in most of a prepared emulsion to ensure a high-energy barrier, which causes the repulsion of adjacent droplets and results in the formation of a stable emulsion.

pH—It has already been shown that the main degradation pathway for a fat emulsion leads to the formation of fatty acids, which gradually reduce the pH of the emulsion.³⁷ The initial pH of the emulsion may decrease progressively with time. However, this pH decrease can be controlled by adjusting the initial pH of the emulsion.⁴³ Provided that the initial adjusted pH is satisfactory, the rate of hydrolysis of phospholipids and triglycerides may be minimized.¹ Therefore, the pH of the emulsion should be monitored continuously over the entire shelf life of the emulsion to detect detrimental free fatty acid formation.

Drug Content—As required for any dosage form, quantitative and sensitive methods of analysis should be applied to evaluate the chemical fate of the active ingredient in the emulsion formulation. In a medicated submicron emulsion, the decomposition of the drug can be accelerated by micellar catalysis.⁶³ Hence, further attention should be given to monitoring of the chemical integrity of drugs in emulsions containing emulsifying agents. Consequently, a thorough

study of the knowledge of the partitioning of a drug among the various emulsion phases is needed. Generally, ultrafiltration techniques are used to achieve such objectives.

Stability Assessment

Accelerated Tests—It should be emphasized that the stability results of accelerated tests based on elevated temperatures generally do not reflect the actual stability of an emulsion stored at normal temperatures. The large discrepancy observed between the predicted values and those obtained experimentally by various authors^{44,64-66} could be explained by the instability of an emulsion and phospholipid decomposition at elevated temperatures. Emulsions subjected to temperature variations undergo dramatic physicochemical changes. Thus, the long-term stability of emulsions and the subsequently induced protection of sensitive drugs to potential hydrolysis cannot be predicted from experiments carried out at high temperatures.

Therefore, of the various accelerated tests reported in the literature,^{1,39,65} steam sterilization, excessive shaking, and freeze-thaw cycles are generally used to predict emulsion shelf life. These tests are considered most relevant to the stress conditions that emulsions may encounter during sterilization, transportation, and aging.

Emulsions are packed in final packaging and subjected to the various accelerated tests described above. The pH, zeta potential, droplet size distribution, and drug content are evaluated before and after testing.

Long-Term Tests—It is routine to determine the shelf life of a new product by storing it for various periods of time at elevated temperatures. The Arrhenius equation is commonly used to predict the shelf life.

Long-term emulsion stability studies are conducted at temperatures ranging from 4–50 °C. The chemical (drug content) and physical (emulsion droplet size, creaming, and pH, etc.) changes that might occur in the emulsion during storage are monitored over long periods of time. However, it must be noted that for emulsions, such monitoring can be erratic, because changes in temperature not only change the rate of the reaction but also can destroy the physical stability of the emulsion.

As suggested by Rieger,⁶⁴ a realistic stability program to assess the normal shelf life of an emulsion should be constructed on the basis of predictions of normal conditions undergone by the specific emulsion formulation.

During the long testing period, samples stored under various conditions should be observed critically for separation and monitored at reasonable time intervals for changes in the following characteristic properties: electrical conductivity, viscosity, particle size distribution, zeta potential, pH, and chemical composition.

In addition to these physical measurements, a shelf life program for an emulsion should include testing of the emulsion for the establishment of sterility and lack of pyrogens by validated, recognized microbiological methods.

In Vitro Release Kinetics Examination

An accurate analysis of in vitro drug release from an emulsion first requires a knowledge of the distribution of the drug in the various phases of the emulsion. This issue is discussed below.

It is difficult to characterize drug release from a colloidal carrier because of the physical obstacles associated with the extremely small size of the dispersed particles. Various techniques have been used to evaluate drug release from colloidal carriers, particularly O/W submicron emulsions.^{67,68} Attempts have been made to elucidate the release mechanism by use of the dialysis sac diffusion technique⁶⁹⁻⁷¹ or diffusion cells.⁷²⁻⁷⁵ Washington⁷⁶ claimed that with these techniques, the carrier is never diluted with the release solution, so the experiment is not performed under sink conditions. As a consequence, the true release rate is

not measured. Levy and Benita⁷⁷ recently proposed the bulk equilibrium reverse dialysis sac technique to avoid the enclosure of the submicron emulsion dispersion in a dialysis sac. However, the kinetic system proposed was only able to differentiate colloidal drug delivery carriers releasing their contents over a period longer than 1 h. Finally, a more rapid stringent test based on a centrifugal ultrafiltration technique in the absence of any physical membrane also has been used.⁷⁸ All these tests were recently used to evaluate clofibrate release from a submicron emulsion¹⁷ to avoid any controversial *in vitro* release kinetics deductions.

Normally, a drug incorporated in an emulsion is liposoluble and exhibits limited aqueous solubility, characteristics that may prevent sink conditions from prevailing.

Use of Human Albumin to Maintain Conditions of Infinite Dilution in a Release Medium—In an *in vitro* investigation of rates of release of drugs from a carrier, the goal is to imitate expected *in vivo* conditions as much as possible. The release profile for a drug from a dosage form intended for *iv* administration is usually tested in aqueous buffered solutions with a pH close to that of blood. Emulsions for *iv* administration are generally used as carriers for lipophilic drugs, which have limited aqueous solubility. The low solubility of such drugs makes it difficult to create conditions of infinite dilution in an aqueous release medium. The addition of organic solvents or surface-active agents definitely improves the solubility of such drugs in release solutions, but it would be erroneous to predict the behavior of the drugs *in vivo* on the basis of their release profiles in such solutions.

Lipophilic drugs bind with a high affinity to blood proteins, mainly albumin.⁷⁹ Recently, Olson et al.⁸⁰ reported the use of human serum albumin to improve the solubility of lipophilic drugs, such as diazepam, in aqueous injectable solutions. The increase in the aqueous solubility of diazepam with albumin was also confirmed in the present study. Increasing the albumin concentration in the aqueous solution enhanced the solubility of diazepam and miconazole in water (Figure 4). Furthermore, human albumin is generally used as a cosolvent in release sink solutions for characterization of the release profiles for lipophilic drugs, particularly from transdermal and *iv* preparations.

Albumin solution as a release medium has a distinct advantage over other cosolvents, because it is a natural component of the prevailing *in vivo* conditions. Albumin at a concentration of 1% is sufficient to ensure the maintenance of conditions of infinite dilution in release media intended for the testing of release profiles for diazepam and miconazole from emulsions.

Dialysis Sac Diffusion Technique—A detailed description of this technique was given elsewhere.^{17,77} A brief description will be reported here.

A given volume of the medicated emulsion should be placed

in the dialysis sac, which should be hermetically sealed and dropped into an appropriate sink solution. The entire system should be kept at 37 °C with continuous magnetic stirring. Samples should be withdrawn from the sink solution at predetermined times and assayed for drug content.

In preliminary studies, various authors^{70,71,73,74} tried first to evaluate the diffusion of a drug dissolved in an appropriate solution through the dialysis membrane. They usually reported that the drug diffused rapidly into the sink solution, a result indicating that the membrane was not a rate-limiting step under the experimental conditions used. However, the release of drugs from emulsions is generally slower than that from the respective drug solutions.

Numerous investigators have pointed out various reasons explaining the drastic decrease in the rate of release of a drug from an emulsion in the dialysis technique.^{17,75–78} It was assumed that the prevalence of a very low drug concentration gradient through the dialysis membrane during the kinetic process was the main factor that drastically reduced the release of the drug from the colloidal carrier, as compared with the results obtained with a high drug concentration gradient.⁷⁷

Bulk Equilibrium Reverse Dialysis Sac Technique—For avoiding enclosing a medicated emulsion in a dialysis sac, the emulsion is placed directly into an appropriate volume of a stirred sink solution. The dialysis sacs, containing 1 mL of the same sink solution, should be equilibrated with the sink solution for a few hours before the experiments.^{17,77} At predetermined times, a dialysis sac and 1 mL of the sink solution should be withdrawn, and the drug contents of the dialysis sac and the sink solution should be assayed. The kinetic experiments should be performed at 37 °C with constant magnetic stirring. Upon drug emulsion immersion in the sink solution, an infinite dilution is achieved, and a new equilibrium is reestablished when the drug is partitioned between the oil droplets and the sink solution, which becomes the external phase of the emulsion without being separated from the oil droplets by any artificial membrane. The diffusion of the drug from the oil droplets to the sink solution is governed by a true and real gradient existing between the oil droplets and the new external aqueous phase. Drugs dissolved in the aqueous phase then permeate into the dialysis sacs.

As previously reported, the percent drug released is calculated from the ratio of drug concentrations measured at predetermined times in the dialysis sacs versus the total concentration of the drug in the sink solution, in which oil droplets are also present.⁷⁷

The release of the drug from the emulsion and the release of the respective product into the sink solution was found to be rapid.^{17,77} Practically all the drug was released into the sink solution within <1 h. No difference in the release profiles for drugs from various dosage forms was observed at a given pH. These observations were expected, because extensive dilution ($\times 500$) with an aqueous phase was performed. Such dilution leads to drug partitioning largely in favor of the aqueous phase, as was confirmed by the apparent partition coefficient data presented by Santos-Magalhaes et al.¹⁷ and Levy and Benita,⁷⁷ who suggested that the overall kinetic process is governed by the oil–water partition rate of the emulsion rather than by the rate of diffusion of the drug through the mixed emulsifying interfacial film.

It is not yet possible to distinguish whether drug release from oil droplets into a sink solution enclosed in dialysis sacs is faster than the rate of permeation of a dissolved drug through a dialysis membrane. This is due to the similarity of the kinetic behaviors of the various pharmaceutical dosage forms.

Attempts were made to identify the actual release profile for a drug from a submicron emulsion by designing an *in vitro* kinetics experiment devoid of any dialysis membrane but based on a centrifugal ultrafiltration technique.

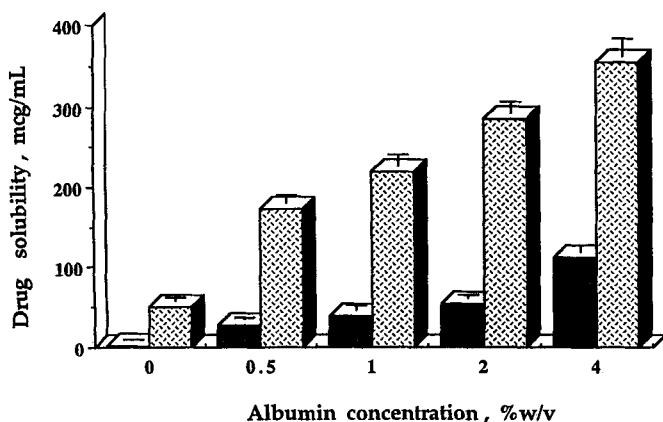


Figure 4—Effect of the human albumin concentration on the aqueous solubility of miconazole (▨) and diazepam (■).

Centrifugal Ultrafiltration Technique—The centrifugal ultrafiltration method was recently developed by Millipore Corp. (Bedford, MA). The device, mainly based on an Eppendorf centrifuge tube separated from an enclosed tube by an ultrafiltration membrane, allows for the separation of nanoparticles from microliter volumes of an aqueous dispersion medium in a centrifuge (Ultra-free MC unit). This technique has been successfully applied by Ammoury⁷⁸ to evaluate the *in vitro* release profile for indomethacin from polylactic acid nanocapsules. Santos-Magalhaes and colleagues¹⁷ used the method to evaluate clofibrate release from a submicron emulsion. One milliliter of a clofibrate emulsion was directly placed in 250 mL of a stirred buffer sink solution at 37 °C. At various times, 400 μ L of the release solution, in which the emulsion was dispersed, was deposited in the Ultra-free MC unit (10 000 NMWL, PLGC-type membrane with a low level of protein binding), which was then subjected to centrifugation at 5000 $\times g$ for 5 min. Fifty microliters of the ultrafiltrate was then withdrawn and assayed for clofibrate content by the HPLC technique. The percent release of clofibrate was calculated from the ratio of drug concentration in the ultrafiltrate to the total concentration of clofibrate in the release solution. This technique yielded rapid *in vitro* profiles for clofibrate release from the emulsion. Seventy-five to 90% of the clofibrate content was released from the emulsion within 15 min, a result confirming that the kinetic process is probably controlled by the oil–water partition rate under perfect sink conditions. However, this technique might suffer from the drawback that the marked centrifugal force needed to separate the ultrafiltrate from the nanodroplets might alter the emulsion integrity and result in a different drug distribution phase profile. Therefore, we attempted to adapt an ultrafiltration technique at a low pressure for the purpose of evaluating drug release profiles for submicron emulsions.

Ultrafiltration Technique at a Low Pressure—A medicated emulsion was placed in a stirred ultrafiltration cell (model 8200; Amicon, Danvers, MA) containing an adequate volume of a sink solution (30–200 mL). At various times, aliquots of the release medium were filtered through an ultrafiltration membrane (Amicon-YM-10 or YM-100) by use of nitrogen gas at <0.5 bar. The lack of adsorption of the drug to the membrane was first established in earlier experiments. The clear filtrate, collected at various times, was then analyzed for drug content by appropriate analytical methods.

It should be emphasized that other types of stirred ultrafiltration cells, such as the UP-110 model from Schleicher and Schuell (Dassel, Germany), could also be used, depending on the final volume of the release sink solution needed. The UP-110 model is suitable for release sink solutions ranging in volume from 200–1000 mL.

This approach was also used to evaluate the release of diazepam in a marketed product, Diazemuls (KabiVitrum, Stockholm, Sweden), from a submicron emulsion, the physicochemical properties of which were previously reported.¹²

The release of diazepam from both emulsions was very rapid (Figure 5). Within a few minutes, most of the drug was released from both emulsions, as expected under perfect sink conditions. Moreover, no change in release from both emulsions and from a hydroalcoholic solution of diazepam was noted, a result clearly indicating that the rate-determining step in the overall kinetic process is the partitioning of the drug in the dispersion system. These results are in agreement with those of clinical studies that revealed no difference in the pharmacokinetic profile of diazepam when the marketed hydroalcoholic solution Valium was compared with the marketed emulsion Diazemuls following *iv* administration.⁸¹ However, authors who compared the pharmacokinetics of cyclosporine in blood after *iv* administration to rabbits as liposomes, an Intralipid emulsion, or a commercially available aqueous solubilized formulation re-

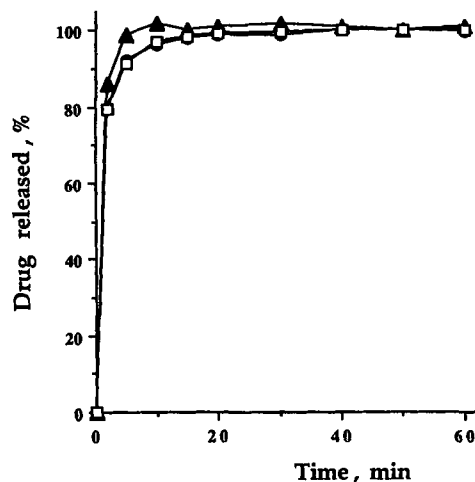


Figure 5—Diazepam release profiles for various dosage forms (Assival, a marketed hydroalcoholic solution, $\blacktriangle$; Diazemuls, a marketed submicron emulsion, $\square$; and a previously described^{12,43} diazepam emulsion, $\bullet$) with the ultrafiltration technique at a low pressure [1 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (pH 7.0)].

ported that the apparent volume of distribution of cyclosporine at the steady state was highly dependent on the administered formulation.⁸² These results are consistent with the clearance kinetics for endogenous lipoproteins recently described by Noguchi.⁸³ Intralipid can reasonably be considered an artificial chylomicron because it contains triglyceride in its core, whereas liposomes, which lack a core lipid, can reasonably be considered artificial high-density lipoproteins. Consequently, the faster clearance of artificial chylomicrons during the initial distribution phase may give rise to a significant increase in the apparent volume of distribution at the steady state.⁸⁴ It is likely that the differences observed between the formulations reflect the extensive interaction between cyclosporine and the different plasma lipoproteins.

Noguchi⁸³ also has reviewed the clearance of drugs associated with chylomicrons. He hypothesized, on the basis of published studies that the release of drugs from chylomicrons or from chylomicron-like emulsions after *iv* administration may be affected by the degree of drug lipophilicity. For compounds with a low log partition coefficient diffusion is probably dominant; consequently drug release is almost independent of chylomicron metabolism. In contrast, for compounds with a high log partition coefficient, such as the lipophilic hydrocarbons and drugs such as testosterone undecanoate, the metabolism of a triglyceride lipid may play a critical role. In this case, in which the highly lipophilic drug is associated with chylomicron lipid, lipoprotein lipase-based lipolysis of the core triglyceride occurs first and is followed by the release of drug. Subsequently, diffusion or transfer of the drug to other lipoproteins may occur. In this case, protein binding is another important factor that may affect drug release and distribution. Compounds having a high protein/lipoprotein binding affinity, such as 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane, may exhibit a more rapid plasma clearance than chylomicron triglyceride.⁸⁵

Therefore, the previously made deductions for *in vitro* kinetics of release, which suggested that drug release from an emulsion was governed mainly by a diffusion partition process, should be restricted to lipophilic drugs with a low log partition coefficient when an attempt to correlate *in vitro* results with *in vivo* results is made. With regard to the *in vivo* kinetics of release of highly lipophilic drugs from emulsions considered to be artificial chylomicrons, the *in vivo* lipolytic metabolism of emulsified triglyceride nanodroplets by lipases is also likely to contribute to drug release. Indeed, no in

vitro-in vivo correlations should be attempted unless appropriate lipases are added to the buffered albumin sink solution used in the ultrafiltration technique at a low pressure.

Distribution Phase Partitioning Study of a Drug in an Emulsion

Ultrafiltration Technique at a Low Pressure—The ultrafiltration technique at a low pressure is generally used to determine the concentration of an unbound drug in the external aqueous phase of an emulsion by measuring drug concentrations in the aqueous ultrafiltrate at various filtrate volumes^{86–88} while maintaining the physical integrity of the dispersed system. The usual technique involves the use of 62-mm YM-10 (Amicon) ultrafiltration membranes, which are soaked in deionized water, with several changes of water, for at least 1 h to remove water-soluble contaminants. The membranes are placed in a stirred ultrafiltration cell (model 8200; Amicon) operated at room temperature. Forty milliliters of a drug solution or emulsion to be filtered is placed in the stirred vessel, and nitrogen at 20–40 psi is applied to begin filtration. Filtrate samples of ~2 mL are collected until 15–20% of the liquid is filtered. Each sample is then assayed for drug content. Before filtration, both the drug solution and the emulsion are also assayed for drug content.

Ultrafiltration Technique Validation—Before the ultrafiltration technique can be used to determine the distribution phase of a drug, the technique must undergo validation, as has already been done by other authors.⁸⁸ Membrane binding and rejection must be accounted for to accurately measure aqueous concentrations of a drug. Ultrafiltration membranes are specifically selected for their exceptionally low levels of nonspecific binding. The effects of membrane binding and rejection of a drug are studied by ultrafiltration of an aqueous solution of the drug, which is maintained at a given concentration. The recovery curve for a drug such as diazepam in an aqueous solution is shown in Figure 6A. The membrane appeared to be nearly saturated after ~25% of the total volume had been filtered, as evident by the leveling off of the curve. The percent recovery was 97–98% of theoretical recovery, a result indicating that rejection was negligible. On the basis of these rejection data, ultrafiltration data for diazepam emulsion formulations required only a slight correction, provided that at least 25% of the total volume was filtered to allow saturation of the membrane.

Phase Partitioning Determination—Usually, drug partitioning should be measured in three distinct phases of an emulsion, namely, the aqueous phase, the oil phase, and the O/W or W/O interface. Determination of the concentration of a drug in the external aqueous phase of an emulsion was carried out by the technique described above. As shown by the data in Figure 6B for a previously described^{12,43} diazepam submicron emulsion and the recovery curve exhibited in Figure 6A, analysis of the separated clear aqueous ultrafiltrate enabled estimation of the amount of diazepam normally located in the aqueous phase of the emulsion. The points at which the filtrate diazepam concentration became constant were used as the appropriate aqueous-phase concentration (Figure 6B). The data were slightly corrected for the low percentage of adsorption observed during filtration of the aqueous diazepam solution. Determination of the drug concentration in the oil phase of the emulsion was performed by subjecting the emulsion to ultracentrifugation with a Beckman LB 50B ultracentrifuge at $115\,000 \times g$ for 30 min, a procedure that resulted in separation of the oil phase from the emulsion. Aliquots of the oil phase were assayed for drug content. It was difficult to directly measure the amount of the drug at the interface. However, indirect determination of the amount of the drug at the interface was made by measurement of the total amount of the drug in the intact emulsion, in the aqueous phase of the emulsion, and in the oil phase after separation of the oil from the emulsion by

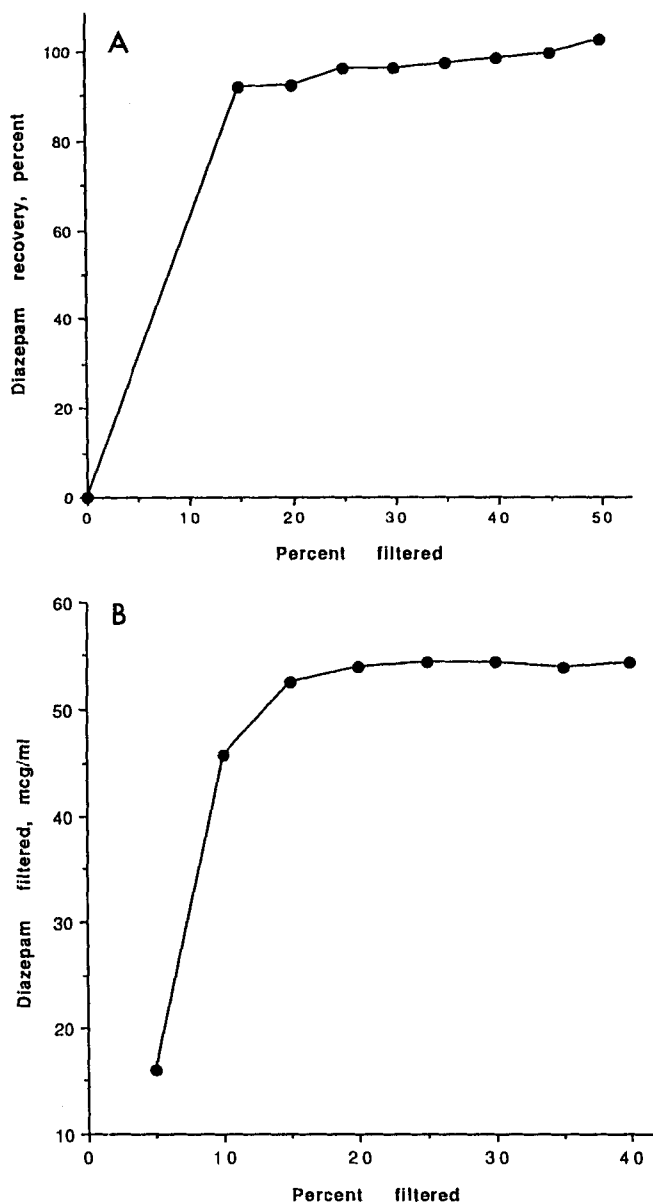


Figure 6—(A) Recovery curve for diazepam in an aqueous solution (50 µg/L) after ultrafiltration at a low pressure. (B) Diazepam concentration in a filtrate after ultrafiltration of a diazepam submicron emulsion (5000 µg/mL) prepared under conditions described in references 12 and 43 and with a 10% soybean oil concentration.

ultracentrifugation as performed by Teagarden and colleagues.⁸⁸ The drug concentration at the O/W interface was then calculated by measuring the difference between the total drug concentration and the concentrations of drug in the oil and water phases. As an example, for the diazepam emulsion, the following drug distribution profile was obtained: 1.3% was dissolved in the aqueous phase, 74.9% was dissolved in the oil phase, and 23.8% of the total amount of the drug was located at the O/W interface.

New Approaches for Emulsion Characterization

Monolayer Studies—Attempts have been made in the last few years to identify the conditions needed for the formation of stable O/W emulsions by estimating the interactions occurring between the surfactants at the O/W interfacial film of the dispersed droplets. For these purposes, surface pressure studies of mixed-surfactant monolayers under dynamic conditions⁸⁹ and under equilibrium conditions^{90,91} have been carried out. Furthermore,

Casas and Baszkin⁹¹ also have characterized the surface potential of mixed surfactant monolayers under different experimental conditions. It should be emphasized that monolayer studies have been shown to be relevant to the study of emulsion stability, especially at high film pressures involving polar lipids, such as lecithin, fatty acids, and monoglycerides.^{16,92-95}

These attempts to gain an overall comprehension of the complex molecular interactions occurring between various components forming an interfacial film around emulsified oil droplets have been reported by some authors to be successful. Santos-Magalhaes and colleagues⁹⁰ have studied the penetration of a polyoxyethylene-polyoxypropylene block copolymer surfactant (poloxamer) into phospholipid monolayers under equilibrium conditions.

The penetration of the poloxamer surfactant into monolayers of soy phosphatidylcholine spread at the air-water interface was monitored by measuring the variations in the surface pressure in a constant area. As revealed by the results obtained at low surfactant concentrations, the adsorption of the surfactant was enhanced by the presence of the phospholipid film. The use of different thermodynamic equations enabled calculation of the quantities of adsorbed surfactant for either the condensed state (102–122 Å² per molecule) or the expanded state (140–230 Å² per molecule) of phospholipid monolayers. In addition, Levy and colleagues⁸⁹ have investigated the interactions of a nonionic surfactant (poloxamer) with mixed phospholipid-oleic acid monolayers under dynamic conditions. These authors found that, whereas the mean surface areas of the mixed phospholipid-oleic acid monolayers corresponded to the additivity rule, the surface pressures corresponded to those of the phospholipids. It was concluded that the nonideal behavior of mixed phospholipid-oleic acid films resulted from the molecular interactions occurring between the hydrophobic parts of the film-forming components and that the double bond of oleic acid was involved in these interactions. The mixing energies and interaction parameters at intermediate and collapse pressures were calculated. Negative values for these parameters indicated that the mixed films were thermodynamically more stable than the films of each component.

The addition of poloxamer into the aqueous subphase yielded films capable of supporting higher compressions, a result that was attributed to the partial penetration of poloxamer molecules into the lipid monolayers. On the basis of these data, models of phospholipid-oleic acid-poloxamer arrangements in the interfacial region were proposed for the expanded and condensed states of the monolayers.⁸⁹ The findings recently were confirmed by data generated in an investigation of the same mixed surfactant monolayers under dynamic conditions.⁹¹ Furthermore, it was reported that oleic acid increased the surface potential of the mixed monolayers. The existence of molecular interactions among phospholipids, oleic acid, and poloxamer would explain the prolonged stability of O/W emulsion formulations comprising the above-mentioned components and triglycerides.⁸⁹

Rubino⁹⁵ used a similar approach of monolayer film studies to investigate the influence of charged lipids on the flocculation and coalescence of O/W emulsions. The influence of Ca²⁺ on the monolayer properties of mixed films containing phosphatidylcholine with either phosphatidic acid or sodium oleate was examined at the air-water interface. Films containing phosphatidylcholine with phosphatidic acid expanded more on a subphase containing calcium than on a subphase not containing calcium. In addition, the compression of films containing phosphatidylcholine with sodium oleate demonstrated two collapse pressures, whereas phosphatidic acid was relatively more miscible in the films. This result suggested that the phase separation of interfacial lipids occurred more easily in systems containing phosphatidylcholine and sodium oleate. These results may help to explain differences in the flocculation and coalescence of emulsions stabilized by lipid films with different compositions.

Microscopic Studies—Transmission Electron Microscopic Examination—The simplest technique used for measuring emulsion particle size and evaluating emulsion stability through the detection of coalescence in emulsions containing droplets <500 nm in size involves an optical microscope; this technique is futile, and it is necessary to use an electron microscope. Du Plessis and colleagues⁹⁶ have developed a new transmission electron microscope (TEM) method for the determination of particle size in parenteral fat emulsions. The technique involves the enclosure of the emulsions in small agar capsules and was used to prepare ultrathin sections of parenteral fat emulsions.

The emulsion-filled agar capsules were placed in small stoppered bottles and fixed for 2 h in a 2% osmium tetroxide solution. Well-defined droplets were detected by this technique; the particle size ranged from 210–260 nm, a result conforming to results reported by other authors using PCS.³⁹ However, the authors defined their TEM technique as being tedious and expensive, as it took about 30 h before results could be obtained. Consequently, a quicker and less expensive method was proposed by Du Plessis and colleagues.⁹⁷ The new TEM method involved diluting the emulsion with distilled water and mixing the dilution with a 2% osmium tetroxide solution. After 15 min of fixation, a thin layer of the fixed emulsion was placed on a Formvar-coated 200-mesh copper grid for TEM examination. Well-defined tiny droplets were observed with this negative-staining technique. It is therefore expected that the simplification and improvement of the TEM examination of submicron emulsions will significantly rationalize research in this area.⁹⁷

Scanning Electron Microscopic Examination—Submicron emulsions are thermodynamically unstable and prone to particle size increases during storage and processing, as previously mentioned.⁵ It was realized that scanning electron microscopy (SEM) could be a valuable tool in the study of droplets in parenteral emulsions.^{98,99}

The three-dimensional appearance of the fat droplets obtained with SEM, in contrast to TEM, could improve the interpretation of results. However, lipids are extremely difficult to fix.⁹⁸ Special attention therefore should be paid to fixation protocols for SEM that will preserve the shape and size of the fat droplets. Hamilton et al.⁹⁹ have reported a technique that combines fixation of an emulsion with osmium trioxide and deposition of the fixed droplets on a Nuclepore filter support (pore size, 0.4 μm); this technique yielded satisfactory results. The shape and size of the fat droplets were clearly visualized. However, very small droplets (<0.4 μm) are unlikely to be retained on the Nuclepore filter support. This technique was recently used by Santos-Magalhaes and colleagues¹⁷ to characterize an O/W clofibride submicron emulsion.

Freeze Fracturing Techniques—Menold and colleagues¹⁰⁰ presented a very accurate method for studying fine polydispersed suspensions and emulsions by use of an electron microscope. Their method, which was also successfully used by other researchers,¹⁰¹ involves the rapid freezing of emulsions, fracturing of the frozen emulsions, and the preparation of carbon replicas. However, this technique is both laborious and time-consuming and requires the use of a special vacuum coater.¹⁰² Nevertheless, this technique is capable of visualizing molecular dimensions and can identify the fine structure of large molecules, such as lipids or other structures, e.g., lamellar or monolayer structures, that are yielded by emulsifiers and that are known to stabilize emulsions. It is possible to detect by this method the presence of vesicles, micelles, liquid crystals, and other structures together with fat droplets.^{50,103-105} Groves and colleagues¹⁰³ reported that liposomal structures are normally present in phospholipid-stabilized emulsions. This finding was recently confirmed by Westesen and Wehler,⁵⁰ and Rotenberg and colleagues also have detected liposomal structures in marketed fat emulsions by using a cryofixation technique instead of freeze fracturing.¹⁰⁴ Because of the sophistication and complexity of the freeze fracturing

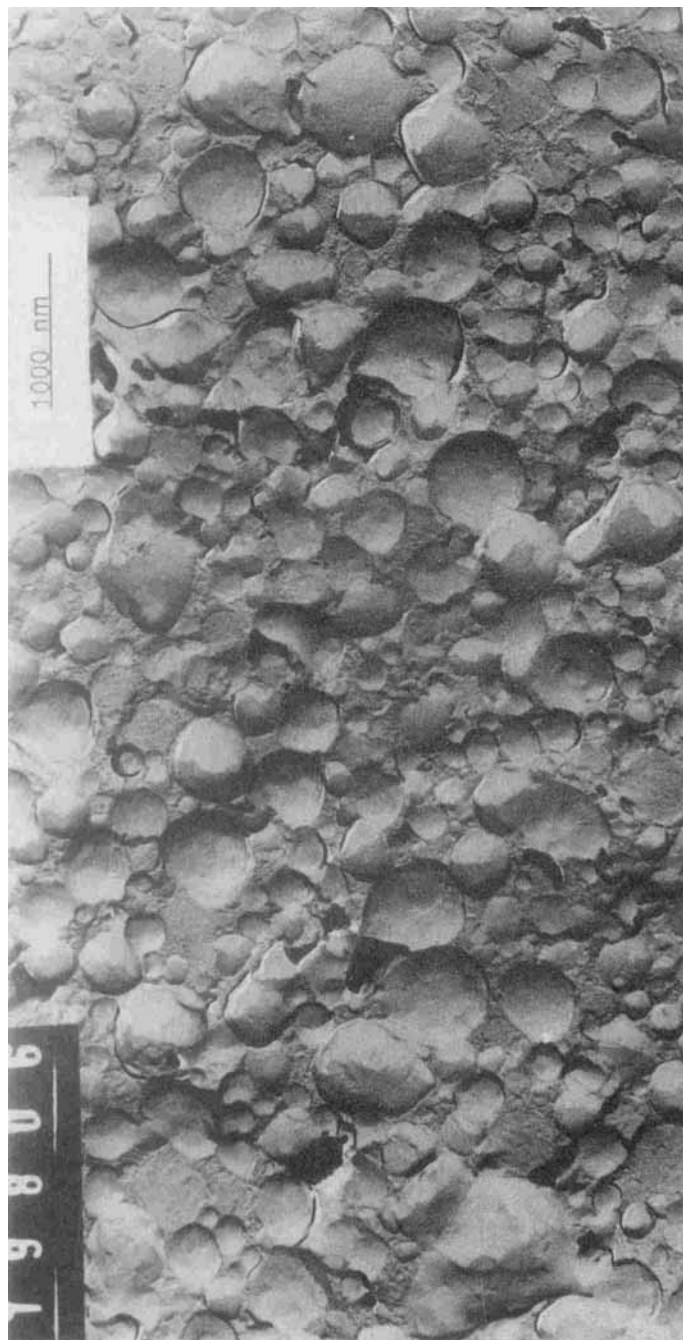


Figure 7—TEM micrograph of an amphotericin B emulsion subjected to the freeze-fracturing technique; the formulation was prepared with the following [percent (weight/weight)]: amphotericin B, 0.075; MCT, 20; phospholipids, 1.0; sodium deoxycholate, 0.5; poloxamer 188, 2.0; α -tocopherol, 0.02; glycerol, 2.25; and water for injection to 100.0.

technique, it is necessary to select optimum specimen preparation methods and to know how the different steps of the preparation, i.e., chemical fixation of samples, cryoprotective pretreatment, cryofixation, freeze fracturing, etching, and replication, may influence the appearance of the different constituents of the emulsions. Such influences were reviewed by Buckheim¹⁰⁵ for food systems. An illustration of this interesting technique applied to an amphotericin B submicron emulsion is shown in Figure 7. This preparation clearly shows a population of dispersed oil droplets with a narrow range distribution, 100–400 nm, for an emulsion prepared under experimental conditions described elsewhere.¹⁰⁶

Early Detection of Emulsion Instability—Emulsion instability is generally characterized by a progressive but moderate increase in droplet size that is very difficult to identify by PCS techniques. Indeed, PCS yields accurate diameter assignments for monodisperse submicron emulsions but fails to describe the size composition for mixed samples, especially when the concentration of a particular size population is low compared with those of other populations. Caldwell and Li¹⁰⁷ showed that the combination of sedimentation field flow fractionation (sedFFF) and PCS was able to characterize the size distribution for polydisperse samples, for which neither technique alone was capable of providing this information. Therefore, this new combined technique is recommended for assessing emulsion stability, especially after perturbation of an emulsion, as suggested by the authors.¹⁰⁷ However, as its name implies, sedFFF is a sedimentation-based method with elution character. Through the sedFFF process, even highly polydisperse samples can be separated into fractions of considerable size uniformity,¹⁰⁸ provided that these fractions are well retained. Such is the case for soybean emulsions, the oil density of which is 0.9–0.92 g/mL, a density significantly different from the density of the aqueous mobile fluid. The uniformly sized particle fractions obtained by this high-resolution separation technique then can be subjected to a PCS analysis for accurate evaluation of particle diameters at a selected elution position. Thus, emulsions submitted to various accelerated tests can be evaluated by the new combined technique, which is capable of identifying subtle changes in the particle sizes of emulsions, changes that forecast later problems.

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