ELSEVIER

Advanced Drug Delivery Reviews 20 (1996) 131–145

advanced
drug delivery
reviews

Stability of lipid emulsions for drug delivery

C. Washington

Department of Pharmaceutical Sciences, University of Nottingham, University park, Nottingham NG7 2RD, UK

Abstract

We discuss the factors influencing the stability of phospholipid-stabilized emulsions, and the effects of drug incorporation on the stability profile. The effects of electrolytes on emulsion stability and techniques for the study of emulsion stability are also reviewed. The application of these principles is illustrated by reference to an emulsion formulation of s-Emopamil. Although emulsion formulations have largely been confined to lipophilic drugs, there are plentiful opportunities for their application to surface-active or ionized drugs, given that the principles of their formulation are understood.

Contents

1. Introduction

The past 30 years have seen enormous activity in drug delivery and targeting research, and in particular the use of colloidal drug delivery systems, for vascular, subcutaneous, intramuscular and oral administration. A wide range of colloidal materials have been studied, including polymeric microspheres and cross-linked proteins such as albumin, which can be used to prepare solid particles of varying sizes. The production of these particles is often complex, involving processes such as solvent evaporation, coacervation and removal of untrapped drug. In contrast, emulsion delivery systems have many advantages. They can be prepared easily in a single dispersion step; for correctly chosen drug candidates, entrapment can be high, and they can be easily administered in liquid form without the need for reconstitution by the user.

Triglyceride lipid emulsions have a number of additional advantages. They have been studied widely for parenteral feeding, and consequently their preparation, stability and biopharmaceutics are well understood [1–10]. The droplet size of triglyceride emulsions normally lies in the range

0169-409X/96/$32.00
SSDI 0169-409X(95)00116-6

ELSEVIER

Advanced Drug Delivery Reviews 20 (1996) 131–145

advanced drug delivery reviews

Stability of lipid emulsions for drug delivery

C. Washington

Department of Pharmaceutical Sciences, University of Nottingham, University park, Nottingham NG7 2RD, UK

Abstract

We discuss the factors influencing the stability of phospholipid-stabilized emulsions, and the effects of drug incorporation on the stability profile. The effects of electrolytes on emulsion stability and techniques for the study of emulsion stability are also reviewed. The application of these principles is illustrated by reference to an emulsion formulation of s-Emopamil. Although emulsion formulations have largely been confined to lipophilic drugs, there are plentiful opportunities for their application to surface-active or ionized drugs, given that the principles of their formulation are understood.

Contents

1. Introduction

The past 30 years have seen enormous activity in drug delivery and targeting research, and in particular the use of colloidal drug delivery systems, for vascular, subcutaneous, intramuscular and oral administration. A wide range of colloidal materials have been studied, including polymeric microspheres and cross-linked proteins such as albumin, which can be used to prepare solid particles of varying sizes. The production of these particles is often complex, involving processes such as solvent evaporation, coacervation and removal of untrapped drug. In contrast, emulsion delivery systems have many advantages. They can be prepared easily in a single dispersion step; for correctly chosen drug candidates, entrapment can be high, and they can be easily administered in liquid form without the need for reconstitution by the user.

Triglyceride lipid emulsions have a number of additional advantages. They have been studied widely for parenteral feeding, and consequently their preparation, stability and biopharmaceutics are well understood [1–10]. The droplet size of triglyceride emulsions normally lies in the range

0169-409X/96/$32.00
SSDI 0169-409X(95)00116-6

200–400 nm, and consequently they are suitable for intravenous administration. The most significant drawback of emulsion delivery systems is that they cannot easily be adapted to the delivery of water-soluble drugs. Oil-soluble drugs can be dissolved in the oil phase of the emulsion, and surface-active drugs can be adsorbed to the emulsion surface; however the only viable approach to the delivery of a hydrophilic drug is to synthesize a hydrophobic prodrug, or analogue. This approach has been used, for example, to formulate emulsions containing hydrophobic mitomycin C derivatives [11].

The stability of colloidal formulations is paramount in their use as drug delivery systems. They must be sufficiently stable to be easily manufactured, sterilized (preferably by terminal autoclaving) and have a shelf life of at least a year, preferably more. Shelf life considerations are particularly important for emulsion systems, since these are generally stored as liquids, and coalescence or aggregation can occur during storage. In contrast, microparticle colloidal systems are stored as freeze-dried solids for reconstitution, and colloid stability problems do not arise in the same way (although that is not to say that such formulations do not degrade physically during storage). There have been limited attempts to store emulsions in other forms, for example, the perfluorocarbon emulsion Fluosol [12–14] is stored frozen (a process which generally causes extensive emulsion coalescence in other systems), and there have been attempts to freeze dry emulsions.

In addition to long-term stability, it is also necessary to understand the way that colloid stability may be influenced by the various additives that may be needed in the final clinical application. The most important such interaction is that with electrolytes, which may be required to adjust tonicity. Electrolytes interact strongly with charge-stabilized colloids, causing severe stability difficulties; for this reason, the tonicity of emulsions is generally adjusted with glycerol. The interaction of electrolytes with phospholipid-stabilized emulsions is quite well understood, since it has been extensively studied due to its importance in the compounding of parenteral nutrition mixtures [15–19].

Further complications may occur if the drug influences the stability of the emulsion. Uncharged hydrophobic species may dissolve in the emulsion oil phase without changing the droplet surface; however, if the drug contains a charged site, it may interact strongly with the emulsifier film, as well as being preferentially located at the interface. We will discuss the formulation of such a drug, s-Emopamil, in Section 8.

2. Physical destabilization processes in emulsions

We can identify two separate destabilization processes in emulsions. These are *flocculation*, in which the emulsion droplets form adherent masses, but retain their individual integrity, and *coalescence*, in which the interface between droplets is lost and they form single, larger droplets. An important distinction is that flocculation is often reversible, while coalescence is not (Fig. 1). These processes are not necessarily exclusive; flocculated systems may undergo coalescence and unflocculated systems may also coalesce. It may be expected that a flocculated system would undergo coalescence more easily than an unflocculated one; in fact, this point has been the subject of some discussion and has not been proven.

These two processes can usually be distinguished visually, since flocculation leads to the formation of a cream layer on the emulsion, while coalescence leads to the formation of large oil droplets or a layer of free oil. The rates at which these processes occur have however misled

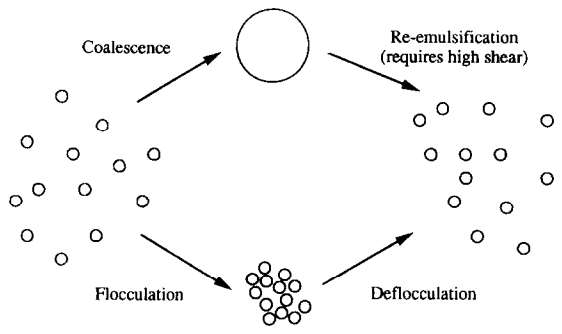

Fig. 1. Flocculation and coalescence in colloidal dispersions.

many workers, particularly in the study of emulsions in parenteral nutrition mixtures, where one often finds descriptions such as "mixture x was stable for 12 h but flocculated after 24 h". In fact, the formation of flocs is often close to diffusion-controlled, and occurs in seconds, as was shown by thin-film microscopic studies [7]. The equilibrium size of the flocs may, however, be such that it takes several hours for them to rise to the top of the container, giving the misleading impression that the formulation was stable for a limited period.

3. Stabilization of emulsions by phospholipids

The phospholipids, or lecithins, used in the preparation of most parenteral emulsions are quite heterogenous materials, being mixtures of phosphatides (Fig. 2). A number of different factors affect the physical behaviour of these materials and these have been reviewed by Cevc and Marsh [20]:

i) The saturation of the acyl chains influences the transition temperatures and the surface behaviour, both in bilayers and in monolayers. Generally, disaturated lipids have transition temperatures above room temperature (e.g. 42°C for dipalmitoyl phosphatidylcholine, DPPC), and introducing unsaturated bonds causes a reduction in transition temperature (e.g. −8°C for dioleyl phosphatidylcholine, DOPC). These temperatures apply to the melting of hydrated bilayers and similar phenomena are seen in lipid monolayers, which undergo solid–liquid type isotherm transitions at ambient temperatures. The extension of these phenomena to the stability of emulsions stabilized by solid- or liquid-phase phospholipids is unclear; anecdotal evidence suggests that saturated phospholipids produce very stable emulsions. The majority of phospholipids from egg or soya lecithin, the commonest sources, are unsaturated, and these materials are widely used as emulsifiers for intravenous use. The degree of unsaturation is normally expressed as an iodine number; 80 is typical for egg lecithin.

ii) The length of the acyl chains influences the transition temperature, with longer chains causing higher melting temperatures (e.g. DPPC, 42°C; Distearoyl, PC 56°C). The majority of natural lecithins have chain lengths from fourteen to twenty carbons, with sixteen to eighteen being predominant.

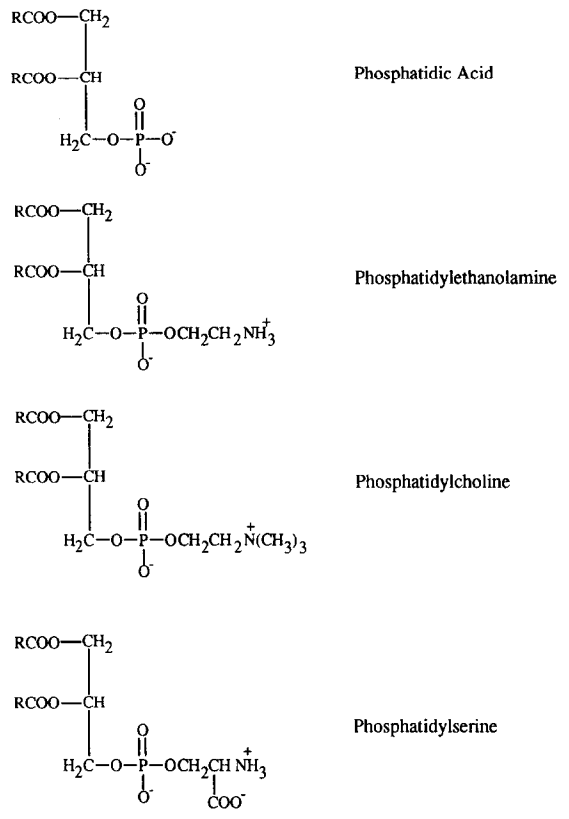

Fig. 2. Phospholipid structures; R is an alkyl chain from 8–24 units long.

iii) The nature of the headgroup is important in determining the ionization behaviour of the lipid. The predominant headgroups are choline and ethanolamine, which produce phosphatides (PC and PE) that are neutral at pH 7. Serine and glycerol produce acidic lipids (PS and PG), which are negatively charged at pH 7, as is the unesterified phosphatidic acid, PA. These acidic lipids are present to a much lower extent than PC and PE in natural egg and soy lecithins, and typically comprise 1–2% of the total bulk. Despite this, they are of major importance in determining the stability of the emulsions prepared from them. Their concentration is suffi-

cient to confer a surface charge of −40 to −60 mV on the emulsion droplets, which results in a long-term stability of several years [15,16,21].

Because of the extensive interest in phospholipids as emulsifiers, and for use as liposomes for drug delivery, a wide range of lecithins is commercially available. These are produced in a number of ways; crude egg or soy lecithin can be refined chromatographically, removing sterols, lysolipids and a range of other contaminants, to produce a parenteral grade of lecithin, such as Lipoid® E80 (Lipoid® AG) or Ovothin® 180 (Lucas-Meyer). These materials are almost fully unsaturated and contain approximately 80% PC: a small amount of acidic lipid leads to a high surface charge at pH 7, and hence, to excellent emulsion stability. Further chromatographic purification leads to a product which is 95–100% pure PC or PE (e.g. Lipoid® E100 or Ovothin® 200), and has very little acidic impurity; this has a low surface charge at pH 7 and thus is not per se a useful emulsifier.

To produce saturated lipids, these materials can be hydrogenated catalytically, which leads to an iodine number of 2–5, compared to 60–80 for the unsaturated lipid. Thus, Lipoid® E100-3 is a nearly pure hydrogenated PC, with a very low level of charged lipids, and Lipoid® E80-3 is a similarly hydrogenated lipid, with a higher level of acidic lipids.

These materials still have heterogenous chain lengths and some variations in headgroups. Pure individual lipids with well-defined chains cannot easily be produced by further fractionation, due to the large number of separate species in the raw feedstock. There are two possible routes to pure phospholipids; one is total synthesis and the other is the use of phospholipases to selectively transesterify chains. Phospholipase A1 and A2 can be used to selectively cleave and replace the acyl chains, while phospholipase D can modify the phosphate ester group. This process is, however, expensive, and the resulting pure lipids cost £20–80 per gram, making them of limited value for the preparation of large volume parenterals. However, they are commercially viable for the preparation of high added value drug emulsions, which normally have a small volume.

4. Theory of emulsion stability

The stability of colloids depends on the forces between them; if these are repulsive, approaching droplets repel and form a stable system. If they are attractive, the droplets clump together and are unstable. The forces themselves can arise from a number of sources [22]:

i) Van der Waals forces. These are attractive forces which exist between all particles. Their magnitude is determined by a factor called the Hamaker constant, which describes the strength of attractive interaction between the disperse and continuous phases.

ii) Electrostatic forces. These are repulsive forces (assuming that all the particles have the same surface charge). They can most easily be quantified using the zeta potential, which is the potential surrounding the droplet at the plane of hydrodynamic shear. The repulsive force depends on the square of the zeta potential (i.e. its sign is irrelevant). Electrolytes modify the zeta potential by adsorbing to the droplet surface and screening the droplet charges.

iii) Solvent forces. These are short-range repulsive forces caused by solvent–surface interactions, and are normally only important at short droplet separations of a few nanometres (i.e. the droplets are nearly "in contact").

iv) Steric forces. These are much less well understood than the previous forces, but are of major importance in many colloidal systems [23]. They arise when long-chain hydrophilic macromolecules are adsorbed or grafted to the colloid surface. They are repulsive in nature, since the overlap of adsorbed macromolecules is energetically unfavourable.

In general, colloids are stabilized either by steric or electrostatic repulsion, depending on the nature of the surfactant. Lecithin-stabilized emulsions are charge-stabilized, and steric forces do not play a significant role [24]. Alternately, emulsions stabilized by block copolymers, such as poloxamers, are sterically stabilized, and charge is of minor importance. Mixed electrosteric systems have also been studied; for example, the perfluorocarbon emulsion, Fluosol, uses a mixture of Pluronic F-68 and lecithin as the emulsifier [25].

5. Electrolyte stability

Although most lecithin-stabilized emulsions have sufficient surface charge to produce excellent long-term stability, they interact strongly with electrolytes, a phenomenon which has considerable clinical significance. The processes occurring are well-understood and are termed the DLVO theory of colloid stability, after its developers [26,27]. Electrolytes interact with charged colloids by two mechanisms; these are termed *nonspecific adsorption* and *specific adsorption*.

Nonspecific adsorption occurs when an ion is attracted to a surface solely due to electrostatic forces. There is no chemical interaction between the ion and the surface. Such an ion is sometimes called an *indifferent* ion for the surface. In such a case, a plot of the zeta potential as a function of electrolyte concentration looks like Fig. 3a. The zeta potential falls as the electrolyte concentration is increased, and asymptotes to zero at high electrolyte concentrations. This behaviour is typical of monovalent cations (e.g. Na^+, K^+) on phospholipid surfaces. As the zeta potential falls, the repulsive force stabilizing the colloid becomes weaker, until at a critical point it is no longer able to overcome the attractive Van der Waals force. At this point the force becomes predominantly attractive, and the colloid flocculates. The concentration of electrolyte required to achieve this is termed the *Critical Flocculation Concentration* or CFC. Further addition of electrolyte causes the repulsive force to decline further, so the flocculation rate increases to a maximum.

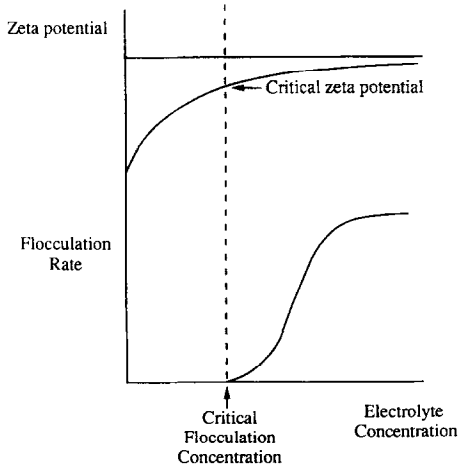

Fig. 3. Zeta potential and flocculation (nonspecific adsorption).

Typical data showing this behaviour in phospholipid stabilized emulsions is shown in Fig. 4 for the flocculation of Intralipid by sodium chloride [7]. The CFC is approximately 0.10 M. This description of the flocculation process is qualitative, but has been put on a quantitative basis [24,28]; The actual interdroplet potentials can be calculated and from them, the flocculation rates. The agreement of the calculated and measured flocculation rates is quite good (Fig. 5).

Specific adsorption occurs when an ion interacts with the surface chemically as well as electrostatically. Examples of this process on phospholipid surfaces are Ca^{2+} (which complexes to the lipid phosphate headgroups) and H^+ (which is involved in acid–base equilibria) [29]. Under these conditions, more ion can adsorb to the surface than is required simply to neutralize the droplet surface charge, and so the surface can acquire a charge of the opposite sign to that which it possessed in the absence of the ion (Fig. 6). This process is called *charge reversal* and is characteristic of the specific adsorption process. Fig. 7 shows the effect of increasing concentrations of Ca^{2+} on the zeta potential and flocculation rate of Intralipid. Initially the charge is neutralized in a similar manner to that observed for a nonspecfically adsorbed ion, and a CFC is seen at a calcium ion concentration of 2 mM. Further addition of calcium causes all the surface charge to be neutralized at 3–4 mM, and this point is called the *point of zero charge*, or PZC. At still higher calcium concentrations, the zeta potential becomes positive.

These changes in the surface potential are reflected in the flocculation rate. The CFC is observed in a manner similar to a nonspecifically adsorbing ion; however, the PZC now corresponds to a well-defined maximum in the flocculation rate. As the surface becomes charge-reversed, it regains some repulsive component to the interdroplet force and as a result, the flocculation rate falls.

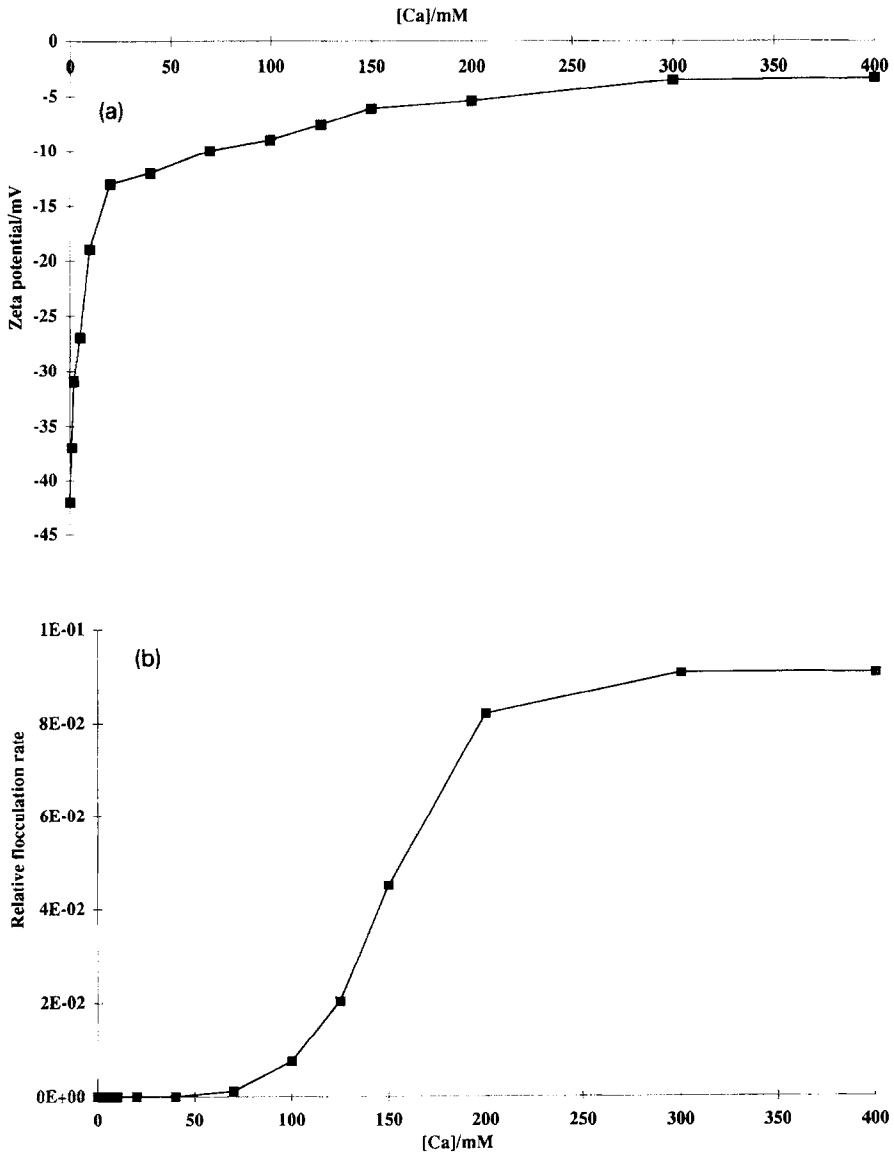

Fig. 4. (a) Zeta potential and (b) relative flocculation rate of Intralipid in sodium chloride solutions.

6. Stability measurement

We have already discussed the existence of two distinct breakdown processes in emulsions; flocculation and coalescence. It is similarly necessary to distinguish two corresponding measurements of stability, which are coalescence rate and flocculation rate. It is important to understand which process is being measured in a particular experiment, much of the literature being confused in this respect.

Flocculation rate. Probably the easiest way to study the flocculation of colloids is by turbidimetry. Although it is possible to relate the turbidity to the absolute rate of aggregation, this is not normally performed, and relative flocculation rates are often quoted simply as the rate of change of absorbance. To perform a turbidimet-

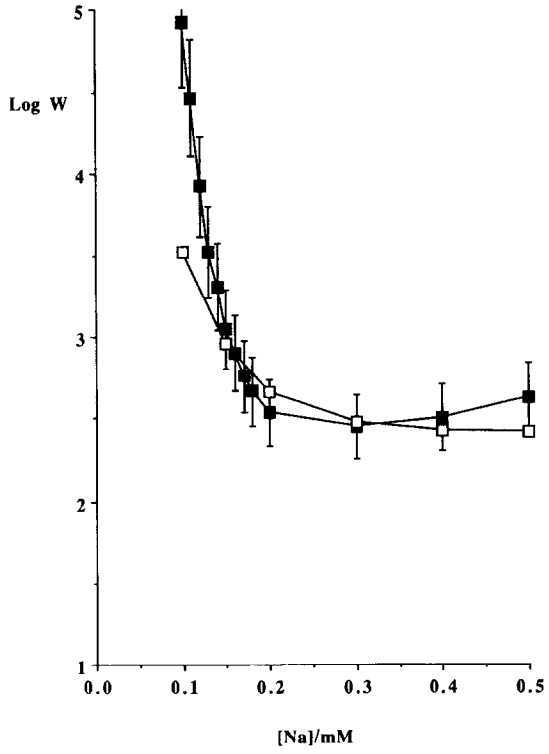

Fig. 5. Measured ■ and calculated □ stability ratios for a triglyceride emulsion in sodium chloride solutions.

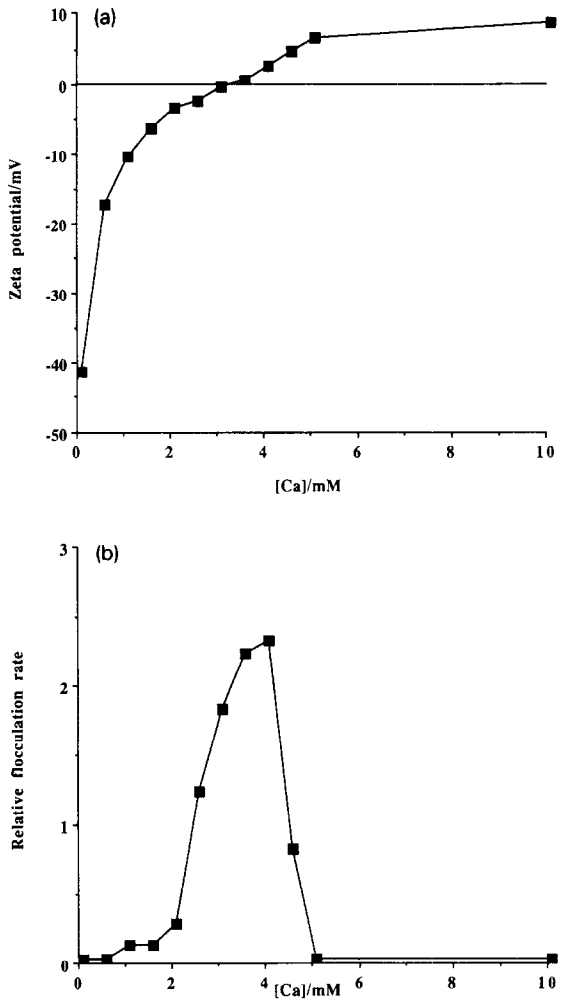

Fig. 7. (a) Zeta potential and (b) relative flocculation rate of Intralipid in calcium chloride solutions.

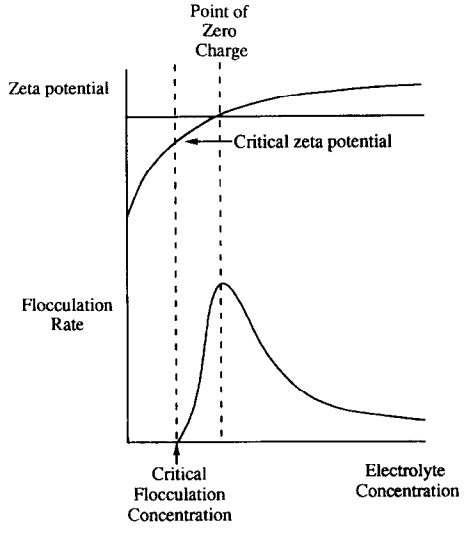

Fig. 6. Zeta potential and flocculation (specific adsorption).

ric experiment, the emulsion must be diluted considerably in order to allow light to pass through it. Measuring flocculation in more concentrated systems is also possible and a number of methods have been used. Flocculation in thin films can be observed microscopically, and quantified by image analysis. A simple variant of this method, called rheoscopy, uses a sample of emulsion between two microscope slides [30]. The sample is sheared, and the flocs disrupted, by moving the top slide, and the resultant kinetic changes in transmission can be measured with a photodetector.

Measurements of cream behaviour are a crude indicator of the presence or absence of flocculation, but it is difficult to relate parameters such as creaming time or cream depth to flocculation rate, because the cream formation is controlled by Stokes' equation. Microscopic studies show that many emulsions flocculate in a few seconds [15]; in contrast, it may take minutes or hours for a cream to form, since the rate at which the flocs rise through the system depends on their size and the local viscosity.

Coalescence rate. Measurements of coalescence are normally performed by particle size analysis. In order to distinguish coalesced droplets from aggregates, it is essential to break up any aggregates prior to measurement. Most particle size analysers require a diluted sample, and this dilution in many cases causes flocs to break up, particularly if flocculation was due to a high electrolyte concentration in the original sample. In addition, it is possible to add a small amount of a dispersant such as Nonidet, Tween, or the more hydrophilic Pluronics. Powerful surfactants can solubilize the oil droplets and are not recommended. It is also unwise to use a sonic bath to disperse the sample, since this can cause major changes in the size distribution. For triglyceride–phospholipid emulsions, a typical protocol is to dilute the sample to a disperse phase volume of 0.1% in 0.1% Pluronic F68 and vortex-mix for 10–20 s. This sample can then be added to the size analyser, as required.

A high degree of sensitivity is valuable in studies of coalescence, due to the small numbers of droplets that are being counted. A typical fat emulsion has a droplet diameter of 0.25 μm, and it would be necessary to coalesce 1000 such droplets to make a 2.5 μm droplet. Thus, unless coalescence is very rapid or extensive, there will either be very few large droplets to count, or only very small changes in the shape of the size distribution.

A wide range of size analysers are available and many are discussed in standard works on the subject [31,32]. The commonest are the Coulter Counter and various forms of light diffraction instrument, such as the Malvern Mastersizer. These instruments both measure the size distribution of droplets larger than about half a micron, and so are sensitive to the larger droplets produced by coalescence. Of the two techniques, the Coulter Counter is preferable, since it counts individual droplets, and is thus more sensitive than diffraction methods [33]. Most diffraction instruments also only report the relative size distribution and cannot easily estimate the absolute amount of material in the sample; thus they can only detect coalescence by virtue of changes in the shape of the size distribution.

Microscopy has been widely used for the study of coalescing emulsions, but is subject to severe statistical problems. The field of view of a microscope at 400 × may contain only a few nanolitres of emulsion, and only a few coalesced droplets may be visible. It is necessary to study many fields to gain accurate counting statistics, and accurate droplet sizing is difficult.

Photon correlation spectroscopy (PCS) can be used for stability measurement, but has a number of disadvantages. Careful technique is needed to measure size distributions from PCS, and if coalescence has caused a few large (>5 μm or so) droplets to form, these may interfere with the measurement, since they will scatter enormous amounts of light on an unpredictable basis. In fact, one of the first indications by PCS of emulsion instability is that the attempts to measure accurate particle sizes become erratic and unreproducible.

7. Drug-containing emulsions

The presence of an additional component in the emulsion can considerably complicate its behaviour. Fortunately, the effect of the drug can often be predicted (at least in terms of DLVO-type emulsion stability) with a knowledge of the physicochemical properties of the drug. For the purposes of emulsion formulation, we can classify drugs on the basis of their solubility characteristics and their ionization properties (Table 1) which, of course, are not completely independent.

Class I drugs are largely water-soluble. In an emulsion, a significant fraction of the drug would be present in the aqueous phase, and conse-

Table 1

Drug	Type	Reference
Amphotericin B	3c	Forster et al. [37]
Biphenylacetic acid esters	2c	Shoji et al. [38]
Bisantrene	1a	Desai et al. [39]
Clarithromycin	1a	Lovell et al. [40]
Clofibride	2c	Santos-Malaghaes [41]
Coenzyme Q10	2c	Kimura et al. [42]
Cyclosporin A	2c	Yanagawa et al. [43]
Dexamethasone palmitate	2c	Mizushima et al. [44]
Diazepam	2a	Von Dardel et al. [45]
Diisopropylphenol	2c	Glen and Hunter [46]
Emopamil	2a	Washington et al. This work
Forskolin	2c	Yamamura et al. [47]
Hexamethylmelamine	2a	Gordon et al. [48]
Lipo-mitomycin C	2c	Sasaki et al. [11]
Lipo-podophyllotoxins	2c	Lundberg [49]
Nitrosoureas	2c	Davignon et al. [50]
Penclomedine	2c	Prankerd et al. [51]
Perilla ketone	2c	Paborji et al. [52]
Pregnanolone	2c	Hogskilde et al. [53]
Prostaglandin A1	2c	Mizushima [54]
Prostaglandin E1	2b	Toyota et al. [55]
Taxol	??	Wheeler et al. [56]

quently such drugs are not good candidates for emulsion formulations.

Class II drugs are predominantly oil-soluble, and in an emulsion would be partitioned predominantly into the oil phase. Such materials are good candidates for emulsion delivery and could normally be formulated by dissolving the drug in the oil prior to emulsification.

Class III drugs are poorly soluble in both water and oil, and can only be loaded into an emulsion by adsorbing to the droplet interface. They may require complex formulation methods due to their unusual solubility characteristics, and it is often necessary to post-load emulsions with them, in order to have a large surface area available for loading.

This is naturally an artificial classification, and boundaries between these areas are indistinct, although many drugs do fall clearly into these classes. The classification of a drug can be changed by chemical means; for example, a hydrophobic (class II) base such as chlorpromazine can be converted into its class I hydrochloride salt. Formulation chemists often do this to increase the water solubility of a drug; emulsion chemists normally want to reverse this process! The boundary between classes I and II cannot be assigned to a particular log P value, although we would suggest that log $P > 4$ would be a useful starting point. A particular problem can arise if the drug has a very slight water solubility and a high oil solubility, since partition can cause the water solubility to be exceeded and the drug will crystallize out into the aqueous phase. The oil phase will dump its drug load as crystals in the aqueous phase until the aqueous phase solubility is not exceeded.

The class II–III boundary is also often indistinct, since most drugs have some surface activity due to polar or ionized groups, whether strongly oil-soluble or not. Consequently we can usefully subclassify drugs into types a, b and c.

Type a drugs are weak bases and thus are positivcly charged at the formulation pH.

Type b drugs are weak acids and are negatively charged at the formulation pH

Type c drugs are uncharged at the formulation pH.

The charge on type a and b drugs leads to surface activity, and so these drugs will normally adsorb, at least partly, to the droplet interface. In doing so, they will contribute some surface charge, which will influence the emulsion stability. Type c drugs may also adsorb to the interface (if, for example, they contain hydroxyl groups) but will not normally influence surface charge. Of course this classification is only valid at a particular or specified pH, which suggests that pH is a major tool to control this aspect of drug behaviour.

We can now consider the effect of these drugs on emulsion stability. Fig. 8 shows the zeta potential vs. pH profile of a triglyceride emulsion stabilized using a typical parenteral phospholipid, such as Intralipid. Phospholipids such as Ovothin® 180 or Lipoid® E80 form similar emulsions, which have a zeta potential of -40 to -50 mV, at pH 7. The surface charge is progressively neutralized at lower pH and declines to zero at pH 3–3.5. Only a little additional charge can be gained by making the system alkaline, since the phospholipid is already quite strongly ionized at pH 7. If we add a type a or b drug to this emulsion, the curve will be shifted in

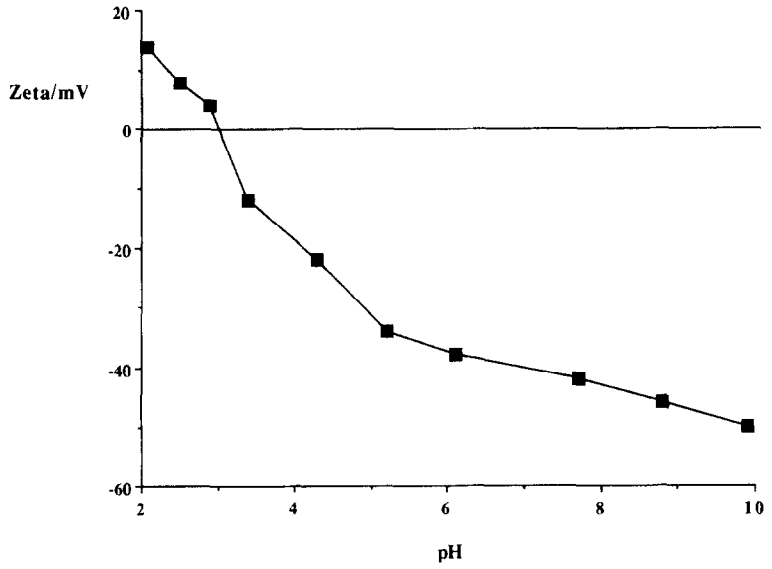

Fig. 8. Zeta potential of Intralipid as a function of pH.

either a positive or negative direction, respectively, over the pH range in which the drug is fully ionized. As we approach the pK of the drug, it will cease to be ionized and then will not contribute surface charge, so the curves converge at these points (effectively the drug changes from class a or b to class c near its pK). This behaviour is illustrated in Fig. 9. The stability of the emulsion as a function of pH can now be predicted from the zeta potential. Adding an acidic type b drug only makes the charge more negative, and so the emulsion stability is not affected, or even becomes more stable. However, a type a drug will contribute a positive charge to the interface, and this will reduce the overall surface charge and the emulsion will become unstable. At sufficiently high pH, it may be possible to restabilize the emulsion using this positive charge, but, depending on the pK of the drug, the pH may then be physiologically unacceptable. We should also note that at extreme pH, the ionized form of the drug may be sufficiently water-soluble to partition extensively into the aqueous phase (i.e. it becomes a class I drug). The ion produced may then back-adsorb to the droplet, leading to rather complex behaviour. This situation has been studied by Lostritto et al. [34] and Silvestri et al. [35].

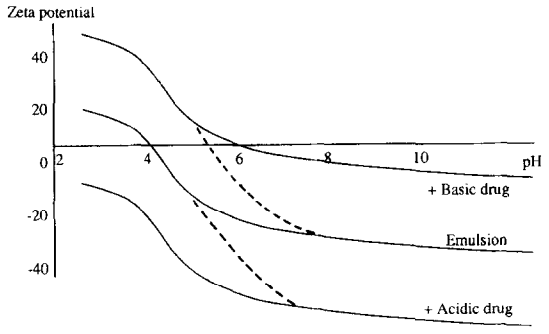

Fig. 9. Hypothetical zeta potential vs. pH for emulsions containing acidic or basic drugs. The dashed lines indicate the transition between ionized and neutral forms of a drug with pK 6.0.

In order to formulate basic drugs, it is necessary to use a different phospholipid emulsifier. In principle, it is possible to increase the level of negatively charged phospholipid to counteract the effect of the drug; however, this is not a particularly elegant or successful approach if the drug contributes a lot of positive charge. It also involves the use of expensive speciality phospholipids. An alternative is to use a slightly purer grade of lecithin, such as Ovothin® 200 or Lipoid® E100, which has very little surface charge at pH 7 (Fig. 10). In the absence of a charging drug species, such an emulsion is nor-

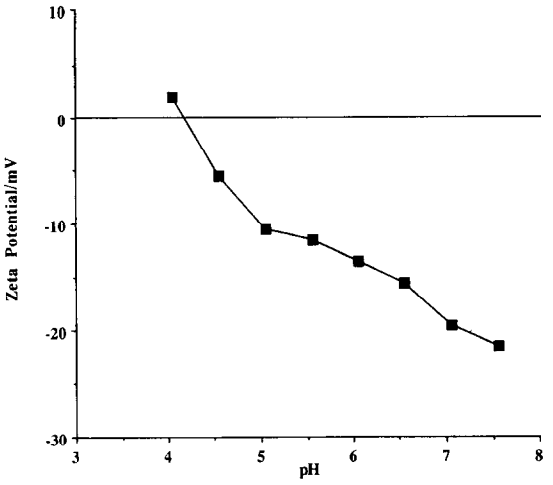

Fig. 10. Zeta potential of an Ovothin 200 stabilized triglyceride emulsion as a function of pH.

mally flocculated, since it has a zeta potential of only −5 to −10 mV. Consequently, the majority of the charge on the emulsion is contributed by the drug, which will then be positive for a type a drug and negative for a type b drug.

It is unfortunate that detailed data on emulsion stability is rarely reported in the literature. Table 1 summarizes some of the existing formulations of drug emulsions according to this classification (at pH 7, unless stated), but in many cases the stability of these emulsions is not precisely known. It is evident that many workers have chosen to adopt emulsion formulation only for neutral oil-soluble drugs, and that the possibilities of this type of formulation have not been widely explored for surface-active or charged drugs.

8. s-Emopamil

The rational design of emulsion formulations is well illustrated by recent studies performed in this laboratory on S-emopamil (Fig. 11), a hydrophobic analogue of Verapamil [36]. Preformulation studies indicated that the free base form, which was a viscous liquid at room temperature, had a pK_a of 8.85, and an octanol–water log P of 5.32 (free base). Consequently, at neutral pH it would be predominantly positively charged. It is also completely miscible with triglyceride oils, and so can be formulated by dissolving in soya oil prior to homogenization in the aqueous lecithin dispersion (1.2%).

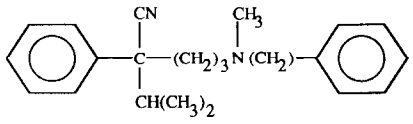

Fig. 11. Structure of Emopamil.

Fig. 12 shows the zeta potential/pH curves of emulsions containing varying amounts of emopamil in 10% soya triglycerides, using a conventional parenteral lecithin emulsifier (Ovothin® 180). The curves are significantly more positive than those for the drug-free emulsion, due to the charge contributed by the ionization of the drug. In fact, this charge is sufficient to completely neutralise the charge induced by the acidic phospholipids at pH 7, so that the zeta potential is very small, and the emulsion flocculates rapidly if formulated at this pH. By taking advantage of the positive charge, it is possible to formulate a more stable system at pH 4–5; in this region the pH is 30–50 mV, sufficient to produce an emulsion with good long-term stability.

A similar set of data using the emulsifier Ovothin® 200 is shown in Fig. 13. This emulsifier produces an emulsion which, in the absence of drugs, has a zeta potential of −10 to −20 mV at pH 7, which is not sufficient to stabilize it, and so it flocculates rapidly. Incorporation of emopamil into this emulsion results in a larger positive zeta potential; in this case the charge generated by the ionization of emopamil at pH 5–6 is sufficient to stabilize the emulsion. Long-term stability experiments on this emulsion indicated that it was stable for two years at room temperature, a performance at least as good as that of conventional negatively charged triglyceride parenteral nutrition emulsions.

The proper selection of pH is thus critical in this type of formulation. If the pH is too high (or low for an acidic drug), the drug will not be ionized, and will not contribute to the zeta potential. In this case, a conventional acidic lecithin emulsifier could be used. In the case of emopamil, the pH would have to be raised to 9–10 to fully suppress drug ionization, and this

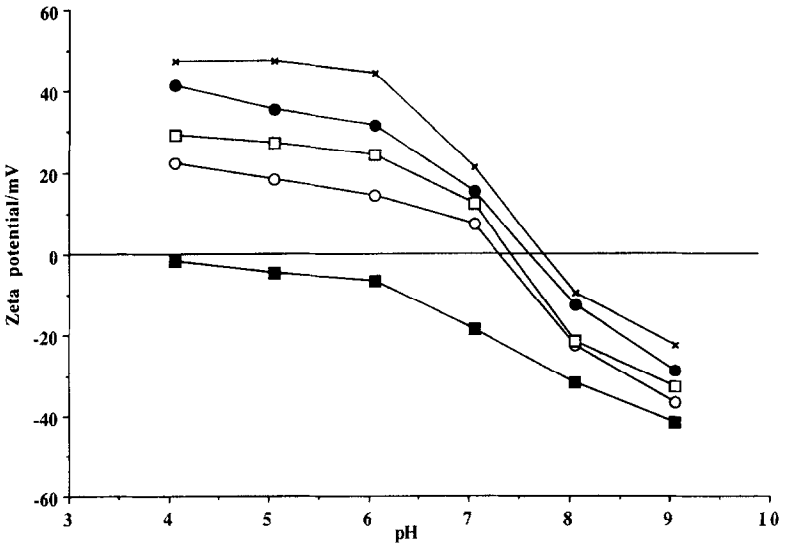

Fig. 12. Zeta potential/pH curves for Emopamil-containing emulsions stabilized by Ovothin 180. ■, 0% Emopamil; ○, 0.2%; □, 0.5%; ●, 1%; ×, 2%.

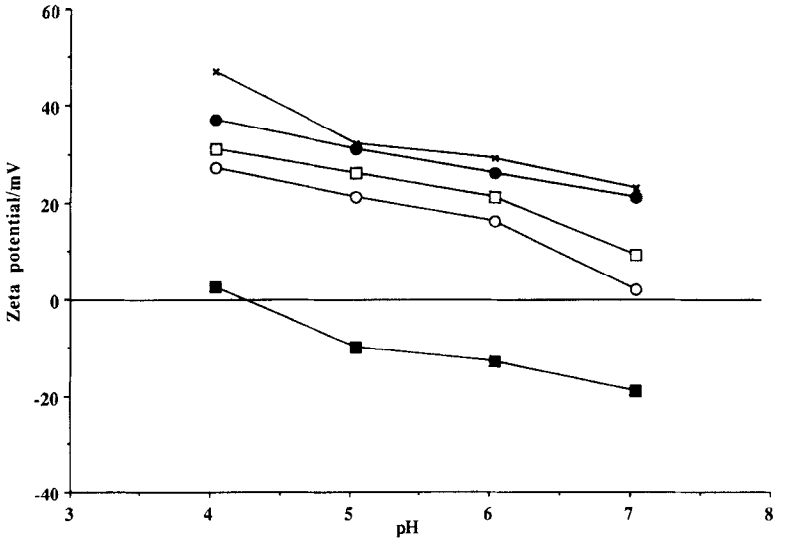

Fig. 13. Zeta potential/pH curves for Emopamil-containing emulsions stabilized by Ovothin 200. ■, 0% Emopamil; ○, 0.2%; □, 0.5%; ●, 1%; ×, 2%.

would not form an acceptable injectable system. Alternately, if the pH was too low, the drug would fully ionize, and this may encourage its dissolution, or micelle formation, in the aqueous medium. This behaviour can be seen in Fig. 14, in which the emopamil content has been assayed in both the oil and aqueous phases as a function of pH. At pH 6–7 the drug is present largely in the oil phase, but as the pH is reduced the ionization of the drug drives it into the aqueous phase and so the "entrapment efficiency" is severely reduced.

This loss, or redistribution, of drug can cause some significant problems when measuring zeta

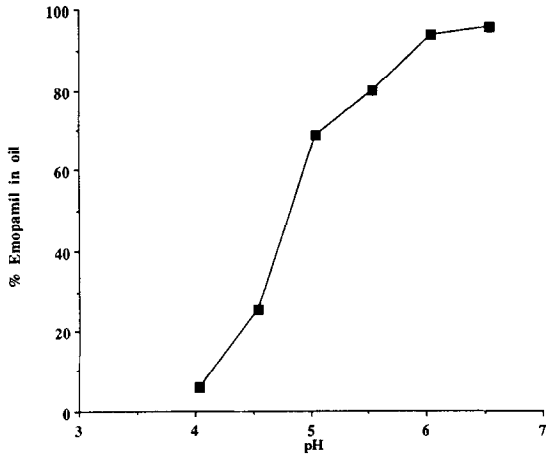

Fig. 14. Distribution of emopamil in emulsion as a function of pH.

potentials. It should first be realized that a positively charged emulsion droplet will adsorb polyvalent anions specifically, so buffers should be selected with care; acetate buffers were used in the present studies, although low dilutions of phosphate buffers did not appear to cause major systematic errors. Normally for zeta potential measurement, it is not necessary to rigorously control the dilution of the colloid, since its surface does not change significantly on dilution. However, in the case of emopamil, the partition of the partially ionized drug at the high dilution

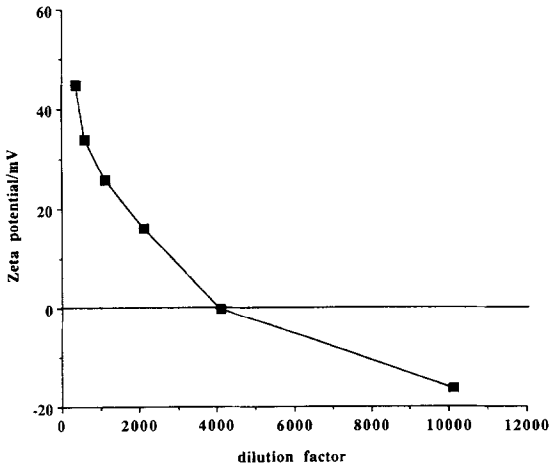

Fig. 15. Measured zeta potential of 2% emopamil in 20% soya oil/Ovothin 200 emulsion as a function of sample dilution (pH 5).

of the electrophoresis experiment depleted the loading of the emulsion droplet, and the measured zeta potential decreased. This behaviour is illustrated in Fig. 15, which shows the decrease in measured zeta potential as the emulsion was diluted. It is possible to extrapolate this data to zero dilution to find the bulk zeta potential (assuming non-interacting droplets); also, one can calculate the drug–oil partition coefficient, although this will not be identical with the bulk partition coefficient, since it will be heavily influenced by surface adsorption and ionization effects.

9. Conclusions

The formulation of drug emulsions has traditionally been regarded as an art rather than a science. We have demonstrated that, given sufficiently detailed studies, the behaviour of drug-containing emulsions can normally be understood or even predicted, leading to rational formulation design.

Acknowledgments

The author would like to thank Leopold Pharma, Graz, Austria, for permission to use the s-Emopamil data.

References

[1] Huang, D.X., Wu, Z.H. and Wu, Z.G. (1992) The all-in-one nutrient solution in parenteral nutrition. Clin. Nutrition. 11, 39–44.

[2] Bock, T.K., Lucks, J.S., Kleinebudde, P., Muller, R.H. and Muller, B.W. (1994) High-pressure homogenization of parenteral fat emulsions — influence of process parameters on emulsion quality. Eur. J. Pharmaceut. Biopharmaceut. 40, 157–160.

[3] Davis, S.S., Washington, C., West, P., Illum, L., Liversidge, G., Sternson L. and Kirsh, R. (1987) Lipid emulsions as drug delivery systems. Ann. NY Acad. Sci. 507, 75–88.

[4] Driscoll, D.F., Bhargava, H.N., Li, L., Zaim, R.H., Babayan, V.K. and Bistrian, B.R. (1995) Physicochemical stability of total nutrient admixtures. Am. J. Hosp. Pharm. 52, 623–634.

[5] Mehta, R.C., Head, L.F., Hazrati, A.M., Parr, M., Rapp R.P. and Deluca, P.P. (1992) Fat emulsion particle-size distribution in total nutrient admixtures. Am. J. Hosp. Pharm. 49, 2749–2755.
[6] Washington, C. and Davis, S.S. (1988) The production of parenteral-feeding emulsions by microfluidizer. Int. J. Pharmaceut. 44, 169–176.
[7] Washington, C. (1990) The stability of intravenous fat emulsions in total parenteral-nutrition mixtures. Int. J. Pharmaceut. 66, 1–21.
[8] Washington, C. (1992) The electrokinetic properties of phospholipid stabilized fat emulsions .6. Zeta potentials of Intralipid 20% in TPN mixtures. Int. J. Pharmaceut. 87, 167–174.
[9] Westesen, K. and Wehler, T. (1993) Particle-size determination of a submicron-sized emulsion. Colloids and Surfaces A — Physicochemical and Engineering Aspects 78, 125–132.
[10] Westesen, K. and Wehler, T. (1993) Characterization of a submicron-sized oil-in-water emulsion. Colloids and Surfaces A — Physicochemical and Engineering Aspects 78, 115–123.
[11] Sasaki, H., Takakura, Y., Hashida, M., Kimura, T. and Sezaki, H. (1984) Antitumour activity of lipophilic prodrugs of mitomycin C entrapped in liposome or O/W emulsion. J. Pharmacodynam. 7, 120–130.
[12] Carr, M. (1984) Status of Fluosol-DA 20%. J. Am. Med. Assoc. 251, 2928–2928.
[13] Riess, J.G. (1992) Fluorocarbon emulsions as injectable oxygen carriers — recent progress and prospects. Revue Francaise de Transfusion et de Hemobiologie 35, 391–406.
[14] Spence, R.K., Norcross, E.D., Costabile, J., McCoy, S., Cernaianu, A.C., Alexander, J.B., Pello, J., Atabek, U. and Camishion, R.C. (1994) Perfluorocarbons as blood substitutes — early years — experience with fluosol DA-20-percent in the 1980s. Artificial Cells Blood Substitutes and Immobilization Biotechnology 22, 955–963.
[15] Washington, C., Chawla, A., Christy, N. and Davis, S.S. (1989) The electrokinetic properties of phospholipid-stabilized fat emulsions. Int. J. Pharmaceut. 54, 191–197.
[16] Washington, C. (1990) The electrokinetic properties of phospholipid-stabilized fat emulsions .2. droplet mobility in mixed electrolytes. Int. J. Pharmaceut. 58, 13–17.
[17] Washington, C. (1990) The electrokinetic properties of phospholipid-stabilized fat emulsions —.3. interdroplet potentials and stability ratios in monovalent electrolytes. Int. J. Pharmaceut. 64, 67–73.
[18] Washington, C., Athersuch, A. and Kynoch, D.J. (1990) The electrokinetic properties of phospholipid stabilized fat emulsions .4. the effect of glucose and of pH. Int. J. Pharmaceut. 64, 217–222.
[19] Washington, C., Connolly, M.A., Manning, R. and Skerratt, M.C.L. (1991) The electrokinetic properties of phospholipid stabilized fat emulsions .5. the effect of amino-acids on emulsion stability. Int. J. Pharmaceut. 77, 57–63.
[20] Cevc, G. and Marsh, D. (1984) Phospholipid Bilayers. Physical Principles and Models, J. Wiley, New York.
[21] Rubino, J.T. (1990) The influence of charged lipids on the flocculation and coalescence of oil-in-water emulsions. 1. Kinetic assessment of emulsion stability. J. Parenteral Sci. Technol. 44, 210–215.
[22] Shaw, D.J. (1980) Introduction to Colloid and Surface Chemistry, Butterworths, London.
[23] Napper, D.H. (1983) Polymeric Stabilization of Colloidal Dispersions, Academic Press, London.
[24] Gamon, B.L., Virden, J.W. and Berg, J.G. (1989) The aggregation kinetics of an electrostatically stabilized dipalmitoyl phosphatidylcholine system. J. Coll. Int. Sci. 132, 125–137.
[25] Riess, J.G. and LeBlanc, M. (1988) Preparation of perfluorochemical emulsions for biomedical use: principles, materials and methods. Blood Substitutes, Ellis Horwood, Chichester, pp. 94–129.
[26] Deryaguin, B.V. (1940) Repulsive forces between charged colloidal particles and the theory of slow coagulation and stability of lyophobic sols. Trans. Faraday Soc. 36, 203–215.
[27] Verwey, E.J.W. and Overbeek, J.T.G. (1948) Theory of Stability of Lyophobic Colloids, Elsevier, Amsterdam.
[28] Washington, C., Ferguson, J.A. and Irwin, S.E. (1993) Computational prediction of stability of lipid emulsions in total nutrient admixtures. J. Pharm. Sci. 82, 808–812.
[29] Macdonald, P.M. and Seelig, J. (1987) Calcium binding to mixed PG–PC bilayers as studied by deuterium nuclear magnetic resonance. Biochemistry. 26, 1231–1240.
[30] Hansrani, P.K. (1980) Ph.D Thesis, Nottingham, UK.
[31] Allen, T. (1990) Particle Size Measurement, Chapman and Hall, London.
[32] Washington, C. (1990) Particle Size Analysis in Pharmaceutics and Other Industries, Ellis Horwood, Chichester.
[33] Washington, C. and Sizer, T. (1992) Stability of TPN mixtures compounded from Lipofundin-s and Aminoplex amino-acid solutions — comparison of laser diffraction and coulter-counter droplet size analysis. Int. J. Pharmaceut. 83, 227–231.
[34] Lostritto, R.T., Goei, L. and Silvestri, S.L. (1987) Theoretical considerations of drug release from submicron oil in water emulsions. J. Parenteral. Sci. Technol. 41, 214–224.
[35] Silvestri, S., Wu, L.L. and Bowser, B. (1992) Release of polyionizable compounds from submicrometer oil-in-water emulsions. J. Pharm. Sci. 81, 413–418.
[36] Hofmann, H.P., Raschack, M. and Unger, L. (1989) S-emopamil, a novel calcium and serotonin antagonist for the treatment of cerebrovascular disorders. Drug Res. 39, 304–308.
[37] Forster, D., Washington, C. and Davis, S.S. (1988) Toxicity of solubilized and colloidal amphotericin-B formulations to human erythrocytes. J. Pharm. Pharmacol. 40, 325–328.
[38] Shoji, Y., Mizushima, Y., Yanagawa, A., Shiba, T. and Takei, H. (1986) Enhancement of anti-inflammatory effect of biphenylacetic acid by its incorporation into lipid microspheres. J. Pharm. Pharmacol. 38, 118–121.

[39] Desai, N.R., Shinal, E.C., Ganesan, M. and Carpentier, E.A. (1989) Emulsion compositions for administration of sparingly soluble ionizable hydrophobic drugs. U.S patent 4 816 247, American Cyanamid Co.

[40] Lovell, M.W., Johnson, H.W., Hui, H.W., Cannon, J.B., Gupta, P.K. and Hsu, C.C. (1994) Less painful emulsion formulations for intravenous administration of clarithromycin. Int. J. Pharmaceut. 109, 45–57.

[41] Santos-Malaghaes, N.S., Cave, G., Seiller, M. and Benita, S. (1991) The stability and in vitro release kinetics of a clofibride emulsion. Int. J. Pharmaceut. 76, 225–237.

[42] Kimura, A., Yamaguchi, H., Watanabe, K., Hayashi, M. and Awazu, S. (1986) Factors affecting the tissue distribution of coenzyme Q10 intravenously administered in an emulsion to rats: emulsifying agents and lipoprotein lipase activity. J. Pharm. Pharmacol. 38, 659–662.

[43] Yanagawa, A., Iwayama, T., Saotome, T., Shoji, J., Takano, K., Oka, H. and Nakagawa, T. (1989) Selective transfer of cyclosporin to thoracic lymphatic systems by the application of lipid microspheres. J. Microencapsulation 6, 161–164.

[44] Mizushima, Y., Hamano, T. and Yokoyama, K. (1982) Use of a lipid emulsion as a novel carrier for corticosteroids. J. Pharm. Pharmacol. 34, 49–50.

[45] Von Dardel, O., Mebius, C., Mossberg, T. and Svensson, B. (1983) Fat emulsion as a vehicle for diazepam. A study of 9492 patients. Br. J. Anaesthesia 55, 41–47.

[46] Glen, J.B. and Hunter, S.C. (1984) Pharmacology of an emulsion formulation of ICI 35 868 (2,6 di-isopropylphenol). Br. J. Anaesthesia 56, 617–625.

[47] Yamamura, K., Nakao, M., Yano, K., Miyamoto, K. and Yotsuyanagi, T. (1991) Stability of forskolin in lipid emulsions and oil–water partition coefficients. Chem. Pharm. Bull. 39, 1032–1034.

[48] Gordon, I.L., Kar, R., Opfell, R.W. and Wile, A.G. (1987) Pharmacokinetics of hexamethylmelamine in Intralipid following hepatic regional administration in rabbits. Cancer Res. 47, 5070–5073.

[49] Lundberg, B. (1994) The solubilization of lipophilic derivatives of podophyllotoxins in submicron sized lipid emulsions and their cytotoxic activity against cancer-cells in culture. Int. J. Pharmaceut. 109, 73–81.

[50] Davignon, J.P., Yang, K.W., Wood, H.B. and Cradock, J.C. (1973) Formulation of three nitrosoureas for intravenous use. Cancer Chemother. Rep. 4, 7–11.

[51] Prankerd, R.J., Frank, S.G. and Stella, V. (1988) Preliminary development and evaluation of a parenteral formulation of penclomedine (NSC 338720; 3,5-dichloro-2,4-dimethoxy-6-trichloromethylpyridine): a novel, practically water insoluble cytotoxic agent. J. Parent. Sci. Technol. 42, 76–81.

[52] Paborji, M., Riley, C. and Stella, V. (1988) A novel use of Intralipid for the parenteral delivery of perilla ketone (NSC 348407), an investigational cytotoxic drug with a high affinity for plastic. Int. J. Pharmaceut. 42, 243–249.

[53] Hogskilde, S., Wagner, J., Carl, P. and Bredgaard Sorensen, M. (1987) Anaesthetic properties of pregnanolone emulsion. Anaesthesia 42, 1051–1056.

[54] Mizushima, Y. (1991) Lipo-Prostaglandin preparations. Prostaglandins, Leukotrienes and Essential Fatty Acids 42, 1–6.

[55] Toyota, T., Hirata, Y., Ikeda, Y., Matsuoka, K., Sakuma, K. and Mizushima, Y. (1993) Lipo-PGE_1, a new lipid-encapsulated preparation of prostaglandin E1: Placebo and prostaglandin E1-controlled controlled multicentre trials in patients with diabetic neuropathy and leg ulcers. Prostaglandins 46, 453–468.

[56] Wheeler, J.J., Wong, K.F., Ansell, S.M., Masin, D. and Bally, M.B. (1994) Polyethylene-glycol modified phospholipids stabilize emulsions prepared from triacylglycerol. J. Pharm. Sci. 83, 1558–1564.