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Physical properties and stability of two emulsion formulations of propofol

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Abstract

We have compared the physical properties of two commercial emulsion formulations of the intravenous anaesthetic propofol, (Diprivan®, AstraZeneca, and Propofol Intravenous Emulsion, Gensia Sicor Pharmaceuticals) which appear to differ primarily in the additive content and formulation pH. Diprivan® contains disodium edetate and has a pH of 7–8.5, while the Gensia product contains sodium metabisulphite and is formulated to a pH of 4.5–6.4. The average zeta potential of Diprivan® at pH 8 was −50 mV while that of the Gensia product at pH 4–5 was −40 mV. This information suggests that the physical stability of Propofol Intravenous Emulsion should be lower than that of Diprivan®. Three random batches of both products were subjected to a range of stability tests, including shaking, thermal cycling, and freeze–thaw cycling, and the emulsion droplet size distribution was then assessed by dynamic light scattering, light diffraction, and electrical and optical zone sensing. Both emulsions initially showed narrow submicrometre particle size distributions. An increased level of droplets larger than 5 μm could be detected in Propofol Intravenous Emulsion after as little as 4 h shaking (300 strokes/min at room temperature) and visible free oil could be detected after 8–12 h shaking. In contrast, Diprivan® showed no increase in the large droplet count after shaking for times up to 16 h. A similar difference in the emulsions was found after one freeze–thaw cycle, with Propofol Intravenous Emulsion exhibiting extensive coalescence, while that of Diprivan® was at the limits of detection. We conclude that these two products have different physical stability characteristics, and that this may in part be due to the reduced zeta potential in Propofol Intravenous Emulsion compared to that of Diprivan®. © 2001 Published by Elsevier Science B.V.

Keywords: Propofol; Diprivan®; Emulsion; Stability

1. Introduction

Propofol (2,6 di-isopropylphenol) is a widely used intravenous anaesthetic (Glen and Hunter, 1984; Cockshott et al., 1990, 1992). Like many anaesthetics, propofol is hydrophobic, having a calculated partition coefficient (Log P_{calc}) of 3.83

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and it presents little opportunity for solubilization through salt formation. As a result it is formulated as an oil-in-water emulsion (Diprivan®, AstraZeneca), in which the disperse phase is soya oil containing dissolved propofol, emulsified using lecithin, and having a mean droplet size of 150–200 nm (VMD).

Emulsions intended for intravenous use should have an extremely small droplet size and be highly stable, since any large droplets placed in the circulation may lodge in the pulmonary capillaries and could potentially lead to an embolism (Illum et al., 1982). The exact size at which this phenomenon becomes important is widely debated and pharmacopoeial limits on particulates in parenterals are vague, although 5 μm is generally accepted as an upper limit. Diprivan®, and intravenous feeding emulsions such as Intralipid, have particle sizes much smaller than this, mean droplet diameters being of the order of 100–300 nm. However, the systems are significantly polydisperse, and droplet counting techniques can detect a small number of particles larger than 1 μm in the formulations. It is also evident that such emulsions must be formulated with adequate physical stability to prevent the droplet size distribution evolving during the storage lifetime, and exceeding clinically safe limits.

Intravenous emulsions such as Diprivan® are stabilized with phospholipids in the form of

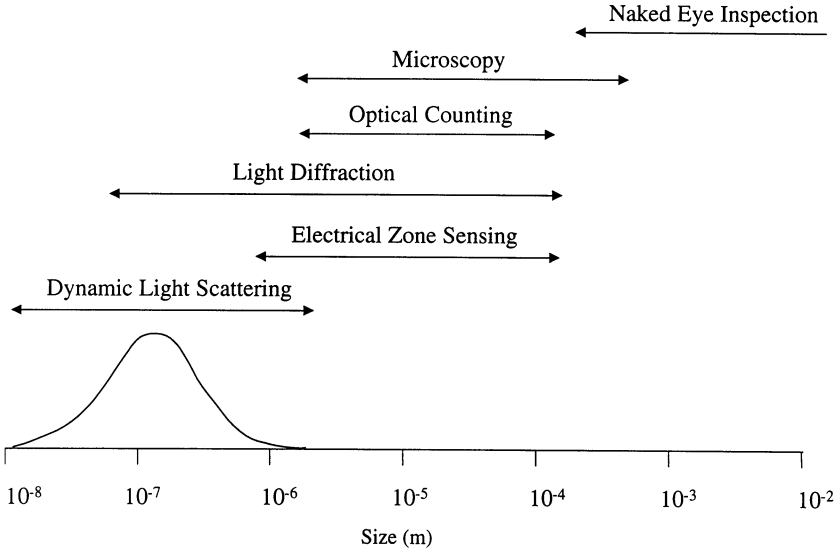

Fig. 1. Sensitivity ranges of the particle characterization techniques used in this study (exact values depend on specific instrument) and the size distribution of a typical intravenous emulsion.

Table 1
Properties of unstressed emulsions

Product	Diprivan®			Propofol intravenous emulsion		
Batch no.	L8140/B	K8179/A	X9015/A	99E301	99E309	99E312
Volume mean diameter (DLS), nm	171 ± 2	177 ± 1	172 ± 2	178 ± 1	184 ± 1	178 ± 1
pH	7.6	7.5	7.5	5.1	5.5	5.6
Droplet count number > 5μm/μl (Coulter Z2)	120	200	160	300	300	220

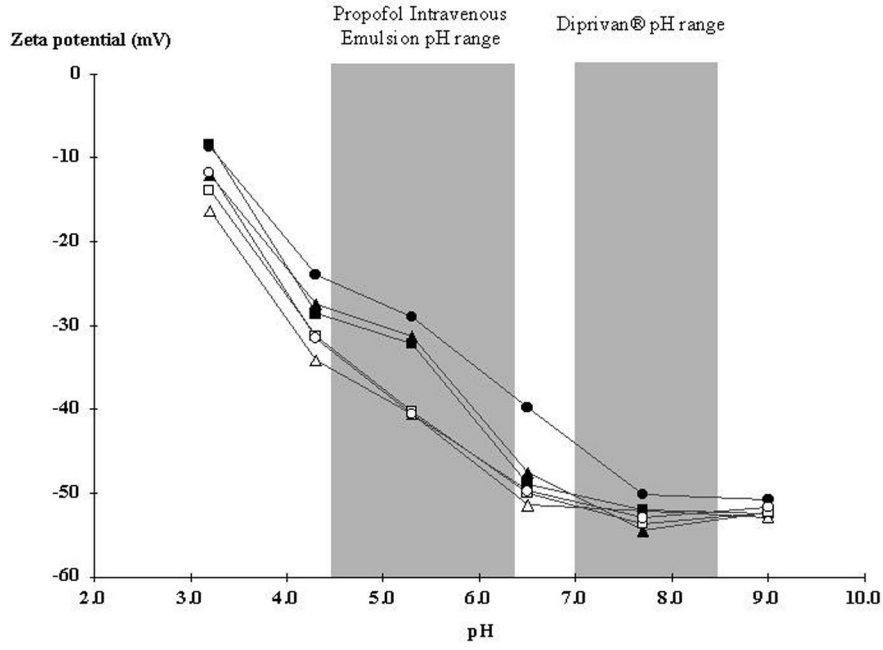

Fig. 2. Zeta potentials of emulsions as a function of pH. Closed symbols: Diprivan® (three batches), open symbols, Propofol Intravenous Emulsion (three batches).

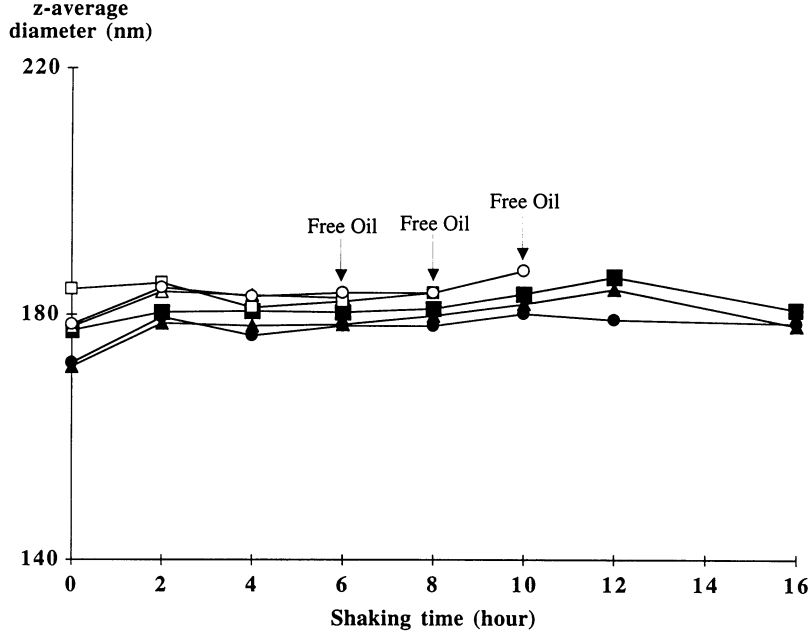

Fig. 3. *z*-average diameter of emulsions by DLS as a function of shaking time. Closed symbols: Diprivan® (three batches); open symbols, Propofol Intravenous Emulsion (three batches).

purified egg lecithins. We and others have previously studied the stability of such emulsions in a number of papers (Black and Popovich, 1981; Burnham et al., 1983; Washington et al., 1989, 1990, 1991, 1992; Washington, 1990a,b,c, 1992a,b, 1996) and it is now well un-

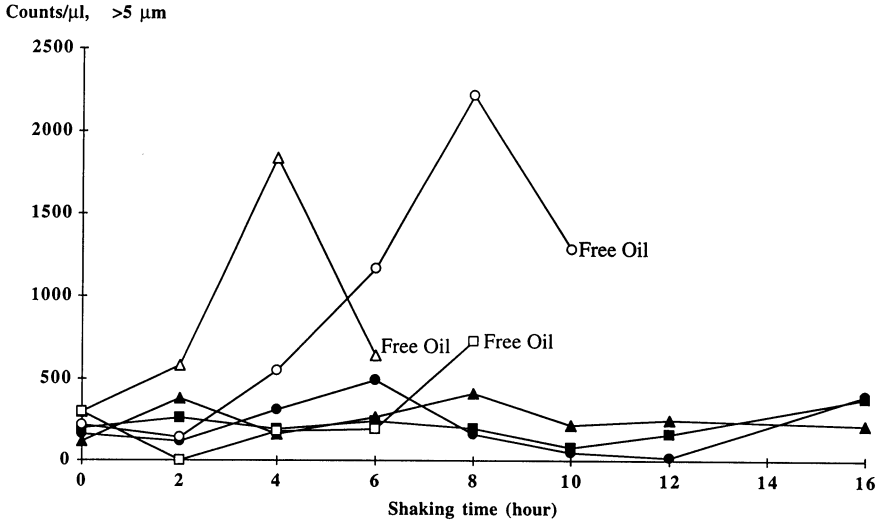

Fig. 4. Count of large droplets in emulsions by Coulter Z2 as a function of shaking time. Closed symbols: Diprivan® (three batches); open symbols, Propofol Intravenous Emulsion (three batches).

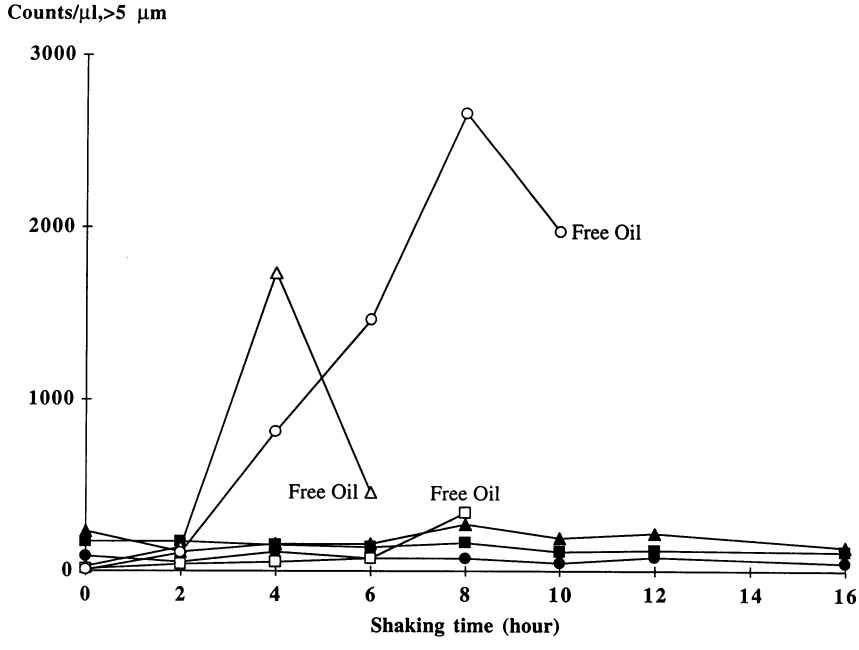

Fig. 5. Count of large droplets in emulsions by Accusizer 780 as a function of shaking time. Closed symbols: Diprivan® (three batches); open symbols, Propofol Intravenous Emulsion (three batches).

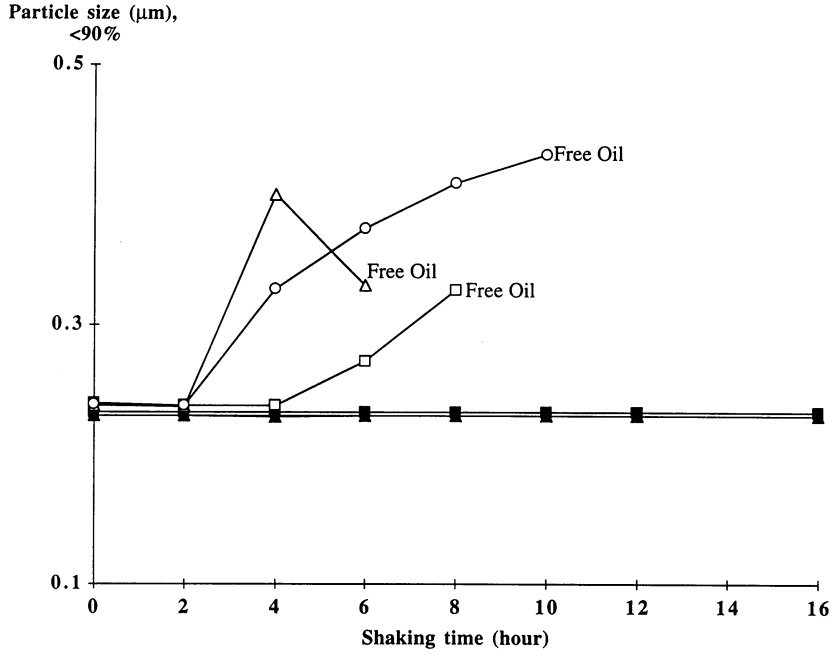

Fig. 6. D90 of emulsions by light diffraction by Coulter LS230 as a function of shaking time. Closed symbols: Diprivan® (three batches); open symbols, Propofol Intravenous Emulsion (three batches).

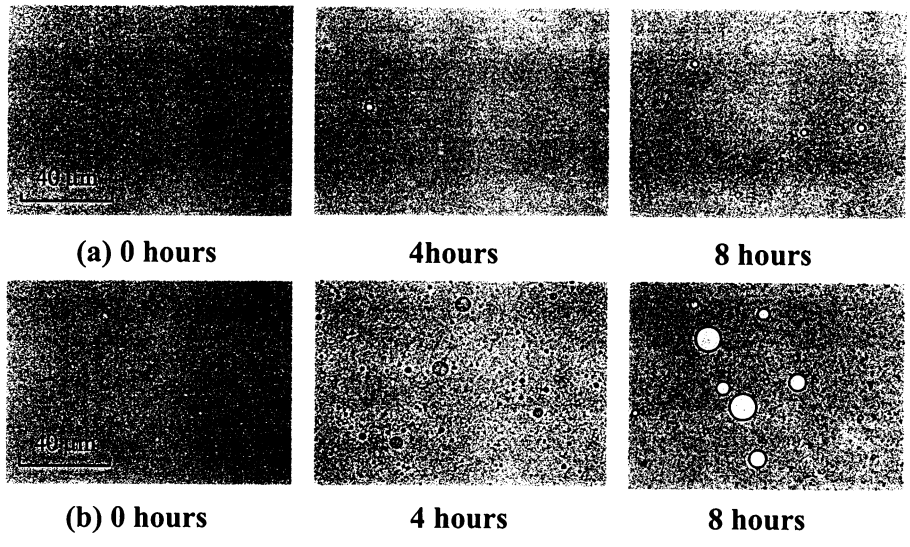

Fig. 7. Photomicrographs of (a) Diprivan® and (b) Propofol Intravenous Emulsion after 0, 4 and 8 h shaking.

derstood that these systems are charge-stabilized with a zeta potential of -40 to -50 mV at pH 8. As a result, they show excellent stability in normal use. However, any factor which lowers the zeta potential may lead to instability and the resultant formation of large oil droplets. Important factors in this regard are electrolytes and pH (Washington, 1990b,c; Washington et al., 1993).

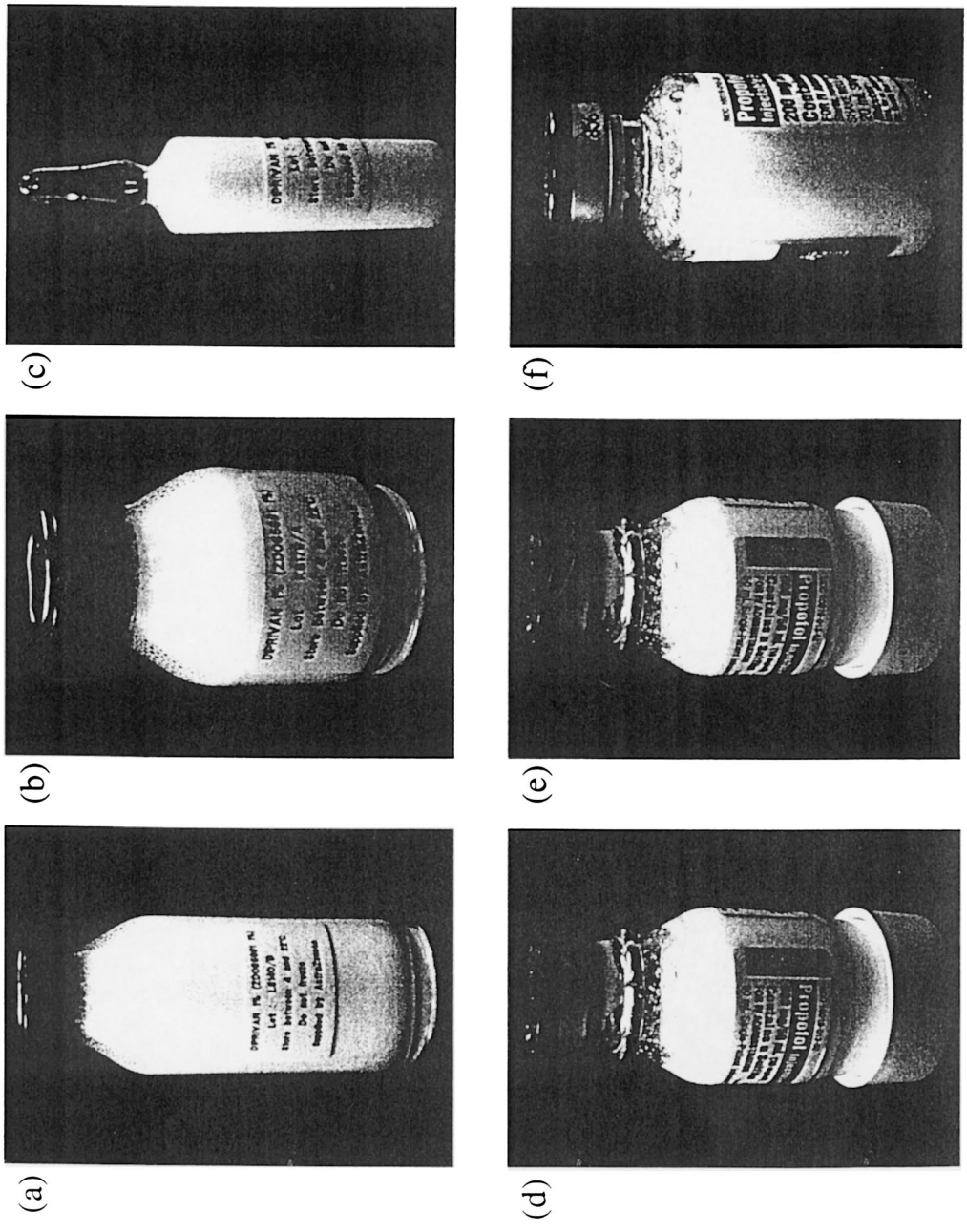

Fig. 8. Pack appearance of (a) Diprivan® and (b) Propofol Intravenous Emulsion after 16 h shaking.

The ionization curve of lecithins reaches a point of zero charge (PZC) at approximately pH 3 and consequently a lowering of pH may reasonably be expected to lead to reduced physical stability.

Recently an alternative propofol formulation to Diprivan® has appeared on the U.S. market (Propofol Intravenous Emulsion, Gensia Sicor Pharmaceuticals). This material is superficially similar to Diprivan® in that it consists of a soya oil based emulsion containing 10 mg/ml propofol and having a mean droplet diameter of 150–200 nm. However the formulation has a pH in the range 4.5–6.4, necessary to ensure the antimicrobial activity of its bisulphite additive. We would predict that, assuming that the lecithin emulsifiers are similar, this emulsion would show a different stability profile to that of Diprivan® and therefore we have performed a comprehensive set of stability measurements to compare the two formulations. To assess the physical stability we used three widely accepted accelerated tests: shaking, freeze–thaw cycling, and thermal cycling. In order to measure properly the effect that these processes had on the droplet size distribution it was necessary to use a number of different particle size measurement techniques. The original emulsions have narrow size distributions which almost wholly lie below 1 μm. However after exposure to the accelerated stability test, the range of droplet sizes may potentially vary from hundreds of nanometres to millimetres, a span of four orders of magnitude. Fig. 1 shows the measurement limits of the techniques used; it will be readily appreciated that a full assessment requires several different measurements. In particular it was felt that the Coulter Counter was an essential technique since it provides droplet volume measurements which are traceable to primary standards. The remaining techniques are all based on optical scattering and as a result may be sensitive to the optical properties of the emulsions.

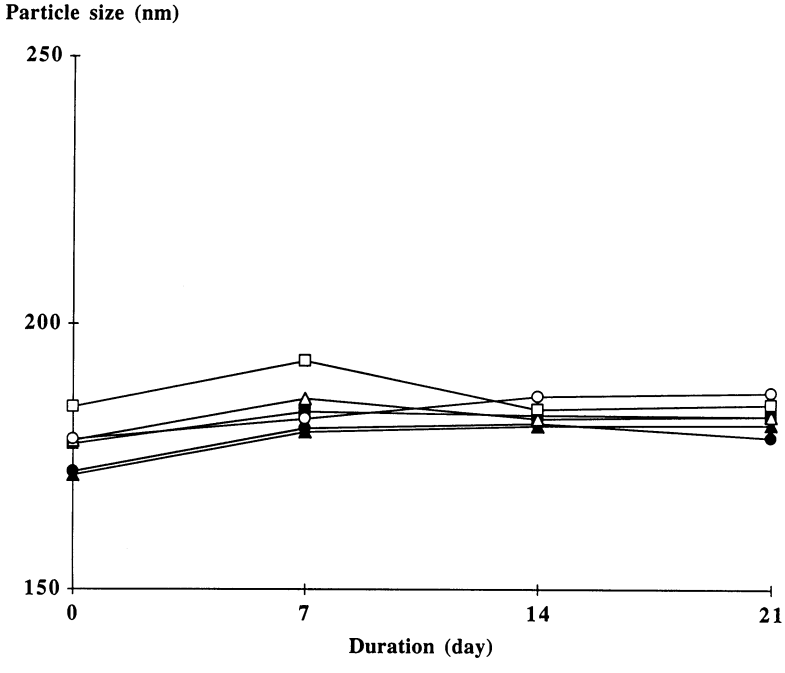

Fig. 9. z-average diameter of emulsions by DLS as a function of thermal cycling time. Closed symbols: Diprivan® (three batches); open symbols, Propofol Intravenous Emulsion (three batches).

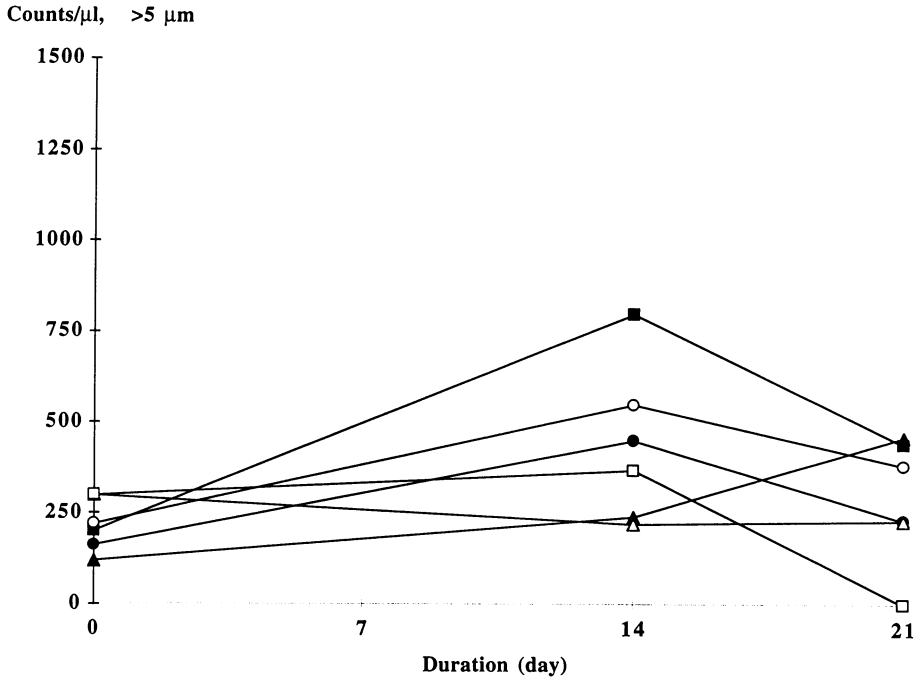

Fig. 10. Count of large droplets in emulsions by Coulter Z2 as a function of thermal cycling time. Closed symbols: Diprivan® (three batches); open symbols, Propofol Intravenous Emulsion (three batches).

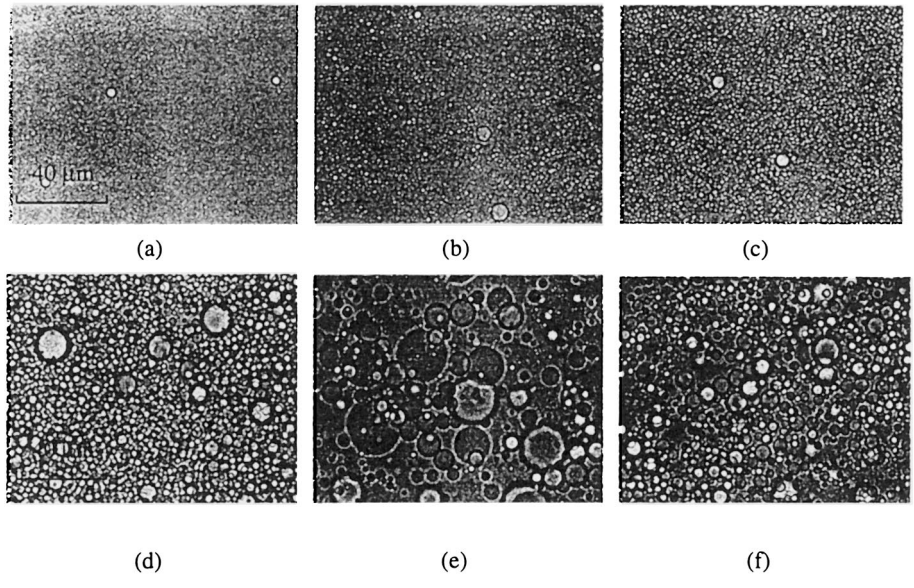

Fig. 11. Photomicrographs of Diprivan® (a–c) and Propofol Intravenous Emulsion (d–f) after one freeze–thaw cycle.

2. Experimental section

2.1. Materials

Diprivan® (Batch nos. L8140/B, K8179/A and X9015/A) was supplied byAstraZeneca Macclesfield (UK). Propofol Intravenous emulsion (Batch nos. 99E301, 99E309, and 99E312, Gensia Gensia Sicor Pharmaceuticals, Irvine, California) was purchased from material on general sale in the U.S. Three batches of each formulation were studied. Isotonic saline was purchased from Beckman Coulter (Luton, UK). All other chemicals used were purchased from Sigma Chemical Company, Gillingham, UK, and were at least of AR grade. All materials were stored in accordance with the manufacturer's instructions.

2.2. Characterization techniques

2.2.1. Zeta potential

Zeta potentials were measured using a Malvern Instruments Zetasizer 4, which is based on laser doppler velocimetry in an electric field. Values quoted are the average of four measurements. The instrument was validated using the manufacturer's polystyrene microsphere transfer standard (quoted -50 ± 5 mV, measured -46 ± 2 mV) which is traceable to the NIST goethite primary standard. The buffers used were: formic acid/potassium hydroxide (pH 3.2); acetic acid/potassium hydroxide (pH 4.3 and 5.3); potassium dihydrogen phosphate/disodium hydrogen phosphate (pH 6.5 and 7.7) and boric acid/borax (pH 9.0). All buffers were made to established formu-

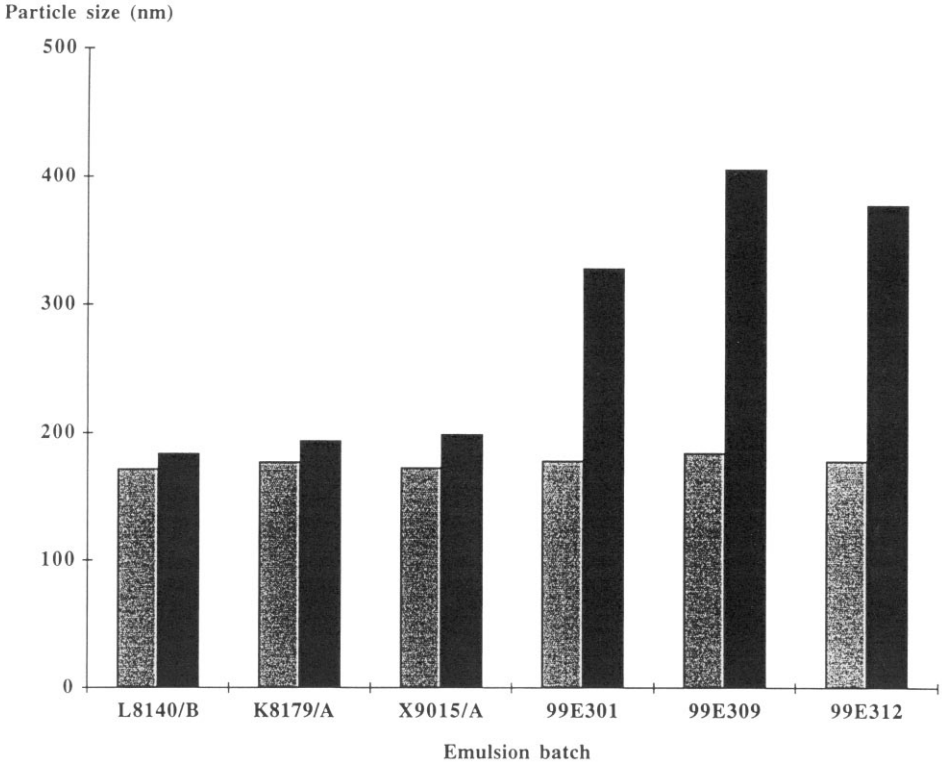

Fig. 12. z-average diameter of emulsions by DLS after one freeze–thaw cycle. Grey bars: Diprivan® (three batches); Filled bars, Propofol Intravenous Emulsion (three batches).

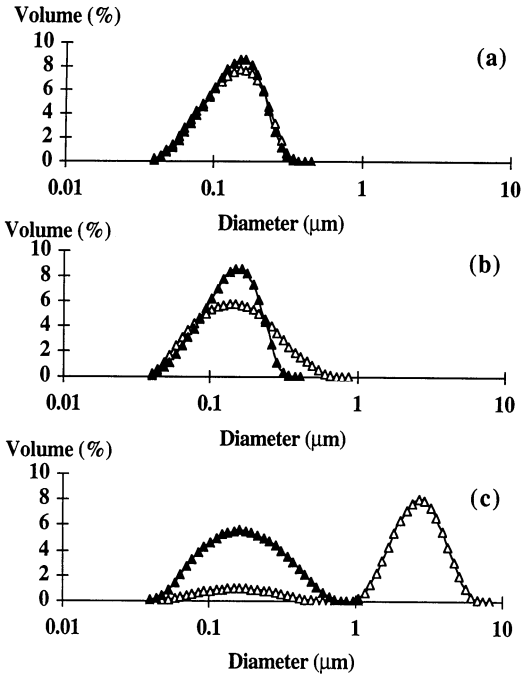

Fig. 13. Droplet size distributions (Coulter LS230) (a) of unstressed emulsions; (b) after 6 h shaking; and (c) after one freeze–thaw cycle. Solid symbols: Diprivan® batch L8140/B; empty symbols: Propofol Intravenous Emulsion batch 99E301.

lae (Dawson et al., 1969) and were diluted to a final cation concentration of 1 mM before use. The pH quoted is that of the diluted buffer measured using a calibrated pH electrode, and not the published value, which is normally incorrect due to the buffer dilution factor. We have previously used these buffers for the study of triglyceride emulsions and found that they do not give rise to detectable errors due to specific adsorption of ions.

2.2.2. Light diffraction

Light diffraction was performed using a Coulter LS230 particle size analyser (Beckman Coulter, Luton, UK) with the polarization intensity ('PIDS mode') included in the analysis, and with an optical model using the refractive index of soya oil. The instrument was validated by the manufacturer immediately prior to the study, and several times during the trial, using polystyrene microspheres (Coulter Corporation, Miami, Florida, 0.293 μm). Sample volumes of 50–200 μl were diluted into the sample recirculator until a PIDS intensity of approximately 50% was achieved. Three sequential measurements (90 s each run) were made on each batch.

2.2.3. Optical particle counting

Particles larger than 1.5 μm were counted with a PSS Nicomp Accusizer 780 in the extinction mode, in which the particle size of a droplet is calculated from the light blockage it produces in an optical sensor. The instrument was validated by the manufacturer immediately prior to the study; calibration was in the form of a calibration file which is matched to the sensor. Proper operation of the instrument was checked using polystyrene microspheres (Duke Scientific, Palo Alto, California) several times during the trial. The lower threshold was set to 1.7 μm; the measurement time was 200 s with a flow rate of 60 ml/min. For the unstressed emulsions a sample volume of 100 μl was used but after the stability tests it was frequently necessary to predilute the samples to avoid coincidences between the numerous large droplets.

2.2.4. Electrical zone sensing

Droplet counting by the electrical zone method was performed using a Coulter Z2 with a 50 μm aperture tube. This instrument provides absolute counts of the number of droplets in a specified size range, typically from 1 to 10 μm. The instrument was validated by the manufacturer immediately prior to the study and the calibration checked against polystyrene microspheres (Coulter Corporation, Miami, Florida 9.932 μm) several times during the trial. The emulsion was diluted to 1:20 with isotonic saline, 40 μl of this mixture was added to 20 ml of isotonic saline in a counting vial, and the droplets in this mixture were counted with a 0.5 ml sample volume. This corresponds to 50 nl of the original emulsion being drawn through the counting orifice. All samples were used immediately after dilution and appropriate backgounds were subtracted.

2.2.5. Dynamic light scattering (DLS)

A Malvern 4700 DLS instrument was used to measure the *z*-average diameter (cumulants method) and droplet size distribution (analysis using CONTIN). The instrument operates by measuring the diffusion coefficient of the droplets from the fluctuations in scattered light (Washington, 1992b) and is most sensitive to particles smaller than 1 μm. The instrument was validated against a polystyrene microsphere standard (98 nm, Interfacial Dynamics Corporation, Portland, Oregon) every day prior to measurement. Samples of emulsion were diluted into cylindrical counting cells until a count rate of $2–4 \times 10^5$ counts per second was obtained. Data were gathered for 30 s counting periods with the correlator in linear sequential mode with automatic correlation time selection, which normally resulted in a fundamental sample time of 10–30 μs.

2.2.6. pH

A Corning Model 7 pH meter was used with a combination glass/SCE electrode to measure pH. The meter was checked and calibrated against standard pH 4 ± 0.01 and 7 ± 0.01 buffers (Scientific Laboratory Supplies, Nottingham). The electrode was thoroughly rinsed, and its calibration checked, after every individual sample in order to avoid errors due to electrode oil contamination.

2.2.7. Physical appearance

The physical appearance of the emulsions was recorded by microscopy (Olympus CH-2 microscope), and by photography of the sealed containers (Fuji DX7 digital camera) as necessary.

2.3. Stability tests

All testing was performed as soon as possible after the termination of the appropriate stress test. All samples were tested in their original unopened containers on a single occasion and then discarded; no container was sampled more than once. Each sample was evaluated by each instrument in those cases where no free oil was visible in the container. Samples showing a film or layer of oil visible to the naked eye without magnification were considered to have broken and were not further measured, since we have found that the statistical precision of such tests is poor. Furthermore, samples containing free oil normally cause subsequent extensive cleaning problems for the particle sizing instruments.

2.3.1. Shaking

Samples in the original unopened containers were subjected to shaking using a Burrell Model 75 wrist-action shaker (Burrell Scientific, Pittsburgh) operating at 300 strokes/min at room temperature (22 ± 4°C). The shaking amplitude was set to its maximum value resulting in a bottle movement of approximately 8 cm. Samples were shaken for 2, 4, 6, 8, 10, 12 and 16 h and the positions of the various batches was randomized on the shaker.

2.3.2. Thermal cycling

Samples were stored in a Sanyo programmable incubator (Model MIR 153, Sanyo Electric Co., Japan) and subjected to a controlled thermal cycle of 8 h at 30°C followed by cooling to 4–8°C for 16 h. This cycle was repeated on a daily basis and emulsion samples withdrawn at 7, 14, and 21 days. The heating/cooling time of the incubator was approximately 30 min and its temperature precision was ± 1°C. Thermal cycles were verified using a recording thermometer with K-type thermocouple probe (Hanna Instruments HI92804C with NAMAS certified thermocouple)

2.3.3. Freeze–thaw

Emulsions were frozen in a conventional laboratory freezer at -20°C. They were agitated gently every 30 min until completely solid and then stored frozen for a total of 8 h (i.e. including the freezing time) then thawed at room temperature.

3. Results and discussion

The trial generated an extensive array of data but space prevents its complete presentation here. The complete data set is available electronically.

Table 1 lists the properties of the original emulsions. Samples from both manufacturers had similar *z*-average diameters and showed a comparable level of large droplets in all batches.

However, Diprivan® had a mean pH of 7.5 and Gensia Propofol Emulsion had a mean pH of 5.3.

Fig. 2 shows the zeta potentials of the six batches of emulsions as a function of pH. The trend is similar to that previously reported for phospholipid-stabilized parenteral emulsions (Washington, 1996) with a value of approximately −50 mV at pH 8 and a gradual decline with decreasing pH. This is due to changes in the ionization of the phospholipids; a sharp surface pK_a is not observed because the lecithin used is made up of a broad range of components with varying pK. Comparing the zeta potential data to the emulsion pH (Table 1) indicates that the mean zeta potential of the emulsion in the original container was −50 mV in the case of Diprivan® and −40 mV in the case of Gensia Propofol Emulsion. This may not appear a particularly large difference and is fairly typical of the level of variation observed in phospholipid-stabilized parenteral feeding emulsions, despite the difference in the formulation pH of the emulsions. It should be noted that Diprivan® is formulated so that its zeta potential does not vary widely over its possible pH range, while the zeta potential of Propofol Intravenous Emulsion can vary substantially over its pH range (Fig. 2). As a result pH variations in Propofol Intravenous Emulsion will lead to much larger changes in zeta potential than corresponding pH changes in Diprivan®

3.1.1. Shaking test

The results from the shaking test (DLS, Coulter Z2, Coulter LS230, PSS Accusizer) are shown in Figs. 3–6. The z-average diameters of all the emulsions were similar and did not change significantly during the trial; all the variations observed were within the limits of long-term experimental error (±5 nm). All three batches of Propofol Intravenous Emulsion showed free oil after 8–12 h shaking, so DLS was not performed on these batches after these times. The apparent insensitivity of DLS to emulsion coalescence is not unusual since this technique is only sensitive to droplets smaller than approximately 1 μm. A small number of larger droplets leads to a poorer quality of data fitting but does not generally influence the z-average diameter despite the fact that such large droplets contain a significant mass of oil.

The remaining instruments are optimised for the detection of droplets larger than approximately 1 μm, and they indicated a rapid increase in the number of large droplets in Propofol Intravenous Emulsion with shaking. In contrast Diprivan® showed no significant change over the duration of the study. Fig. 7 shows photomicrographs of all six batches of emulsion after 8 h shaking. The appearance of the Diprivan® samples was similar to that before shaking, while Propofol Intravenous Emulsion showed a significant number of large droplets. After 16 h Propofol Intravenous Emulsion showed extensive free oil while Diprivan® showed only a foam (Fig. 8). Foam formation is characteristic of emulsions in which no oil separation has occurred, since oil surface films invariably lead to rapid foam breakage (Bikerman, 1973).

3.1.2. Thermal cycling

None of the emulsion batches showed a significant change in either z-average droplet size or large droplet count after 21 days thermal cycling. Typical data (z-average diameter and Coulter Z2 large droplet count) are shown in Figs. 9 and 10, respectively. The variations in z-average diameter are typical of the long-term precision of the technique (±5 nm) and the variations in the droplet counts are consistent with the random statistical variation in the droplet counting.

3.1.3. Freeze–thaw

The behaviour of the emulsions under freeze–thaw testing paralleled that observed during shaking, with a single freeze–thaw cycle causing a large increase in the droplet diameters of the Propofol Intravenous Emulsion batches and only a very small change in the appearance of the Diprivan® samples. Typical micrographs are shown in Fig. 11. It is immediately obvious that the destabilization caused by freeze–thawing was far more extensive than that caused by shaking, since a large fraction of the Propofol Intravenous Emulsion appeared to be made up of large droplets of broken emulsion. This is in contrast to the coalescence caused by shaking, and provides some insight into the mechanisms of destabilization. In the case of freeze–thawing, the majority of the oil droplets are compressed together between the advancing ice

crystals as the continuous phase of the emulsion freezes, and it is not surprising that under these conditions most of the oil droplets have the opportunity to coalesce. In contrast shaking only seems to lead to the formation of a few rather large oil droplets, which ultimately become free oil. In this case it seems reasonable that the coalescence is confined to a small population of droplets, possibly at the large end of the size distribution, which act as nucleation centres. This view is supported by the size measurements. DLS (Fig. 12) showed a major increase in z-average diameter of Propofol Intravenous Emulsion after freezing, confirming that a majority of the droplets had undergone coalescence. This is in contrast to the behaviour after shaking, which is characterized by an unchanged z-average diameter, indicating that the coalescence was confined to a small population of large droplets.

A similar picture was provided by the diffraction data (Fig. 13) which illustrates the droplet size distributions of the two emulsions after shaking and freeze–thawing. These distributions were initially similar, but shaking of Propofol Intravenous Emulsion led to the formation of a small tail at the large end of the distribution, while freeze–thawing led to a major depletion of the original small droplet population in favour of large coalesced droplets. Freeze–thawing of Diprivan® led to a small increase in the z-average diameter, although this was minor compared to the change seen in Propofol Intravenous Emulsion. These data exemplify the difference in stability between the two emulsions; Propofol Intravenous Emulsion exhibits a coalesced large-droplet tail in the size distribution after shaking, following which free oil is rapidly formed. It is possible that free oil formation in this manner is nucleated by the formation of a few large oil droplets which then act as nucleation centres for coalescence. In contrast Diprivan® shows no significant changes in droplet size distribution on shaking. The same trend is shown by the freeze–thaw test, in which substantial coalescence is observed for Propofol Intravenous Emulsion, with Diprivan showing lesser changes.

The large difference in the stability of the emulsions is to some extent surprising since their surface potentials are not very dissimilar. Average values of -40 and -50 mV are within the range which we have observed for parenteral fat emulsions from a variety of sources, all of which display satisfactory long-term stability (Washington, 1993). Consequently it is possible that some additional factor may contribute to the rapid breakage of Propofol Intravenous Emulsion during the shaking and freeze–thaw tests. This may be connected to the interaction of the bisulphite additive with the emulsion droplets, or its sodium counterion (the ionic strength of the emulsions has not been measured). It is also possible that there may be differences in the phospholipid emulsifiers used, despite the similarity of the zeta potential profiles of the two emulsions.

4. Conclusions

The two emulsions tested show different stability profiles during the shaking and freeze–thaw tests, despite their similar initial properties. The reasons for this are still not clear but are probably connected to the differences in additives used and differences in pH.

Acknowledgements

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