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Research Article

Influence of Incorporation Methods on Partitioning Behavior of Lipophilic Drugs into Various Phases of a Parenteral Lipid Emulsion

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Abstract. The purpose of this study was to investigate the effect of drug incorporation methods on the partitioning behavior of lipophilic drugs in parenteral lipid emulsions. Four lipophilic benzodiazepines, alprazolam, clonazepam, diazepam, and lorazepam, were used as model drugs. Two methods were used to incorporate drugs into an emulsion: dissolving the compound in the oil phase prior to emulsification (*de novo* emulsification), and directly adding a concentrated solution of drug in a solubilizer to the emulsion base (extemporaneous addition). Based on the molecular structures and determination of the oil and aqueous solubilities and the partition coefficients of the drugs, the lipophilicity was ranked as diazepam > clonazepam > lorazepam > alprazolam. Ultracentrifugation was used to separate the emulsion into four phases, the oil phase, the phospholipid-rich phase, the aqueous phase and the mesophase, and the drug content in each phase was determined. Partitioning of diazepam, which has the highest lipophilicity and oil solubility among the four drugs, was unaffected by the drug incorporation method, with both methods giving a high proportion of drug in the inner oil phase and the phospholipid-rich phase, compared to the aqueous phase and mesophase. Partitioning of the less lipophilic drugs (alprazolam, clonazepam, and lorazepam) in the phases of the emulsion system was dependent on the method of incorporation and the drug solubility properties. Emulsions of the three drugs prepared by *de novo* emulsification exhibited higher drug localization in the phospholipid-rich phase compared to those made by extemporaneous addition. With the latter method, the drugs tended to localize in the outer aqueous phase and mesophase, with less deposition in the phospholipid-rich phase and no partitioning into the inner oil phase.

KEY WORDS: benzodiazepine drugs; drug distribution; drug partitioning; incorporation methods; lipid emulsion; phase separation.

INTRODUCTION

Intravenous lipid emulsions are heterogeneous systems in which vegetable oil droplets in the submicron size range are dispersed in the aqueous phase and stabilized by phospholipids. Such emulsions have been widely used in medicine for parenteral nutrition as a source of essential fatty acids and calories for patients unable to ingest food. The use of lipid emulsions as vehicles for parenteral delivery of poorly water-soluble drugs has been extensively investigated and has led to a number of successful products. Lipid emulsion systems offer many appealing properties as drug carriers for parenteral administration, including greater solubilization for poorly water-soluble drugs, stabilization, and reduced drug irritation or toxicity (1).

In general, lipid emulsions consist of an oil phase, an interface or phospholipid monolayer, and an aqueous phase. However, addition of excess phospholipids causes formation of complex multiple phases that are capable of incorporating a large volume of water and acting as mechanical barrier for emulsion stability. Several studies have proposed that the excess phospholipids form unilamellar liposomes dispersed in the continuous aqueous phase of the lipid emulsion: namely, a mesophase that can be separated by ultracentrifugation (2–5). Thus, drugs incorporated in a lipid emulsion may localize not only in the oil droplets or interface but also in phospholipid structures in the aqueous phase. Localization of drugs in various phases of the emulsion may influence the physicochemical properties of the emulsion; for example, interactions with the emulsifier layer may reduce the emulsion stability and the stability of the incorporated drug (6–8).

Drug distribution in phases of lipid emulsions has not been studied extensively, but the activity of antioxidants in emulsion systems has been shown to depend on localization (9–12). Hydrophobic antioxidants tend to be located in the oil phase and at the oil–water interface, whereas hydrophilic antioxidants remain in the aqueous phase and are less efficient (11–12). The studies indicate that both the lipophilicity of the incorporated substance and its oil or water solubility can influence the distribution of the compound in the emulsion system.

The method of drug incorporation into an emulsion system also has to be considered. Two approaches are

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generally used to incorporate drugs into parenteral lipid emulsions: *de novo* emulsification, in which the drug is dissolved in the oil phase prior to emulsification; and extemporaneous addition, in which a concentrated solution of drug in an appropriate solvent is added directly to a formulated emulsion base (13). Both methods differ in terms of the primary phase of the emulsion that contacts the drug and this may influence the partitioning of the drug into various phases of the system. Therefore, the purpose of this study was to examine the effect of incorporation methods on partitioning behavior into phases of a parenteral lipid emulsion, using the benzodiazepines alprazolam, clonazepam, diazepam, and lorazepam as model drugs.

MATERIALS AND METHODS

Materials

Parenteral grade soybean oil (Fluka, Buchs, Switzerland) was used in the study. A phospholipid mixture (Epikuron® 200) was obtained from Lucas Meyer GmbH and Co., Germany; glycerol was purchased from Sigma Chemical, St. Louis, USA; alprazolam, clonazepam, diazepam and lorazepam were kindly supplied by Siam Pharmaceutical Ltd., Bangkok, Thailand; and dimethyl isosorbide (Arlasolve® DMI) was supplied by the East Asiatic Co. (Thailand), Bangkok, Thailand. All solvents for drug analysis were HPLC grade (supplied by Lab Scan Ltd., Bangkok, Thailand).

Methods

Drug Solubility Determination

The solubilities of the drugs were determined in oil and aqueous media. An excess of each drug was added to each medium and the mixture was shaken in a control temperature water bath shaker at 25±1°C. During shaking the samples were withdrawn at various time intervals, and the time to reach equilibrium was found to be 7 days. The mixture was filtered through a 0.45-μm membrane before analysis. The concentrations of lorazepam and diazepam in the aqueous medium were assayed spectrophotometrically at a wavelength of 254 nm after appropriate dilution with distilled water, which was also used as a blank.

The concentrations of alprazolam and clonazepam in aqueous solution were determined using HPLC (SCL-10 A VP system controller, LC-10 AD VP pump, SPD-10 A VP UV-VIS detector, SIL-10 AP VP auto-injector, Shimadzu, Tokyo, Japan) with a C18 column (3.9×300 mm, Bondclone 10 C18, Phenomenex, USA) and a detection wavelength of 254 nm. Diazepam was used as an internal standard. The mobile phase consisted of methanol and ultra-pure water (70:30% *v*/*v*), with a flow rate of 1.0 ml/min and an injection volume of 20 μl.

Determination of the concentration of drugs in an oil medium was performed in two steps: sample preparation and quantification. Solid phase extraction (3 ml and 200 mg, Extra-Sep™ H.L C18, USA) was used to extract the benzodiazepine drugs from the oil phase prior to HPLC analysis. This procedure was modified from that described previously for extraction of preservative substances in emulsions (14). A cartridge was conditioned by consecutive treatment with 5×1.0 ml of methanol and 5×1.0 ml of double distilled water. Both solvents were filtered through a 0.45-μm membrane filter and degassed before use. The oil solution (0.5 ml) was then applied to the cartridge. The solvent was pulled through the cartridge and the drug and impurities were retained in the adsorbent. The cartridge was washed with 1.0 ml of hexane to remove the unwanted compounds, and finally the drug was eluted from the adsorbent with 5×1.0 ml of ethanol. The eluate from the sample preparation step was analyzed by HPLC using the parameters given above. Diazepam was used as the internal standard for lorazepam, alprazolam and clonazepam analysis, and lorazepam was used as the internal standard for diazepam analysis. Solubility determinations were carried out in triplicate.

Oil–Water Partition Coefficient Determination

Each drug was dissolved in soybean oil at a concentration of 50 μg/ml. Aliquots of 5 ml of drug solution and distilled water were transferred to a 25-ml Erlenmeyer flask and shaken at 25±1°C. To determine the equilibration time, samples from the aqueous phase were withdrawn at various time intervals up to 10 days. Analysis of these samples indicated that 7 days were required to reach equilibrium. The drug concentration in each phase was assayed using HPLC as described above, and determinations were performed in triplicate.

Emulsion Preparation

Drug-containing emulsions were prepared using either *de novo* emulsification or extemporaneous addition. For *de novo* emulsification, lipid emulsions composed of 10% w/w soybean oil (oil phase), 1.2% w/w phospholipids (emulsifier), 2.25% w/w glycerol (osmotic agent) were prepared in sufficient distilled water to make a 100% w/w product. The drug was incorporated by being dissolved in soybean oil at 70% w/w of its oil solubility, and the phospholipids were dispersed in the glycerol aqueous phase. Both phases were separately heated up to 80°C and the aqueous phase was then added to the oil phase. The mixture was passed through a high pressure homogenizer (Emulsiflex™-C5, Avestin, Canada) at a pressure of 1000 psi for 1 cycle to form a coarse emulsion. Subsequently, this emulsion was passed through the high pressure homogenizer at 15,000 psi for 6 cycles.

In the extemporaneous addition approach, the emulsion base was prepared using a similar method to that for *de novo* emulsification. The same amount of each model drug was dissolved in dimethyl isosorbide (0.45% w/w of formulation) and then mixed with the submicron emulsion base with stirring using a magnetic stirrer at 25°C for 2 h.

Characterization of the Emulsion Base and Drug-containing Emulsions

The droplet size of emulsions was determined using photon correlation spectroscopy, which covers a size range of 20 to 1000 nm (Zetaplus™, Brookhaven Instruments Corp., Holtsville, New York, USA.). Each sample was determined in triplicate at 25°C. Data are shown in terms of effective mean

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diameter and the polydispersity index (PI), which reflects the width of the particle size distribution. The charge on the emulsion droplets was measured at 25°C using the moving boundary electrophoresis technique (Zetaplus™). Electrophoretic mobility was converted into zeta potential using the Helmholtz–Smoluchowski equation. Measurements were performed in a 1 mM KCl aqueous solution with a conductivity of 50 μS/cm and a pH of 5.5–6. Five readings were recorded for each sample. The pH value of all submicron emulsions was determined at 25°C (Thermo Orion™, Boston, USA). The droplet size, charge on dispersed droplets, and pH of the emulsions were measured after preparation and after storage for 7 days.

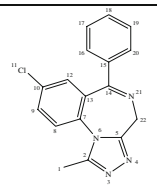
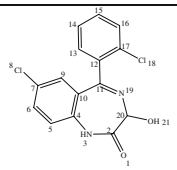
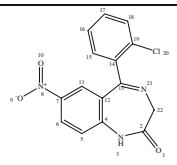
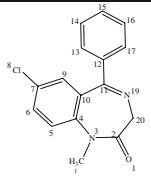
Phase Separation by Ultracentrifugation

After storage for 7 days at ambient condition (25–29°C), the drug-containing emulsions were fractionated using ultracentrifugation. Each emulsion preparation was accurately weighed into polycarbonate tubes and centrifuged at 25°C using a fixed angle rotor (90 Ti type, Beckman, USA) at 60,000 rpm for 6 h. After centrifugation, the supernatant (a clear yellowish oil phase) was collected with a needle and syringe. The lower layer, a creamy interface and the aqueous phase, was gently poured into a container and then the aqueous phase was withdrawn using a needle and syringe. The mesophase remained at the bottom of tube. The amount of drug in the oil phase was assayed using solid phase extraction for sample preparation prior to HPLC analysis. For drug analysis in the other three phases, each phase was dissolved in an appropriate volume of isopropanol before dilution with mobile phase and injection into the HPLC apparatus.

RESULTS AND DISCUSSION

The oil solubility, aqueous solubility, and partition coefficient of the drugs are shown in Table I. The oil solubility of diazepam was approximately 13, 20, and 35 times higher than that of lorazepam, alprazolam and clonazepam, respectively. The aqueous solubilities of the drugs were very low, but those of lorazepam and alprazolam were about 2 and 13 times higher than diazepam and clonazepam, respectively. The chemical structures of the benzodiazepines (Table I) indicate that substitution of a pyrazole moiety in the 1,4 benzodiazepine of alprazolam gives a more polar molecule

Table I. Aqueous Solubility, Oil Solubility, and Oil–Water Partition Coefficient of the Drugs (n=3)

Compound	Chemical structure	Molecular weight	Aqueous solubility (mg/ml) Mean±SD	Oil solubility (mg/g) Mean±SD	Oil-water Partition Coefficient (Log $P_{o/w}$) Mean±SD
alprazolam		308.77	0.116 ± 0.002	0.669 ± 0.005	0.54 ± 0.02
lorazepam		321.17	0.117 ± 0.001	1.035 ± 0.030	0.99 ± 0.02
clonazepam		315.72	0.008 ± 0.173	0.377 ± 0.010	1.46 ± 0.07
diazepam		284.75	0.051 ± 0.000	13.718 ± 0.046	2.23 ± 0.03

than the hydroxyl, nitro and methyl substituents in lorazepam, clonazepam and diazepam, respectively, resulting in increased aqueous solubility but decreased oil solubility of alprazolam. The partition coefficient ($P_{o/w}$) of the drugs in a soybean oil and distilled water mixture (50% w/w) were in the order diazepam > clonazepam > lorazepam > alprazolam. The data indicate that diazepam is more lipophilic than clonazepam, lorazepam and alprazolam, which is consistent with the molecular structures. However, the oil solubility and aqueous solubility of clonazepam were not correlated with its partition coefficient. This may be due to the resonance structure at the nitro group of clonazepam leading to an altered distribution of electrons and strong intramolecular interactions. In turn, this may weaken the interaction between clonazepam and solvent molecules, giving aqueous and oil solubilities of clonazepam that are lower than the expected respective values (15).

Oil droplet sizes and polydispersity indices of emulsions containing alprazolam, lorazepam, clonazepam, and diazepam prepared by the two incorporation methods are shown in Fig. 1. Data are shown at beginning and after storage at room temperature for 7 days. The effective mean diameter of the oil droplets in all preparations ranged from 200–300 nm. The difference in the droplet sizes of emulsion bases employed for extemporaneous addition of each compound is due to the different lots of preparation. The particle size of drug-containing emulsions prepared by *de novo* emulsification was smaller than that of the emulsion base without drugs, whereas those of drug-containing emulsions prepared by extemporaneous addition appeared to be in the same size range as that of the emulsion base. In the *de novo* method, the whole drug-containing emulsion is passed through the homogenizer together with other components, and the drug is likely to be located at the oil–water interface with the phospholipid monolayer, thus causing a reduction of interfacial tension and resulting in a smaller oil droplet size. Pongcharoenkiat (1999) reported that methylparaben reduces interfacial tension between the aqueous and oil phases, based on the assumption that this compound is localized at the interface (16). This is supported by the drug distribution data presented later in the paper, which indicates that emulsions prepared by *de novo* emulsification have greater drug localization in the phospholipid-rich phase, compared to those prepared by the extemporaneous method. In contrast, in extemporaneous addition a concentrated drug solution is added and stirred without intensive mixing and less drug molecules may reside at the oil/water interface.

After storage for 7 days, the effective mean diameter of the droplets slightly increased in all preparations, compared with the size soon after preparation. The polydispersity indices of all preparations indicated that the width of the size distributions were within the acceptable range (a value of 0.100 indicates a relatively narrow size distribution, and the value for a parenteral fat emulsion should not be > 0.25) (17). The results indicate that emulsions prepared by both methods had a consistent size distribution.

The zeta potential of emulsion bases and drug-containing emulsions prepared using different incorporation methods were all negative (Fig. 2). The initial average zeta potentials of emulsion bases before drug incorporation by extemporaneous addition ranged from −14 to −25 mV. The variation of the zeta potential among the different lots of emulsion bases

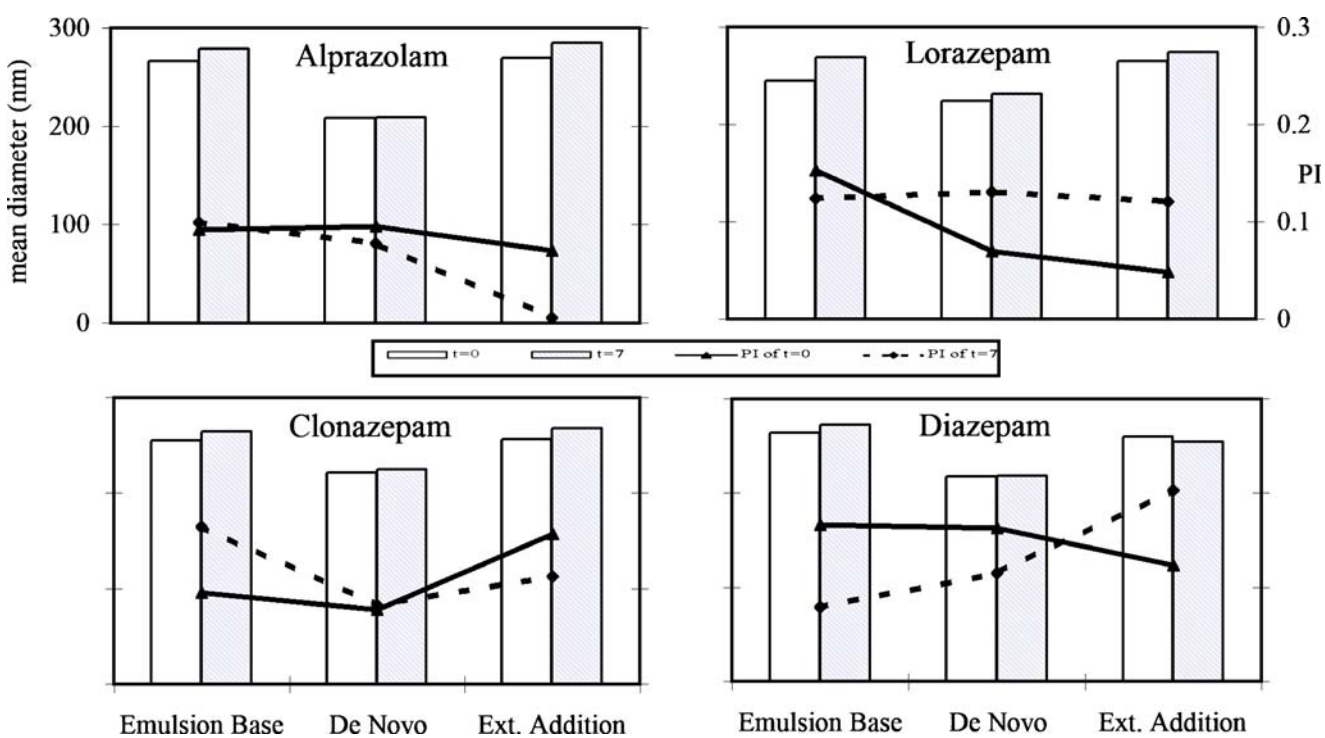


Fig. 1. The droplet sizes and polydispersity indices (PI) of drug containing emulsions prepared by different methods in comparison with emulsion base without drug determined at initial (t=0) and after keeping for 7 days(t=7)

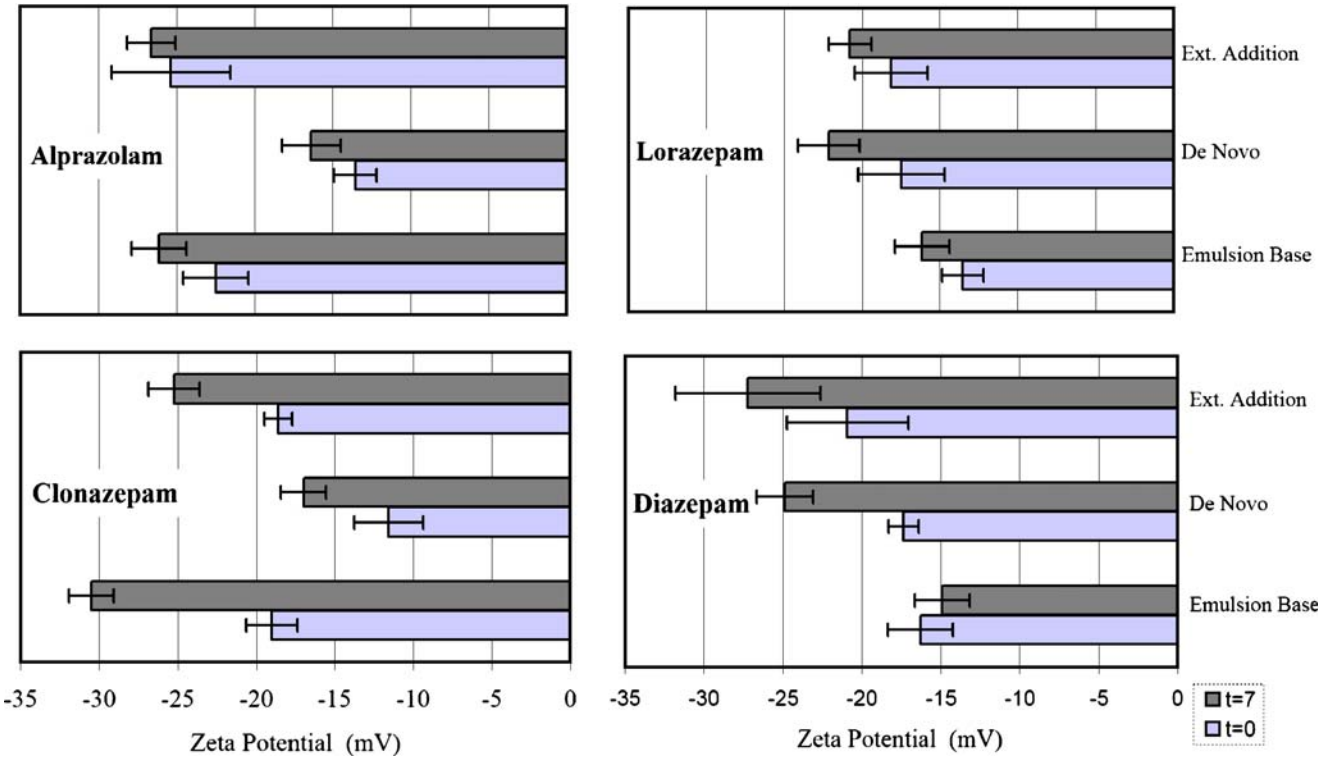


Fig. 2. Zeta potential of drug containing emulsions prepared by different methods in comparison with emulsion base without drug determined at initial (t=0) and after keeping for 7 days (t=7)

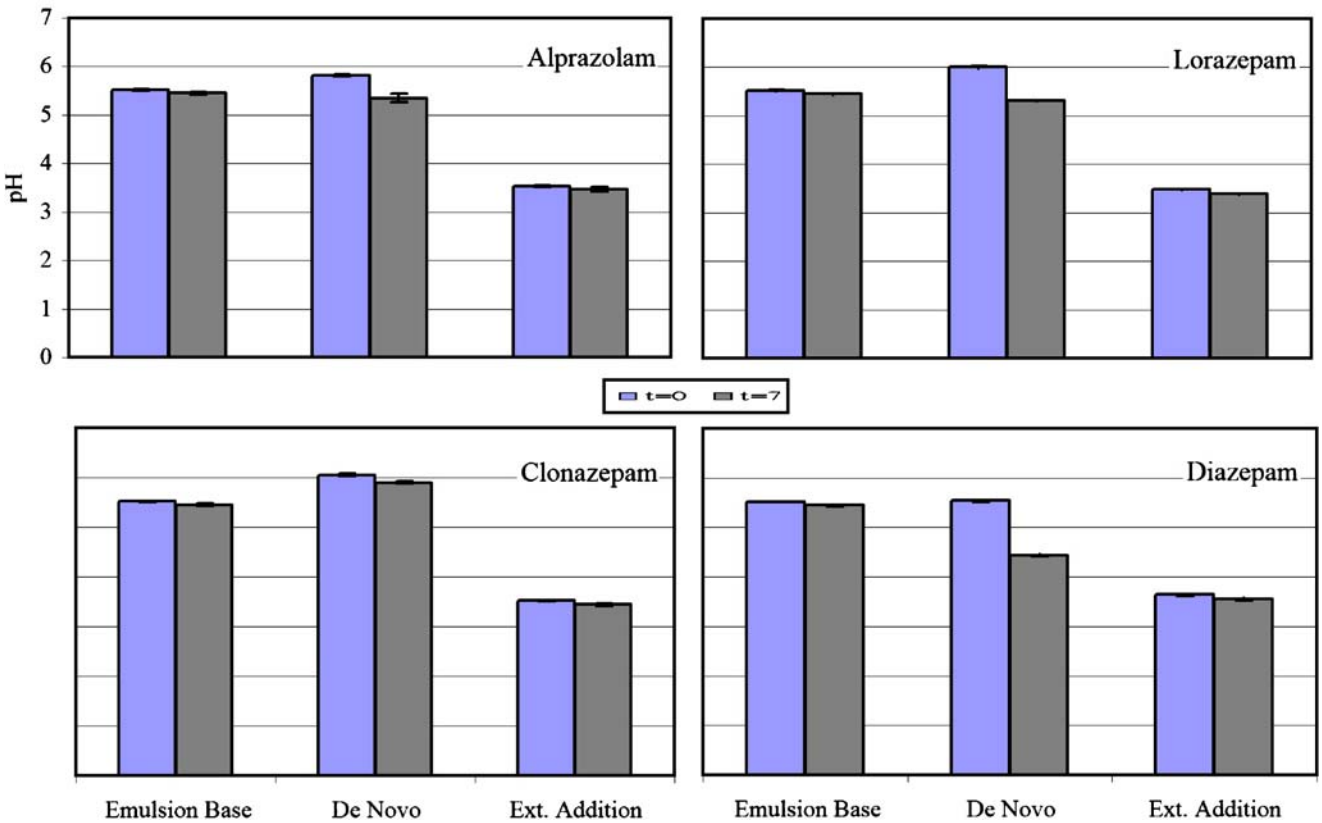


Fig. 3. pH of drug containing emulsions prepared by different methods in comparison with emulsion base without drug determined at initial (t=0) and after keeping for 7 days (t=7)

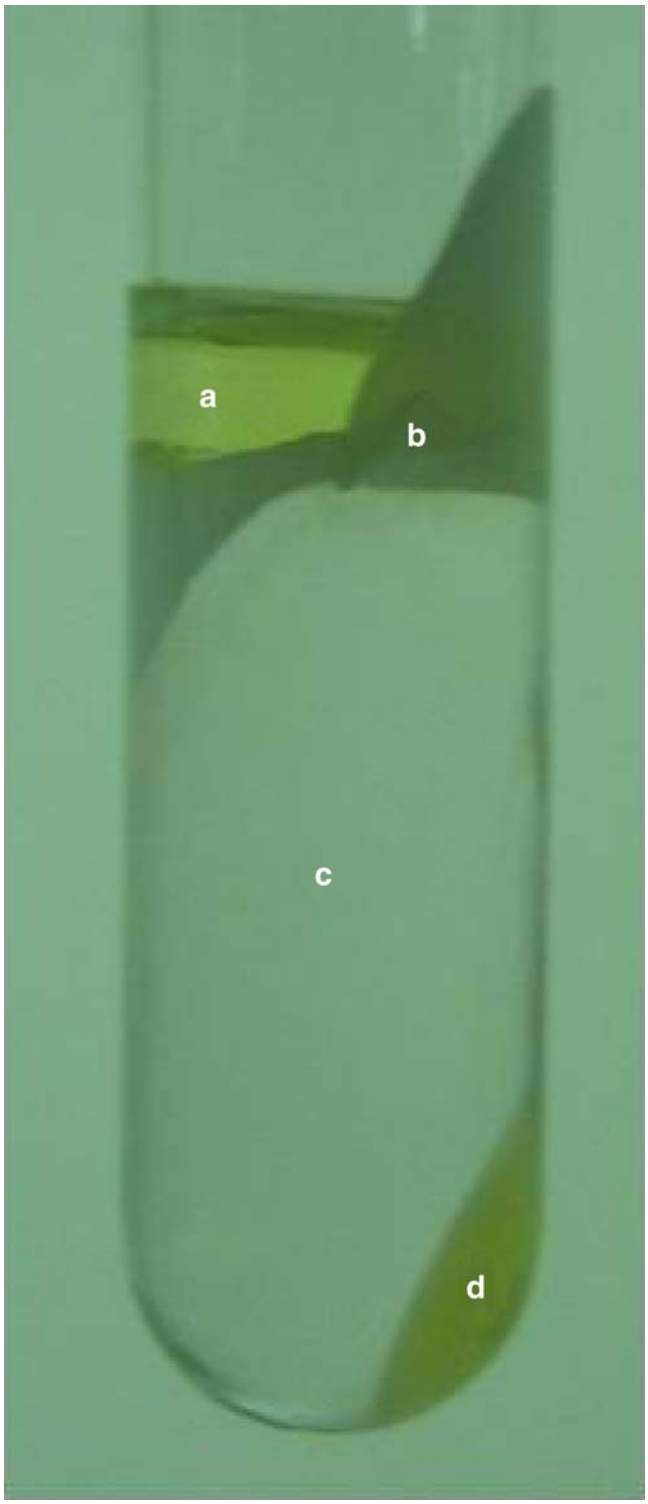


Fig. 4. Phase separation of an emulsion after ultracentrifugation, a: oil phase, b: phospholipid-rich phase, c: aqueous phase, d: mesophase

might be attributable to different degrees of hydrolysis of phospholipids and/or contamination of metal ions due to corrosion of the contact surface during several passages of the emulsion through the high pressure homogenizer. A significant difference in zeta potential has also been reported for commercial intravenous lipid emulsions with the same components and content (18). The zeta potential of drug-containing emulsions prepared by both methods also ranged from −14 to −25 mV. The zeta potential of all preparations became more negative with storage for 7 days except for the emulsion base used for extemporaneous addition of diazepam, and this change corresponded with the decrease in pH (Fig. 3). This also suggests that the oil component and/or phospholipids were hydrolyzed, leading to formation of free fatty acids. Such fatty acids may interfere with electrical conductivity, causing an increased (more negative) zeta potential and a reduced pH of the emulsions (7,19,20). However, all preparations remained stable without marked changes in physical properties in the 7-day storage period. The pH values of emulsions made by extemporaneous addition were lower than those of emulsion bases and those prepared by *de novo* emulsification. This may be due to the acidity of dimethyl isosorbide used for drug solubilization, even though only a small amount was used.

After 7 days, the drug-containing emulsions were fractionated by ultracentrifugation to separate the oil and aqueous phases. Several authors described this technique as the method for separation of oil droplets and most of the aqueous phases (4,21,22). It was in agreement with those previous reports, after prolonged centrifugation, an emulsion was found to separate into four phases: an oil phase, a phospholipid-rich phase, an aqueous phase and a mesophase (Fig. 4). The yellowish oil phase floated to the top of the centrifuge tube, while the creamy layer (phospholipid-rich phase) was in the middle between the oily and aqueous phases. The separated creamy layer may contain both phospholipids that formed a layer at the interface between the oil droplets and aqueous phase and excess phospholipids vesicles dispersed in the emulsion system. Wabel (1998) reported that either multivesicular or, more likely, small vesicles were evident in the creamy layer after centrifugation of a model lipid emulsion in a transmission electron micrograph and polarized light microscopy (22). In this present study, it was also found the presence of intact vesicles in separated creamy layer when observed under polarized-light microscope.

At the bottom of the centrifuge tube, a sedimentary mass (referred to as a pellet) was present (23), and this had a multilamellar structure when examined by cross-polarized-light microscopy as also reported by Wabel (1998) (22). With time, separation of a yellow layer from the creamy layer was observed, with settling on the bottom of the centrifuge tube to form a pellet. This may be due to concentration of liposomes from the aqueous supernatant by high speed centrifugation, and the vesicle structures in the pellet appeared to be formed from excess phospholipids (21). The structure of the pellets indicated that excess phospholipids are present in the emulsion system, and this phase is referred to as a mesophase (24). This observation is in agreement with previous descriptions of the infrastructure of phospholipid-stabilized emulsions (2–5,20,25), in which an excess of phospholipids were found to have formed unilamellar liposomes in the *i*nfranatant or aqueous phase of the emulsion and could be separated by high speed centrifugation. Formation of small unilamellar vesicles instead of liquid crystalline phases occurs because the

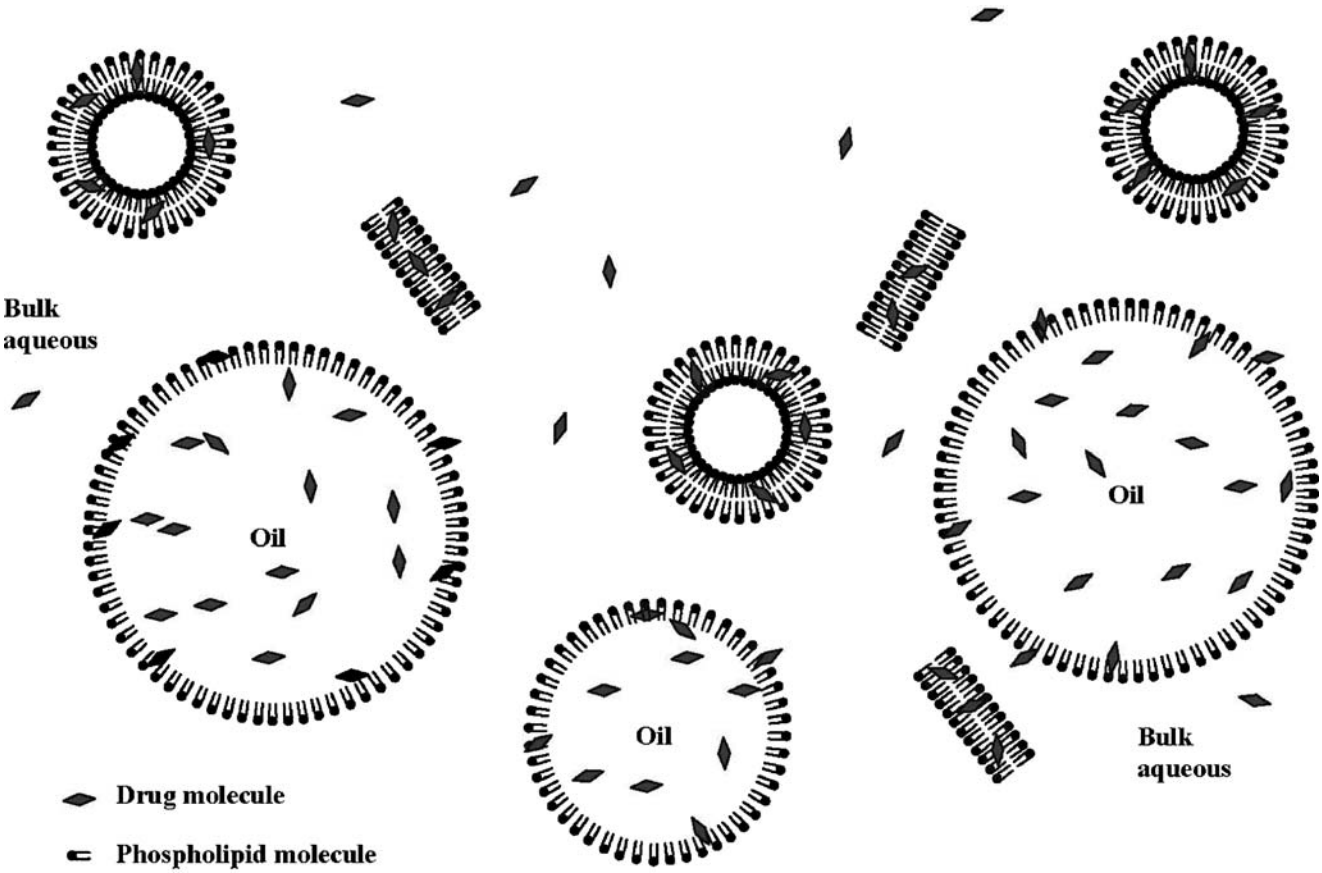


Fig. 5. Schematic illustration of emulsion infrastructure showing the different structures originated from the excess phospholipids and drug localization in various phases

energy input during homogenization is high enough to disrupt and separate large phospholipid bilayers in the aqueous phase (25). Therefore, drugs in a lipid emulsion may distribute through various phases of the system, as described above. A schematic illustration of the structure of a drug-containing lipid emulsion showing the localization of incorporated drug could be proposed as depicted in Fig. 5.

Figure 6 illustrates the percentage distribution of drugs in various phases of emulsions prepared with different drug incorporation methods. The partitioning behavior of the model compounds in the emulsion system apparently depended on their relative polarities and solubilities. The two incorporation methods differ in the direction of drug partitioning: in *de novo* emulsification, drugs partition from the oil phase to the outer aqueous phase, whereas the drug partitions in the opposite way in extemporaneous addition. Diazepam is more lipophilic and much more oil soluble than the other three drugs, and the two methods of drug incorporation gave similar drug distribution patterns for diazepam. In extemporaneous addition, the primary phase of the emulsion system in contact with the drug molecules is the outer aqueous phase, and a similar percentage of diazepam was able to partition into the inner oil phase to that with direct addition to the oil phase prior to emulsification. Therefore, for a drug with strong oil affinity, simple stirring may be sufficient to facilitate drug partitioning into various phases, without a need for high intensity agitation.

The drugs with lower lipophilicity (alprazolam, lorazepam and clonazepam) appeared to be localized more to the phospholipid-rich phase when incorporated by *de novo* emulsification, compared with extemporaneous addition. The percentage distribution of clonazepam in the phospholipid-rich phase was higher than those for alprazolam and lorazepam due to its low aqueous and oil solubilities and partition coefficient. The clonazepam-containing emulsion was prone to destabilization by coalescence compared with emulsions containing alprazolam and lorazepam, which might be caused by clonazepam deposition at the interface interfering with stable formation of the phospholipids film around the oil droplets. The three drugs with lower lipophilicity were not detected in the oil phase of emulsions prepared by extemporaneous addition, indicating that they are preferentially deposited in the aqueous phase and mesophase. This suggests difficulty of partitioning into the inner oil phase of drugs with low lipophilicity, and indicates that for such drugs the preparation procedure may give different distributions of drug in the phases of emulsion. In contrast, lipid emulsions of a highly lipophilic drug are likely to have similar distributions of drug when prepared by either *de novo* emulsification or extemporaneous addition.

Theoretically, the final drug distribution in various phases of a system should be identical regardless of the method of drug incorporation. The velocity of drug partitioning between phases may be important when the drug is

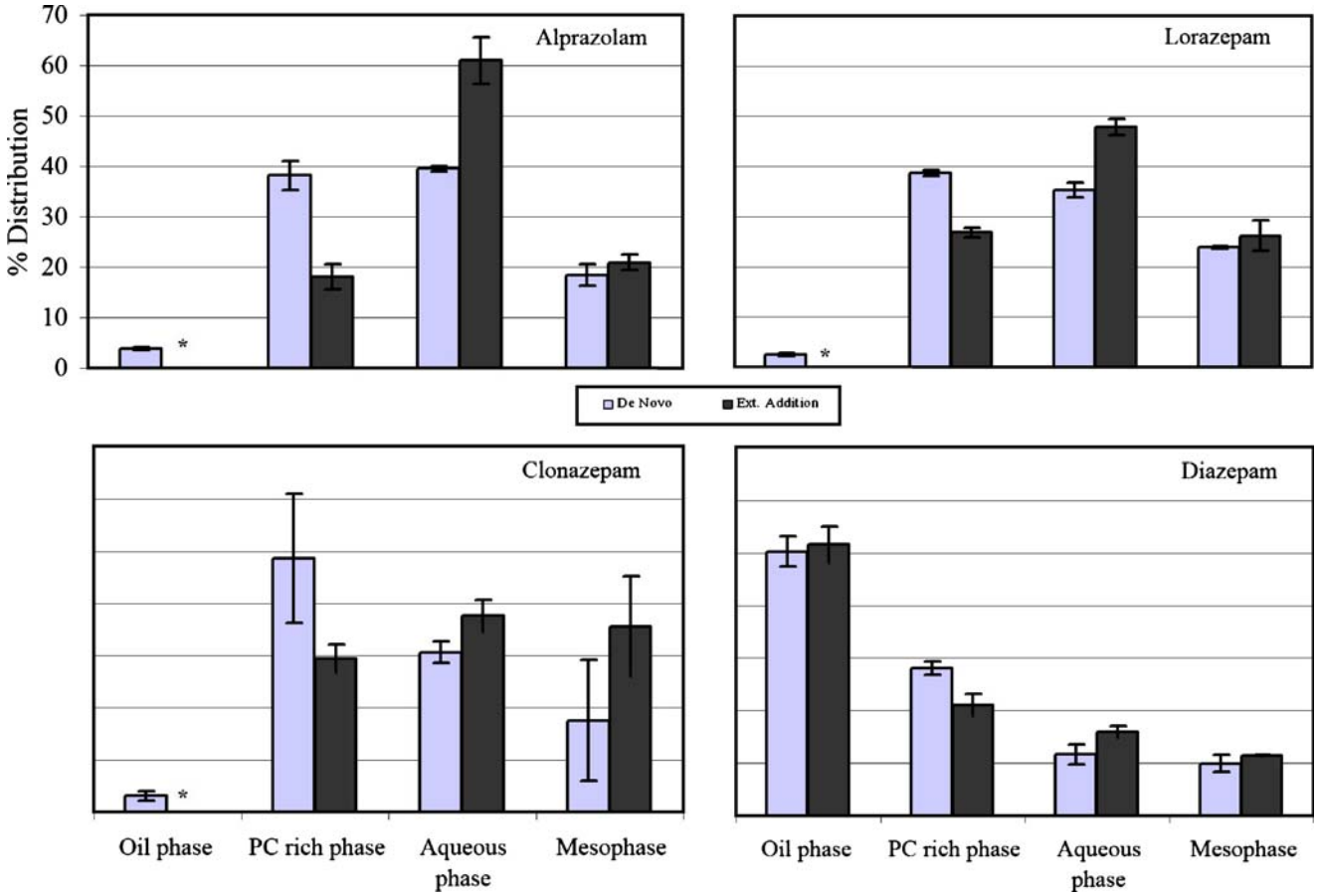


Fig. 6. Percentage distribution of the drugs in various phases of emulsions prepared by different methods. (The mark * denotes no drug was detected in oil phase for extemporaneous addition method)

initially added to different phases during preparation, since partitioning may be delayed if no further agitation force is applied to the system after completion of preparation. The velocity of partitioning is likely to depend on the hydrophilicity-lipophilicity properties of the drug. For diazepam, the fractions in each phase were similar for the two drug incorporation methods, suggesting that partitioning of diazepam may be a rapid process. In contrast, emulsions containing the other three drug compounds had significantly different drug distributions among the emulsion phases when prepared using different drug incorporation methods, suggesting a slow partitioning velocity for these drugs.

Han and Washington (2005) have recently investigated the comparative partitioning of various antimicrobial additives in an intravenous emulsion (26). The preservative was dissolved in a minimum volume of water, and added directly to the emulsion and allowed to equilibrate for 24 h without shaking. Centrifugation was used to determine the distribution of preservatives in the four phases, and the results indicated quite different partitioning of the additives depending on hydrophilicity. In our study, the solutions of drugs were mixed by shaking with the emulsion base for 2 h and then kept for 7 days before phase separation by centrifugation. Therefore, the time for partitioning in the system after preparation and before drug determination was longer, but different drug distributions in different phases were still observed for drug-loaded emulsions prepared by different processes. This indicates that a lengthy period may be required to reach the same drug distribution using different preparation methods.

CONCLUSIONS

This study suggests that different procedures for drug incorporation in preparation of a medicated lipid emulsion might give different distributions of the drug in various phases of the system. This may have effects on the product integrity and the clinical outcome of the administered drug (26–27). Therefore, during the development phase of a drug-loaded lipid emulsion, procedures for drug loading should be considered and the resulting drug distribution behavior should be assessed.

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REFERENCES

1. R. J. Prankerd, and V. J. Stella. The use of oil in water emulsions as a vehicle for parenteral drug administration. *J. Parenter. Sci. Technol*. **44**:139–147 (1990).
2. M. Rotenberg, M. Rubin, A. Bor, D. Meyuhas, Y. Talmon, and D. Lichtenberg. Physico-chemical characterization of Intralipidä emulsions. *Biochim. Biophys. Acta*. **1086**:265–272 (1991).
3. K. Westesen, and T. Wehler. Physicochemical characterization of a model intravenous oil-in-water emulsion. *J. Pharm. Sci*. **81**:777–786 (1992).
4. J. Ferezou, N.-T. Lai, C. Leray, *et al*. Lipid composition and structure of commercial parenteral emulsions. *Biochim. Biophys. Acta*. **1213**:149–158 (1994).
5. J. Li, and K. D. Caldwell. Structural studies of commercial fat emulsions used in parenteral nutrition. *J. Pharm. Sci*. **83**:1586–1592 (1994).
6. T. D. Egito, H. M. F. Appel, G. Barrat, P. Legrand, J. Bolard, and J. Devissaguet. A morphological study of an Amphotericin B emulsion-based delivery system. *Int. J. Pharm*. **145**:17–27 (1996).
7. C. Washington. Stability of lipid emulsions for drug delivery. *Adv. Drug Deliv. Rev*. **20**:131–145 (1996).
8. N. Pongcharoenkiat, G. Narsimhan, R. T. Lyons, and S. L. Hem. The effect of surface charge and partition coefficient on the chemical stability of solutes in o/w emulsions. *J. Pharm. Sci*. **91**:559–570 (2002).
9. E. N. Frankel, S.-W. Huang, R. Aeschbach, and E. Prior. Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol, and rosemarinic acid, in bulk oil and oil-in-water emulsion. *J. Agric. Food Chem*. **44**:131–135 (1996).
10. S.-W. Huang, E. N. Frankel, and J. B. German. Antioxidant activity of alpha and gamma tocopherols in bulk oils and in oil-in-water emulsions. *J. Agric. Food Chem*. **42**:2108–2114 (1994).
11. S.-W. Huang, E. N. Frankel, K. Schwarz, R. Aeschbach, and J. B. German. Antioxidant activity of carnosic acid and methyl carnosate in bulk oils and oil-in-water emulsions. *J. Agric. Food Chem*. **44**:2951–2956 (1996).
12. S.-W. Huang, A. Hopia, K. Schwarz, E. N. Frankel, and J. B. German. Antioxidant activity of alpha tocopherol and trolox in different lipid substrates: Bulk oils *vs.* oil-in-water emulsions. *J. Agric. Food Chem*. **44**:444–452 (1996).
13. A. G. Floyd, and S. Jain. Injectable emulsions and suspensions. In H. A. Lieberman, M. M. Rieger, and G. S. Banker (eds.), *Pharmaceutical Dosage Forms Disperse System*. 2 vol., 2nd ed., Marcel Dekker InC., New York, 1996, pp. 261–318.
14. N. Pongcharoenkiat, A. Wittayanukulluk, and S. L. Hem. Determination of methylparaben in o/w emulsions by solid-phase extraction and high-performance liquid chromatography. *J. Cosmet. Sci*. **54**:47–52 (2003).
15. L. Montanari, F. Ciluzo, L. Valdo, *et al*. Gamma irradiation effects on stability of poly (lactide-co-glycolide) microspheres containing clonazepam. *J. Control Rel*. **75**:317–330 (2001).
16. N. Pongcharoenkiat. The effect of surface charge and drug distribution on the chemical stability of paraben esters in intravenous lipid emulsions. Ph.D. Thesis, *Department of Industrial and Physical Pharmacy*. W. Lafayette: Purdue University; 1999.
17. R. H. Muller, and B. H. L. Bohm. Nanosuspensions. In R. H. Muller, and B. H. L. Bohm (eds.), *Emulsions and Nanosuspensions for the Formulation of Poorly Soluble Drugs*, Medpharm Scientific, Stuttgart, 1998, p. 153.
18. H. Komatsu, A. Kitajima, and H. Okada. Pharmaceutical characterization of commercially available intravenous fat emulsions: estimation of average particle size, size distribution and surface potential using photon correlation spectroscopy. *Chem. Pharm. Bull*. **43**:1412–1415 (1995).
19. C. Washington, and S. S. Davis. Ageing effects in parenteral fat emulsions: the role of fatty acids. *Int. J. Pharm*. **39**:33–37 (1987).
20. S. Benita, and M. Y. Levy. Submicron emulsions as colloidal drug carriers for intravenous administration: comprehensive physico-chemical characterization. *J. Pharm. Sci*. **82**:1069–1079 (1993).
21. M. J. Groves, M. Wineberg, and A. P. R. Brain. The presence of liposomal material in phosphoipid stabilized emulsions. *J. Dispers Sci. Technol*. **6**:237–243 (1985).
22. C. Wabel. Influence of lecithin on structure and stability of parenteral fat emulsions., Ph.D. Thesis, Nurnberg: Erlangen University, 1998.
23. New RRC. Liposomes—a practical approach: IRL Press, 1990.
24. G. M. Eccleston. Multiple-phase oil-in-water emulsions. *J. Soc. Cosmet. Chem*. **41**:1–22 (1990).
25. K. Westesen, and T. Wehler. Particle size determination of a submicron-sized emulsion. *Colloids Surf. A Physicochem. Eng. Asp*. **78**:125–132 (1993).
26. J. Han, and C. Washington. Partition of antimicrobial additives in an intravenous emulsion and their effect on emulsion physical stability. *Int. J. Pharm*. **288**:263–271 (2005).
27. G. S. Robert, and D. A. Bradley. Solubilization and stabilization of an anti-HIV Thiocarbamate, NSC 629243, for parenteral delivery, using extemporaneous emulsions. *Pharm. Res*. **10**7: 1076–1082 (1993).