Design and Evaluation of an Emulsion Vehicle for Paclitaxel. I. Physicochemical Properties and Plasma Stability

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Purpose. The current formulation of paclitaxel contains ethanol and Cremophor EL and has been reported to cause serious adverse reactions. The purpose of the present work was to develop an improved emulsion vehicle for paclitaxel and to study the physicochemical properties of such a system.

Methods. Emulsions were prepared by either microfluidization or sonication method and the droplet size characterized by dynamic light scattering and light microscopy.

Results. Stable emulsions could be made using mixtures of lecithin/sodium deoxycholate as the emulsifiers. The formulation was further improved by using a combination of free acid and the sodium salt. Paclitaxel could be loaded into the emulsions at 2.5 mg/ml without the formation of drug crystals. While these emulsions were stable on storage, they flocculated when mixed with plasma. Steric stabilization of the emulsion droplets with poloxamer 188 increased the stability of the emulsions in plasma but promoted the crystallization of paclitaxel. The crystallization tendency could be reduced by using PEG5000PE (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-*N*-[poly (ethylene glycol) 5000]), a less water-soluble stabilizer

Conclusions. Emulsions with good stability characteristics containing 2.5 mg/ml paclitaxel could be made using bile salt/acid and lecithin, and the excellent stability of these emulsions in plasma was achieved by steric stabilization using PEG5000PE.

KEY WORDS: block copolymer; emulsion; paclitaxel; pegylated phospholipids; steric stabilization.

INTRODUCTION

Paclitaxel is a promising anticancer drug against a wide range of tumors (1). However, the poor aqueous solubility of the drug makes the design of intravenous paclitaxel formulations a challenging task. The current clinically-used formulations (Taxol, Bristol-Myers Squibb, New York, NY, USA and equivalent generics Onxol (Ivax, Miami, FL, USA) and Anzatax (Faulding Pharmaceuticals, Adelaide, Australia) contain paclitaxel 6 mg/ml in a vehicle of polyethoxylated castor oil (Cremophor EL) and ethanol (50:50). It must be diluted before use, and in addition to ethanol ic intoxication (2) there has been report of serious life-threatening hypersensitivity reactions (3).

Effort has been made to formulate more biologically acceptable delivery systems. A high-throughput combinatorial approach involving a liquid dispenser has been used by Chen *et al.* (4) to screen large number of combinations of ingredient solutions efficiently for the incorporation of paclitaxel. Although Cremophor EL free, the formulations developed still retained alcohol. This method works well for formulations which require simple mixing of relatively transparent ingredient solutions (so that paclitaxel crystal could be detected by changes in transparency) but will be difficult to apply to more complicated systems such as emulsions.

A mixed micellar formulation based on bile salts and phospholipids has been described by Alkan-Onyukse *et al.* (5). They found that while the polarity and conjugation of the bile salt did not significantly change the potential of the micelles to solubilize paclitaxel, solubilization increased significantly with increasing molar ratio of phosphatidylcholine to bile salt. More recently, a sterically stabilized phospholipid mixed micelles has been developed and the loading of paclitaxel has been shown to be higher than those containing phospholipids/bile salt micelles (6). However, no stability study was reported. Diblock copolymers of poly(DL-lactide)-*block*-methoxy polyethylene glycol have also been studied as micellar carriers of paclitaxel. Zhang *et al.* (7) suggested that polymers with higher poly (DL-lactide) content and higher molecular weight solubilized more paclitaxel because of an increase in hydrophobic regions available for hydrophobic interaction with the paclitaxel skeleton. However, so far as we are aware, this system has not been evaluated *in vivo*.

Paclitaxel has also been incorporated into liposomal formulations (8) which were better tolerated then those formulated in Cremophor/ethanol. Unfortunately, others have reported that their experience with liposomes of similar lipid compositions had been far less promising (9), with paclitaxel crystals being observed in all cases. They concluded that because of its large size, paclitaxel may not be accommodated within the phospholipid bilayers in liposomes (9). The stability of sterically stabilized liposomes containing paclitaxel has been reported to be less than 1 day at 4°C (10).

Emulsion systems offer potential advantages for the delivery of poorly water soluble drugs because of the high volume fraction of the oil phase that can be used. In the case of paclitaxel, the drug would be carried in the oil phase since it is poorly soluble in water and does not possess the amphiphilicity need for it to be localized at the oil-water interface. A clinically acceptable oil is required that can dissolve the drug. Unfortunately, soybean oil emulsions which are widely used are unsuitable, since paclitaxel solubility in soybean oil is low [0.18 mg/g (11)]. Lundberg (12) has reported that the paclitaxel solubilization capacity of triolein phospholipid stabilized emulsions, was the same as the equivalent phospholipid liposome systems, without the triolein oil phase. Paclitaxel exhibits good solubility in triacetin and an emulsion formulation has been investigated by Tarr *et al.* (13). Satisfactory loadings of paclitaxel were achieved, but the emulsions were toxic when administered to mice. An emulsion with cholesterol ester as the oil phase has also been developed with a good drug loading (5.4–6 mg/ml) but it was stable for only 8 days at 4°C (14). Other water-immiscible liquids that have been investigated include benzyl benzoate, tributyrin,

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tricaproin and tricaprylin (11,15) but apparently none has shown sufficient promise for clinical evaluation. A microemulsion formulation has also been studied (16) but no stability data were reported and the formulation still retained Cremophor EL (albeit to a lesser extent).

Constantinides *et al.* (17) have reported the use of a vitamin E emulsion consisting of α-tocopherol, α-tocopheryl-polyethyleneglycol-1000 succinate, poloxamer 407, PEG 400, paclitaxel and water for injection. The compositions varied widely and it is difficult to ascertain the nature of the final optimized system, but loadings of 8.8 mg/ml paclitaxel were claimed. Since vitamin E is a good solubilizer of paclitaxel, it offers considerable potential for improved delivery (18). The aim of this work was therefore to further investigate parenteral vitamin E emulsions and to study their physicochemical properties with respect to paclitaxel incorporation.

MATERIALS AND METHODS

Materials

Egg lecithin (Lipoid E 80, 78% phosphatidylcholine, 8% phosphatidylethanolamine) was obtained from Lipoid KG (Ludwigshafen, Germany). Deoxycholic acid, sodium deoxycholate, sodium oleate, polysorbate 80, poloxamer 188, glycerol, vitamin E ((±)-α-tocopherol), concentrated hydrochloric acid and sodium hydroxide were purchased from Sigma Chemical Company Limited (Poole, Dorset, UK). Paclitaxel was kindly provided by Bristol-Myers Squibb (New Jersey, USA). Methanol (far UV HPLC grade) was obtained from Fisher Scientific UK Limited (Loughborough, Leicestershire, England). Glycerol (BP) was purchased from William Ransom & Son plc. (Bancroft, Hitchin, Hertfordshire, England). PEG5000PE (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-*N*-[poly (ethylene glycol) 5000]) was purchased from Avanti Polar Lipids, Inc. (Alabaster, Alabama, USA). Mouse plasma, rat plasma and sheep plasma and serum were kindly supplied by Dr M Hinchcliff, DanBioSyst Ltd., (Nottingham, Nottinghamshire, UK), and stored at −70°C before use.

Preparation of Vitamin E Emulsions

Paclitaxel, vitamin E, lecithin, and deoxycholic acid were co-dissolved in methanol, which was then removed by evaporation under a stream of nitrogen, followed by vacuum desiccation overnight at room temperature. The oily paste that remained was used as the oil phase of the emulsion. Sodium deoxycholate, sodium oleate, and glycerol (a tonicity adjusting agent) were dissolved in distilled water to provide the aqueous phase of the emulsion. Emulsification was accomplished either by sonication or microfluidization. In the sonication method, the oil phase and water phase were directly emulsified using an ultrasonic probe (Dawe 7532B, Dawe Instruments Limited, Middlesex, UK) in a small water bath at room temperature to prevent the samples from over-heating. This method was used for initial tests on the loading of paclitaxel emulsions because it is suitable for the production of small samples (2 ml). In the microfluidization method, a coarse emulsion was first prepared using a Laboratory Mixer Emulsifier (Serial No. 17231, Silverson Machines Ltd., Bucks, UK) with at a stainless steel stationary outer blade and a rotary inner blade at a speed of 10,000 rpm. This emulsion was then passed through a Microfluidizer (Model 110T, Serial 7018, Microfluids Corporation, Newton, MA, USA) six times to produce a fine emulsion. The microfluidizer was operated at room temperature using compressed air 100 psi coupled with a compressor tank. Although this method requires larger sample size (minimum 45 ml), in our experience it produces better emulsions than the sonication method and therefore was used to prepare all the samples except for the initial loading study.

Physical Stability of Vitamin E Emulsions

The stability of vitamin E emulsions was determined by measuring particle size, examining the physical appearance and identifying the formation of paclitaxel crystals. Emulsion particle size was measured using a Malvern S4700 PCS system (Malvern Instruments Limited, Malvern, UK), equipped with a Uniphase Model 2213-75SLYV argon laser (San José, California, USA). The formation of paclitaxel crystals were examined under an Olympus model CHS microscope (Model 2C0002, Olympus Optical Co. Ltd., Tokyo, Japan) equipped with polarizers. The emulsions were filled with N_2 and sealed in glass bottles which was then wrapped in aluminum foil and stored in darkness at room temperature (22 ± 2°C) unless otherwise indicated. Autoclaving of the emulsions was undertaken by heating the samples to 121°C for 15 min in 50 ml sealed glass bottles in a Series 400V Autoclave (Model 400V, Boxer Laboratory Equipment Ltd., Hertfordshire, UK).

Stability of Vitamin E Emulsions in Plasma

It was anticipated that future *in vivo* evaluations would be carried out in variety of animal models. Emulsion stability was therefore studied *ex vivo* in mouse, rat and sheep plasma and serum, as an important preliminary screen. 0.05 ml aliquots of emulsions were mixed with 0.2 ml plasma or serum, examined under a microscope for flocculation, and incubated at 37°C in a water bath for 24 h. The particle size was then measured using PCS and compared with the original emulsions. Photomicrographs were taken using a video camera attached to the microscope and recorded directly into a computer as digital image files using QuickImage Release 1.6 software.

Paclitaxel Assay

The paclitaxel content of the emulsions was measured at room temperature using a HPLC method. The equipment consisted of an Perkin-Elmer series 10 Liquid Chromatograph (Perkin-Elmer Corporation, Norwalk, CT, USA), Perkin-Elmer LC-90 UV spectrophotometric detector, a Spectra-Physics ChromJet Integrator, Rheodyne 7125 injector and a Hichrom H5ODS- 5327 (0.4 × 10 cm) column. The mobile phase was acetonitrile-water (47:53 v/v), the flow rate 1.0 ml/min, and detection was at 227 nm. Emulsions were directly dissolved in acetonitrile prior to injection.

Titration of Sodium Deoxycholate and Sodium Oleate Solutions

Emulsions stabilized with sodium oleate were easily destabilized by a reduction in pH but emulsions stabilized with sodium deoxycholate were not. These differing pH sensitivities were investigated by titrating 5 ml aliquots of 0.4% sodium oleate, sodium cholate or sodium deoxycholate solution

with 0.1N HCl added in 20 μl aliquots. The solution was shaken after each addition and the pH was measured with a Corning model no. 7 pH meter (R. W Jennings & Company Limited, Nottingham, UK). Turbidity was measured immediately afterward using a DU-600 Spectrophotometer (Beckman Instruments, Inc., Fullerton, CA, USA) as an absorbance at 700 nm.

Data Analysis

All formulations were produced in a minimum of three batches to ensure reproducibility. The particle size of each batch was analyzed six times and the mean ± SD (n = number of analysis) are shown in all cases [except in Fig. 3 and Fig. 4 where mean + SD (n = number of analysis) are shown]. For the paclitaxel-containing emulsions developed in this study, data for three different batches of the formulation are presented individually in Table IV, where the batch-to-batch variability can be compared. For the remainder, the average of six analyses on a single batch is shown. For the study of stability in plasma, a minimum of three batches of each formulation was tested and each batch was analyzed six times. Data for a single batch in different plasma sample are presented to best reveal the stability of the formulation without incorporating batch-to-batch variations.

RESULTS AND DISCUSSION

Investigation of Suitable Emulsifier Systems

Phospholipids are usually the first candidates selected as emulsifiers because of their biocompatibility and application in commercial intravenous fat emulsions, in which vegetable oil is used as the oil phase. Table I (sample A1) shows how egg lecithin Lipoid E 80 was not capable of emulsifying vitamin E alone. This may be a result of the phospholipids having higher solubility in vitamin E than in vegetable oil, and thereby being less available at the oil-water interface. A hydrophilic co-emulsifier, with low vitamin E solubility, was therefore more likely able to create a stabilized interface and consequently a range of water-soluble emulsifiers (polysorbate 80 (HLB 15), sodium oleate (HLB 19), sodium deoxycholate (HLB 24) and polyoxyethylene-polyoxypropylene block copolymer, poloxamer 188 (HLB 29) were tested as emulsifiers suitable for parenteral submicron emulsions (19).

Table I shows the effectiveness of these emulsifiers in preparing 10% vitamin E emulsions. Polysorbate 80 did not produce stable emulsions and the incorporation of Lipoid E80 further reduced the emulsifying power (A2–A4). Poloxamer 188 improved emulsification in comparison with Lipoid E80 alone, but the emulsions had large droplet sizes and poor stability (A5).

Emulsions co-emulsified with sodium oleate (A6) and sodium deoxycholate (A7) had the smallest mean particle sizes and lowest polydispersity values and visual examination confirmed that both emulsions appeared more stable since no creaming was observed within the period studied. Overall, sodium oleate and sodium deoxycholate were more effective than polysorbate 80 and poloxamer 188, perhaps because of the ionic charge imparted to the surface of the droplets by the ionisable emulsifiers. Charge repulsion has long been known to be an important factor for good emulsion stability (20). The mean droplet size of an intravenous emulsion is generally required to be smaller than 1 μm and no droplets should be larger than 5 μm to avoid possible embolism of the lung capillaries (19). Commercial intravenous fat emulsions usually have mean droplet sizes between 100 and 500 nm. Therefore the mean droplet sizes of sodium deoxycholate and sodium oleate stabilized emulsions (A6 and A7) were suitable for intravenous applications.

Unfortunately, emulsions made using sodium oleate had a high pH of 9.5 (apparently as a result of sodium oleate being alkaline in aqueous environment), and could be unsuitable for parenteral use. On reducing to pH 8 by the addition of hydrochloric acid, the emulsion became unstable and oil drops were seen on the emulsion surface. A further reduction to pH 7 resulted in rapid phase separation and obvious cracking of the emulsion. This pH sensitivity was further examined by turbidimetric titration of emulsifier solutions with 0.1 M HCl (Fig. 1). It can be seen that sodium oleate solution rapidly developed turbidity below pH 8.5 (paralleling the pH sensitive behavior of the emulsions) whereas sodium deoxycholate solutions retained transparency until below pH 6.8, corresponding approximately with the pK_a of deoxycholic acid at 6.58. The reported pK_a of oleic acid varies depending

Table I. The Effectiveness of Selected Emulsifiers in Vitamin E Emulsions*

Formulation	Emulsifiers	Particle size† (nm)	Polydispersity†	Visual appearance and stability
A1	Lipoid E 80 2%	ND‡	ND‡	The oil phase could not be dispersed. The aqueous phase was clear.
A2	Polysorbate 80 0.5% + Lipoid E80 1.5%	ND‡	ND‡	The oil phase could not be dispersed. The aqueous phase was turbid.
A3	Polysorbate 80 1% + Lipoid E80 1%	916 ± 452	0.84 ± 0.19	Poor homogeneity. Creaming occurred within a few days.
A4	Polysorbate 80 2%	563 ± 74	0.61 ± 0.04	Poor homogeneity. Creaming occurred within a few days.
A5	Poloxamer 188 1% + Lipoid E80 1%	1325 ± 71	0.59 ± 0.04	Poor homogeneity. Creaming occurred within a few days.
A6	Sodium oleate 1% + Lipoid E80 2%	177 ± 2	0.17 ± 0.02	Homogenous. No creaming observed.
A7	Sodium deoxycholate 1% + Lipoid E 80 2%	163 ± 2	0.17 ± 0.04	Homogenous. No creaming observed.

* 10% vitamin E, 2.25% glycerol.
† Mean (n = 6) ± SD.
‡ Not determined because the oil phase could not be dispersed.

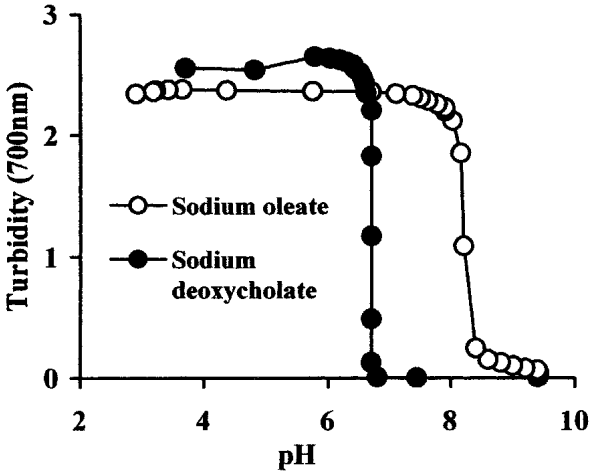

Fig. 1. Turbidity changes of sodium deoxycholate and sodium oleate solutions when titrated with 0.1 N HCl.

on the environment but Cistola *et al.* (21) have reported values of 8.0–8.5 in water. Instability of the emulsions appears to be simply related to ionization of the emulsifier. Sodium deoxycholate, with the ability to remain ionized at neutral pH, was therefore selected for further study.

Emulsifier Ratio and Salt Form

As a strategy to further improve the formulation, mixtures of deoxycholic acid and sodium deoxycholate were used in combination. Deoxycholic acid was added to the oil phase, and the sodium salt added to the aqueous phase before emulsification. It was predicted that this would kinetically facilitate the rapid formation of a stable emulsion since the bile salt/acid could "approach" the oil-water interface from both phases. The vitamin E content was also raised to 20% which would be advantageous for a high loading of paclitaxel. Table II shows the particle size and pH of emulsions prepared with different ratios of the salt and acid, with an emulsion (B4) prepared from the salt as a comparator. Although B4 had the smallest mean droplet size, it sometimes creamed within a few days after production. Using the salt/acid mixtures (B1 to B3), no creaming was observed and the particle sizes were within the acceptable range for intravenous applications. No appreciable changes in droplet size were detected in samples stored up to 300 days. It can be seen that a higher salt acid ratio resulted in a higher pH of the emulsion and that at low ratios, a neutral pH was obtained. This was another advantage of using salt and acid mixtures. The mean particle sizes of the four formulations were not significantly affected by autoclaving (121°C, 15 min).

Whilst all salt: acid ratios produced stable emulsions in terms of droplet size, emulsion B1 was rejected because the emulsification process appeared slower when making the coarse emulsion initially. B4 sometimes creamed and also had a high pH (8.5), and therefore it was not considered further. An emulsifier salt: acid ratio of 1:1 to 3:1 was chosen for further study as within this range stable emulsions were produced with acceptable pH values (7.3–7.8).

Lower concentrations of total sodium deoxycholate and deoxycholic acid were also evaluated. Table III shows how stable emulsions could be made at an emulsifier concentration of 0.5% and that the pH, particle size and polydispersity were not affected by autoclaving, and did not change significantly over 270 days. The pH of these emulsions remained near neutral.

Incorporation of Paclitaxel Initial Assessment of Drug Loading

The initial assessments were carried out using the sonication method because it was suitable for small samples (2 ml), and avoided excessive consumption of paclitaxel. It was found that paclitaxel was soluble in vitamin E up to 40 mg/g (Determined by mixing paclitaxel solutions in methanol with vitamin E and removing the solvent under a stream of N_2 followed by desiccation over night at room temperature. The drug loaded mixtures were then examined for the formation of crystals by the naked eye and under the optical microscope). In a 20% (g/ml) vitamin E emulsion, this solubility is equivalent to a loading of up to 8 mg/ml of emulsion. However, whilst this loading was possible in an emulsion, it was found that above 3 mg/ml, crystals of paclitaxel in the aqueous phase were detected under a microscope in a few days (at 4 ± 2°C and room temperature). The solubility of paclitaxel in emulsified vitamin E may be reduced, possibly because of the saturation of the oil phase with water. Plainly, crystals in an intravenous formulation are not acceptable, and the drug loading was set at a lower level of 2.5 ± 0.1 mg/ml. No crystal was subsequently detected at this loading level.

Preparation of Paclitaxel Vitamin E Emulsion Using Microfluidization

The properties of three batches of paclitaxel-containing emulsions are shown in Table IV. Good reproducibility in

Table II. Particle Size and pH of Emulsions* Containing Different Ratios of Sodium Deoxycholate and Deoxycholic Acid

			0 day		120 days†		300 days†	
Emulsion	Salt:Acid	pH	Size‡ (nm)	Polydispersity‡	Size‡ (nm)	Polydispersity‡	Size‡ (nm)	Polydispersity‡
B1	1:3	6.9	216 ± 5	0.19 ± 0.03	216 ± 4	0.20 ± 0.04	221 ± 3	0.22 ± 0.03
B2	1:1	7.3	199 ± 4	0.18 ± 0.03	195 ± 4	0.18 ± 0.02	199 ± 3	0.20 ± 0.03
B3	3:1	7.8	197 ± 4	0.19 ± 0.02	195 ± 3	0.18 ± 0.03	195 ± 3	0.20 ± 0.01
B4	1:0	8.5	177 ± 2	0.21 ± 0.03	176 ± 4	0.2 ± 0.02	178 ± 2	0.20 ± 0.03

* Formulations: 20% vitamin E, 2% Lipoid E 80, 2.25% glycerol and 1% sodium deoxycholate and deoxycholic acid (in total). The sodium deoxycholate:deoxycholic acid ratios are shown in the table.

† Stored at room temperature (22 ± 2°C).

‡ Mean (n = 6) ± SD.

Table III. Particle Size of Emulsions* Containing 0.5% Bile Salts

Sample	pH	0 day		90 days†		270 days†	
		Size‡ (nm)	Polydispersity‡	Size‡ (nm)	Polydispersity‡	Size‡ (nm)	Polydispersity‡
Original	6.8	177 ± 2	0.15 ± 0.03	174 ± 2	0.13 ± 0.02	ND§	ND§
Autoclaved	6.7	178 ± 3	0.14 ± 0.03	176 ± 2	0.14 ± 0.03	190 ± 2	0.15 ± 0.01

* Formulation: 20% vitamin E, 2% Lipoid E 80, 0.2% deoxycholic acid, 0.3% sodium deoxycholate, 2.25% glycerol.
† Stored at room temperature (22 ± 2°C).
‡ Mean (n = 6) ± SD.
§ Not determined.

particle size and polydispersity was achieved. There is a pH difference of 0.2 between C_1 and the other two batches, but regarded acceptable. Drug loadings in C_1 and C_2 (2.26 mg/ml) were lower than the target (2.5 mg/ml). This was because the small volumes made the processing losses relatively significant. In sample C_3, to ensure a minimum loading of 2.5 mg was achieved, sufficient paclitaxel was added to raise the theoretical drug content to 2.6 mg/ml and the batch size was increased from 50 to 170 ml.

A small increase in particle size was detected over the 90 day period studied but the size remained at the smaller end of that reported for commercial intravenous emulsions (22). No change in the polydispersity was observed and visual examination confirmed that no creaming occurred over the period studied; an important indication that the integrity of the emulsion had been maintained.

Stability of Emulsions in Plasma

Stability of Bile Salt Stabilized Emulsions

Emulsion stability in plasma is clearly an important factor in intravenous applications. The possible flocculation and potential subsequent coalescence of emulsions in plasma and serum may cause adverse effects, including blocking the lung capillaries (23). As it was envisaged that the next stage study would be carried out using different animal models, the stability of the emulsion in mouse, rat and sheep plasma and serum was studied. Figures 2a and 2b show photomicrographs of a bile salt stabilized vitamin E emulsion, before and after mixing with mouse plasma. Photomicrographs of the original emulsion appear clear because individual droplet size is beyond the resolution of an optical microscope. However, when admixed with mouse plasma, a flocculation network was observed. The phenomenon was also observed in rat plasma and sheep plasma and serum (micrographs not shown). The flocculation was probably caused by the adsorption of plasma proteins onto the emulsion droplets, as it is well known that adsorbed polymers can lead to flocculation in a number of mechanisms including bridging and charge neutralization (24). Since the plasma is a complex of mixture, it is difficult to ascertain which component is causing the flocculation.

Stabilization of Bile Salt Stabilized Emulsions Using Poloxamer 188

To stabilize the emulsion in plasma, poloxamer 188, a PEO-PPO-PEO *co*-block polymer (approved for parenteral application), was added to the bile salt stabilized formulations as a steric stabilizer. Emulsions containing 2% poloxamer 188 did not aggregate when mixed with mouse plasma, rat plasma and sheep plasma and serum (Figs. 2c and 2d). After incubation in plasma and serum at 37°C for 24 h, the droplet size and polydispersity of the poloxamer stabilized emulsions remained unchanged while those not containing poloxamer 188 increased dramatically (Fig. 3). Large droplets (up to 40 μm) and droplet clusters were seen in emulsions incubated in the absence of poloxamer 188 while no change could be detected in poloxamer-containing emulsions. Increased stability was probably a result of the steric barrier formed by the PEO chains of the poloxamer rejecting the adsorption of proteins, as there have been numerous reports that surface coatings with PEO containing polymers can reduce the proteins adsorption (24–27).

Stabilization of Bile Salt Containing Emulsions Using PEG5000PE

There has been considerable interest in the use of pegylated phospholipids as steric stabilizers for parenteral drug

Table IV. Properties of Paclitaxel-Containing Emulsions* Prepared by Microfluidization

Sample	Volume (ml)	pH	Loading of paclitaxel (mg/ml)		0 day		30 days†		90 days†	
			Theoretical	Achieved‡	Size§ (nm)	Poly¶	Size§ (nm)	Poly¶	Size§ (nm)	Poly¶
C_1	50	7.2	2.5	2.26 ± 0.15	204 ± 3	0.20 ± 0.04	210 ± 3	0.22 ± 0.02	230 ± 3‖	0.20 ± 0.05‖
C_2	50	7.4	2.5	2.26 ± 0.02	202 ± 4	0.23 ± 0.02	208 ± 5	0.21 ± 0.03	218 ± 2	0.22 ± 0.02
C_3	170	7.4	2.6	2.59 ± 0.03	194 ± 4	0.23 ± 0.03	206 ± 2	0.22 ± 0.04	211 ± 2	0.18 ± 0.02

* Formulation: Vitamin E 20%, Lipoid E80 2%, deoxycholic acid 0.3%, sodium deoxycholate 0.4%, glycerol 2.25% and paclitaxel as indicated in the table.
† Stored at 4 ± 2°C.
‡ Measured by HPLC, mean (n = 3) ± SD.
§ Mean (n = 6) ± SD.
¶ Polydispersity, mean (n = 6) ± SD.
‖ Determined at 120 days.

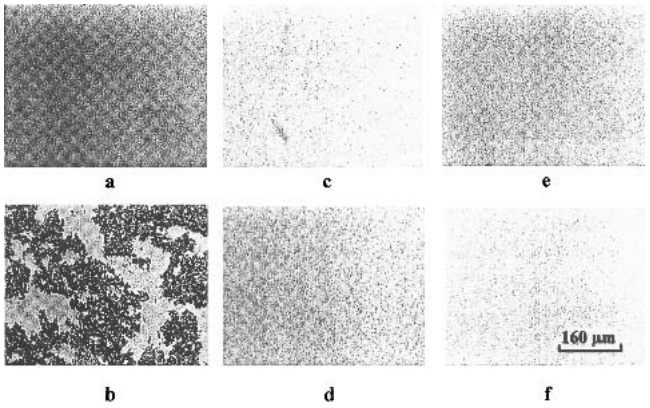

Fig. 2. Photomicrographs of vitamin E emulsions before (a, c, e) and after (d, e, f) mixing with mouse plasma. Formulation: a, b – C_3 in Table IV excluding paclitaxel; c, d – C_3 in Table IV excluding paclitaxel plus 2% poloxamer 188; e, f – C_3 in Table IV excluding paclitaxel with Lipoid E80 replaced by 2% PEG5000PE.

delivery systems such as liposomes (10) and it has been shown that a longer PEG chain within the polymer provides a better protein-rejecting effect (28). A pegylated phospholipid (PEG5000PE (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-[poly (ethylene glycol) 5000]) which has one of the longest PEG chains available, was tested. Figuree 2e and 2f show that an emulsion containing 2% PEG5000PE remained unflocculated in mouse plasma and the particle size and polydispersity remained unchanged after incubation in different plasma and serum at 37°C for 24 h (Fig. 4). PEG5000PE containing emulsions showed good stability at 4°C with no significant increase in droplet size detected in 180 days.

Crystal Growth in PEG5000PE and Poloxamer 188 Stabilized Emulsions

While both poloxamer 188 and PEG5000PE prevented the flocculation of vitamin E emulsions in plasma and serum, poloxamer 188 facilitated crystal growth in drug loaded emulsions. In emulsions containing 2% poloxamer 188 at paclitaxel loading of 2.5 mg/ml, crystals could be detected in a few days. We suspect this was possibly related to the micelle forming properties of the block copolymer. Poloxamer 188 is freely soluble in water but it is generally accepted that PEO-PPO-PEO copolymers form micelles in aqueous solutions above certain concentrations and temperatures (29). It is suggested that these micelles may increase the drug solubility in the aqueous phase and therefore facilitate the transfer of the drug from the oil phase into the aqueous phase and the conversion of the solubilized drug into crystals. Another possibility is that the poloxamer 188 may interact with the oil phase and the oil/water interface so that the drug solubility in the internal phase and the release rate through the interfacial film are altered.

Crystals were not detected at the same loading of paclitaxel in PEG5000PE stabilized emulsions which be a result of its different lipophilicity. The lipophilic segment of two fatty acid chains is more hydrophobic than the PPO segment of poloxamer 188. This will tend to increase the adsorption of the PEG5000PE on to oil droplets and reduce the overall

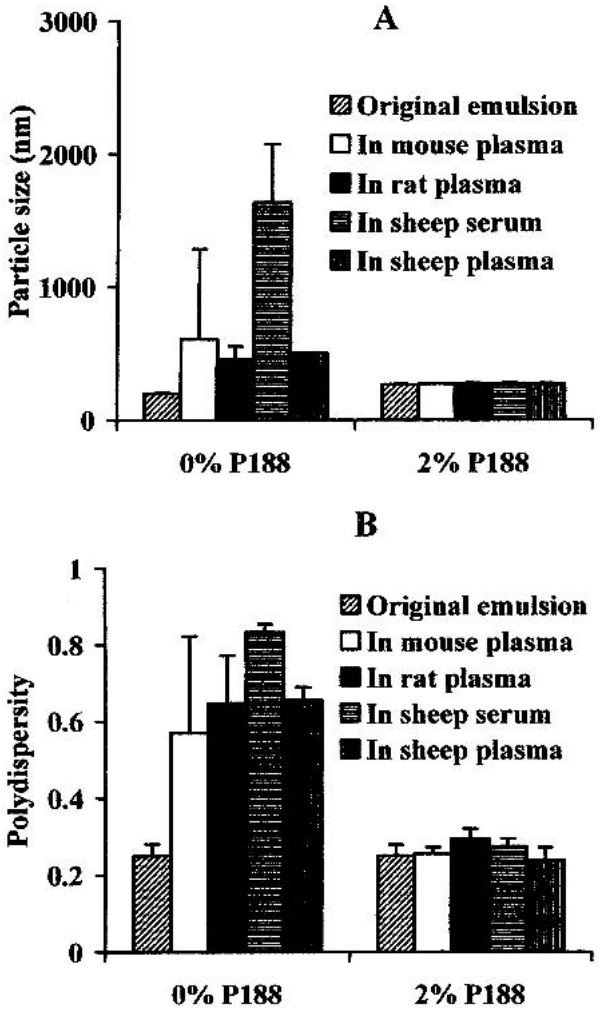

Fig. 3. Stability of vitamin E emulsions in plasma and serum with and without poloxamer 188 (P188) after 24 h incubation at 37°C. Formulation: 0% P188 – C_3 in Table IV excluding paclitaxel; 2% P188: C_3 in Table IV excluding paclitaxel plus 2% poloxamer 188. Mean (n = 6) + sd.

solubility of PEG5000PE in water. Future work is required to ascertain the mechanisms.

CONCLUSIONS

Among a range of parenteral emulsifiers studied, mixtures of lecithin/sodium oleate or lecithin/sodium deoxycholate as the emulsifiers produced the most stable vitamin E emulsions in terms of particle size. However, emulsions made with sodium oleate had high pH values unsuitable for parenteral applications and were unstable near neutral pHs. Bile salt stabilized emulsions did not show such pH sensitivities and formulation of sodium deoxycholate stabilized emulsions could be further improved by using a combination of free acid (added to the oil phase) and the sodium salt (added to the aqueous phase). Paclitaxel could be loaded into the emulsions at 2.5 mg/ml without the formation of drug crystals.

However, while such emulsions were stable under storage conditions, they flocculated when mixed with plasma. The incorporation of poloxamer 188 or a pegylated phospholipid, PEG5000PE, effectively stabilized the vitamin E emulsion

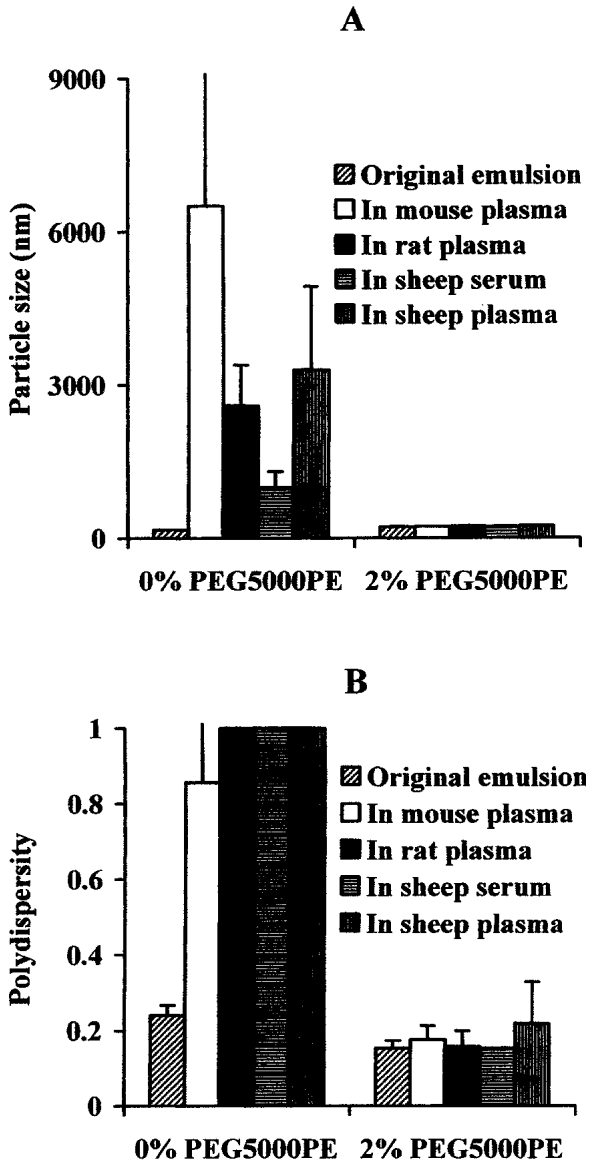

Fig. 4. Stability of vitamin E emulsions containing 2% PEG5000PE in plasma and serum after 24 h incubation at 37°C. Formulation: 0% PEG5000PE – C_3 in Table IV excluding paclitaxel; 2% PEG5000PE – C_3 in Table IV excluding paclitaxel with Lipoid E80 replaced by 2% PEG5000PE. Mean (n = 6) + SD.

against plasma and serum, possibly as a result of the steric barrier of PEO chains being able to reduce plasma protein adsorption. Unfortunately poloxamer 188 also facilitated the crystallization of paclitaxel, possibly a result of the micelle-facilitated transport of paclitaxel from the oil phase to the aqueous phase. PEG5000PE, on the other hand, did not facilitate the crystallization of paclitaxel.

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