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The physical stability of thermally-stressed phospholipid-based emulsions containing methyl, propyl and heptyl parabens as model drugs

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Abstract

The objectives of the studies presented herein was to investigate the mechanisms of emulsion instability under thermal stress (121 °C) by evaluating the effects of a lipophilic drug dissolved in the internal phase of an oil-in-water (o/w) emulsion on growth rate suppression and the apparent microviscosity. Model drugs used were methyl, propyl and heptyl paraben. The o/w emulsions were prepared using medium chain triglycerides as an internal phase in aqueous glycerol solutions emulsified with phospholipids. Concentrations of paraben in the internal phase varied from 0.2–0.8 M. Microfluidization was used to reduce the droplet size to the submicron range. Microviscosity was calculated from the measured anisotropy of a fluorophore probe (1,6-phenyl-1,3,5-hexatriene) using a modified Perrin's equation. Emulsion aliquots were subjected to thermal stressed at 121 °C the droplet growth rate was determined from periodic measurements of the mean droplet diameter using photon correlation spectroscopy. The growth rate decreased in the presence of parabens. Maximal growth suppression occurred at paraben concentrations of 0.4 M. However in deference to theoretical predictions of the effects of increasing co-solute concentrations based on Ostwalt ripening, the droplet growth rates increased at concentrations greater than 0.4 M. The logarithm of the growth rate was linearly correlated to the interfacial rigidity (inverse microviscosity) of the emulsion which suggests that coalescence rather than molecular diffusion was primarily responsible for emulsion instability under the conditions studied.
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1. Introduction

Phospholipid-based emulsions have been the subject of a number of studies evaluating their potential as drug delivery systems (Simamora et al., 1998; Prankerd et al., 1988; Stella et al., 1988; Levy and Benita, 1989, 1991). Oil-in-water (o/w) emulsions can be used for the delivery of hydrophobic and lipophilic drugs. The latter partition into the internal oil phase when incorporated into the emulsions. It has been reported that the inclusion of drugs at relatively low concentration may not affect the physical stability of the emulsion, for example, Levy and Benita (1989) reported that the emulsion system containing 0.018 M diazepam has similar physical properties to drugless

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emulsion. Other investigators (Yamamura et al., 1991) found that forskolin-loaded emulsion at concentration of 2.44×10^{-4} M is stable up to at least 30 days at room temperature.

Irreversible emulsion instability involves droplet growth and phase separation. Two mechanisms are primarily associated with droplet growth: coalescence and molecular diffusion (Oswald ripening).

Coalescence is the process by which the interfacial liquid film between two droplets ruptures causing the droplets to merge. The process begins by adhesive contact between the two droplets. The interfacial film drains, ruptures and the droplets fuse and merge.

Film rupture can be viewed as a process induced by the self-propagation of a disturbance (Tadros and Vincent, 1983; Walstra, 1996) externally induced or induced by surface waves. Film rupture usually starts at a specific turbulent location. The stability of the film is also kinetically associated with thermal or mechanical fluctuations. The influence of emulsifier concentration and properties on film thinning and the duration of droplet encounters have attracted theoretical studies (Ivanov et al., 1979; Ivanov, 1980). Several researchers have attempted to correlate emulsion stability to interfacial tension and interfacial viscosity (microviscosity). Dickinson et al. (1988) studied the coalescence of oil droplets at a planar *n*-hexadecane/water interface by measuring their coalescence time as function of the surfactant used and interfacial tension. No direct correlation existed between interfacial tension and emulsion stability. A similar conclusion was also made by other researchers (Lee and Tadros, 1982a,b).

There is strong evidence that interfacial viscosity plays a significant role in determining the coalescence rate of emulsion droplets (Dickinson et al., 1988; Jones and Wilson, 1978). The higher the interfacial viscosity, the lower the coalescence rate and the more stable the emulsion. This observation can be explained by the effect of interfacial viscosity in determining the drainage and stability of the thin liquid films. High interfacial viscosity lowers the rate of drainage of the film, which results in increased stability of the droplets.

The rate of coalescence is proportional to the number of oil droplets collisions. The coalescence rate (v) of the oil droplets in o/w emulsion is expressed by the following equation (Lawrence and Mills, 1954):

$$v = \frac{\mathrm{d}\bar{V}}{\mathrm{d}t} = \left(\frac{4\phi kT}{3\eta_{\mathrm{w}}}\right) \mathrm{e}^{-E/RT}$$

where $\bar{V}$ is the mean volume of the droplets, ϕ is the phase volume ratio, k is Bolzmam constant, and η_{w} is viscosity of external phase. The pre-exponential term reflects the frequency of collision between droplets and is proportional to the concentration (i.e. volume fraction) of the internal phase and inversely proportional to the viscosity of the external phase. The exponential term denotes the fraction of interacting droplets that have sufficient energy for coalescence. The exponential coefficient, E, is the interfacial energy barrier to coalescence. Besides the phase volume ratio and the external phase viscosity, the energy barrier determines the rate of coalescence of droplets, and hence, the stability of the emulsion. The energy barriers include the electric double layer and steric repulsion attributed to adsorbed surfactants when droplets approach each other. The barrier of 20 kT can result in a half-life of 4 years of an emulsion, which is sufficient for pharmaceutical applications (Fridberg et al., 1996). Yamaguchi et al. (1995a,b) studied the coalescence of parenteral phospholipid emulsions in the temperature range of 100–180 °C. The activation energy of phospholipid-based emulsions estimated from an Arrhenius treatment of isothermal coalescence rate constants was in the range 90–195 kJ/mol.

Ostwald ripening is a diffusional process in which molecules of the dispersed phase diffuse from small droplets to large droplets causing the large droplets to grow while smaller droplets shrink. The chemical potential of the internal phase material is inversely related to the radius of droplet curvature so small droplets tend to dissolve more readily into the external phase and diffuse and re-deposit onto a larger droplet causing in an overall increase in the mean diameter of the emulsion (Kabalnov and Shchukin, 1992). It has been experimentally demonstrated that Ostwald ripening occurs in fluorocarbon and hydrocarbon emulsions (Davis et al., 1981; Kabalnov et al., 1987a,b, 1990).

Equations which describe the rate of Ostwald ripening in dilute systems have been developed by Lifshitz and Slyozov (1961) and by Wagner (1961). According

to LSW theory

$$\omega = \frac{d\bar{r}^3}{dt} = \frac{8\gamma DCV_m}{9RT}$$

where ω is the Ostwald ripening rate, γ is the interfacial tension at the o/w interface, D, C, and V_m are the diffusion coefficient, the bulk solubility, and the molar volume of the dispersed phase, respectively, R is the gas constant and T is the absolute temperature. This model was first proposed for saturated solid solutions and then was verified for emulsions (Kabalnov and Shchukin, 1992; Kabalnov et al., 1987a; Higuchi and Misra, 1962).

For an emulsion in which the dispersed phase is composed a single component, the chemical potential is greater in small droplets. According to Raoult's law, the vapor (capillary) pressure in the small droplet decreases. Consequently, the pressure difference (ΔP) across the emulsifier film increases and the smaller droplet dissolves. Raoult's law can be expressed as:

$$\mu_A = \mu_A^o + RT \ln x_A$$

where μ_A and μ_A^o are chemical potential of component A in mixture and pure component A, respectivly, and x_A is molar fraction of component in mixture.

Ostwald ripening can be slowed by adding a second component which has a low solubility in the external phase. Higuchi and Misra (1962) have shown that if one of the components in the dispersed phase is very insoluble in the external phase, then even a small amount of this component tends to decrease the Ostwald ripening rate. Mass transfer from smaller droplets to large ones changes their composition. It increases the molar fraction of the poorly soluble component in small droplets and decreases the molar fraction of this component in large droplets. Thus, the chemical potential drop of the second component in the small droplets is less than that in large droplets. This effect compensates for the chemical potential drop of the first component in the droplets. When the concentration effect completely compensates for the chemical potential, the mass transfer terminates and the rate of the Ostwald ripening becomes equal to zero. The following equation can be used to calculate Ostwald ripening rate in a two-composition system:

$$\omega = \frac{d\bar{r}^3}{dt} = \frac{8\gamma D_2 C_2 V_{m2}}{9\phi_2 RT}$$

where D_2, C_2, and ϕ_2 are the diffusion coefficient, the bulk solubility, and molar fraction of the second component. V_{m1} is molar volume of the first component. If the solubility of the two components is significantly different, then the total ripening rate is given by

$$\omega = \frac{1}{(\phi_1/\omega_1) + (\phi_2/\omega_2)} \quad (1)$$

where ω_1, ω_2 and f_1 and f_2 refer to the ripening rate and molar fraction of components 1 and 2, respectively and the presence of the second component slows the overall Ostwald ripening rate. Several experiments have confirmed the decrease in the ripening rate on addition of a second component to the dispersed phase (Kabalnov et al., 1987a; Buscall et al., 1979).

1.1. Study objectives

The volume-based mean droplet size as determined using photon correlation spectroscopy (PCS) linearly increases in phospholipid-based emulsions subjected to thermal stress (Yamaguchi et al., 1995a; Zhang and Kirsch, 2003). The droplet growth rate correlates to microviscosity as measured by fluorescence polarization (Zhang and Kirsch, 2003) which suggests that the predominant instability mechanism under extreme thermal stress (>120 °C) is coalescence rather than molecular diffusion.

The effects of the addition of a second component (such as a lipophilic drug) to the internal phase may be useful in distinguishing between coalescence- or molecular diffusion-mediated emulsion instability. According to the molecular diffusion models described by Higuchi and Misra (1962), the second component in the internal phase may decrease the droplet growth rate in accordance with the effects of Raoult's law whereby the mass transfer of the major component from small to large droplets caused by the difference in the capillary pressure increases the second component concentration in the small droplet and decreases it in the large ones. This results in a compensation of the chemical potential drop between the droplets.

The objectives of the studies presented herein are to test the hypothesis that droplet growth in emulsions containing model drugs occurs primarily by a molecular diffusion. In addition, the apparent microviscosity of these emulsions were determined to investigate the effects of incorporating drugs on the interface rigid-

ity. Model drugs used were methyl, propyl and heptyl paraben.

2. Methods and materials

2.1. Materials

p-Hydroxybenzoic acid methyl ester (methyl paraben), 1,6-phenyl 1,3,5-hexatriene (DPH) and *p*-hydroxybenzoic acid propyl ester (propyl paraben) were purchased from Sigma Chemical Co. (St. Louis, MO). *p*-Hydroxybenzoic acid heptyl ester (heptyl paraben) was obtained from Pfaltz & Bauer, Inc., Waterbury, CT. Acetonitrile (HPLC grade), tetrahydrofuran (THF), hydrochloric acid (0.10 N, certified), sodium hydroxide (0.10 N, certified), sodium phosphate dibasic, 85% phosphoric acid, and glycerin (certified, ACS.) were purchased from Fisher Scientific, Pittsburgh, PA. Medium chain triglycerides (MCT) were donated by Croda Inc., Parsippany, NJ. The phospholipid (Ovothin 160) was donated by Lucas Meyer Inc., Decatur, IL. Water for injection (WFI) was purchased from Abbott Laboratories, North Chicago.

2.2. Emulsion preparation

O/W emulsions were prepared using 20% (w/w) MCT as oil phase in 2.21% (w/w) glycerin solutions emulsified with 1.2% (w/w) phospholipid. Parabens and phospholipids were dissolved in MCT oil at concentrations of 0.2, 0.4, 0.6, or 0.8 M by warming at 70 °C and stirred using a laboratory hot plate/stirrer. Glycerin was dissolved in water for injection (WFI). Both solutions were filtered and then a coarse emulsion was formed using a homogenizer at 3000 rpm for 3 min. Microfluidization (Model MT 100, Microfluidics, Newton, MA) was used to reduce the droplet size to the submicron range. Twelve microfluidization cycles were used at 7000 psi. The initial pH of the emulsions was adjusted with 0.1 N NaOH or HCl to pH 7.4 or 4.0. The emulsions were sealed in 1-ml glass ampoules. The compositions of the emulsions are listed in Table 1.

2.3. Analytical methods

The droplet growth rate was measured by placing emulsion aliquots in 1-ml glass ampoules which were immersed in a thermally equilibrated oil bath at 121 °C. Ampoules were removed periodically and rapidly cooled to room temperature with ice. The volume-based mean diameter of emulsion oil droplets was measured by PCS (Nicomp Model 380/ZLS) detecting scattered light at 90 °C relative to an incident HeNe laser source ($\lambda = 632.8$ nm). The instrument was calibrated with polystyrene microspheres. The emulsion sample was briefly mixed and diluted 500

Table 1
Summary of emulsion composition, microviscosity measurements and the effects of thermal stress (121 °C) on the emulsion droplet growth rate for a series of emulsion composed of 20% (w/w) MCT dispersed in 2.21% (w/w) glycerin solutions emulsified with 1.2% (w/w) phospholipid

Paraben	Paraben concentration (M)	pH	Droplet growth rate (μm^3/min)	Microviscosity ± S.D. (cP)
None	0	4.0	2.33×10^{-5}	43.98 ± 0.89
		7.4	2.29×10^{-6}	45.63 ± 1.22
Methyl	0.4	4.0	6.03×10^{-6}	46.51 ± 0.50
		7.4	1.38×10^{-6}	45.64 ± 0.20
Propyl	0.2	4.0	6.44×10^{-6}	43.90 ± 0.28
	0.4	4.0	3.4×10^{-6}	47.14 ± 0.25
		7.4	6.75×10^{-7}	43.90 ± 0.28
	0.6	4.0	9.15×10^{-5}	39.62 ± 0.59
Heptyl	0.2	4.0	3.63×10^{-6}	43.43 ± 0.56
	0.4	4.0	1.36×10^{-6}	47.40 ± 0.95
		7.4	6.03×10^{-7}	47.14 ± 0.25
	0.6	4.0	1.05×10^{-4}	39.93 ± 0.78
	0.8	4.0	5.76×10^{-4}	35.36 ± 0.50

times with the filtered de-ionized and double-distilled water before each measurement. The oil droplet growth rate was estimated from the slope of the linear regression of the mean volume-based radius versus time.

Microviscosity was determined by mixing 1 μl of emulsion and 10 μl of 1 mM DPH solution with 10 ml of double distilled filtered water and agitating for 10 min. Fluorescence intensities of DPH in the diluted emulsions were measured in triplicate at the excitation and emission wavelengths of 354 and 427 nm using a spectrofluorometer (Kontron SFM 25, Kontron Instruments Spa, Milano, Italy). Temperature of the sample holder in the spectrofluorometer was controlled at 25 °C with a water bath. Anisotropy (r) was calculated from the fluorescence intensities using

$$r = \frac{I_{VV} - (I_{HV}/I_{HH})I_{VH}}{I_{VV} + 2(I_{HV}/I_{HH})I_{VH}}$$

where I_{VV} is represented as vertically polarized excitation and vertically polarized emission, I_{VH} as vertically polarized excitation and horizontally polarized emission, I_{HV} as horizontally polarized excitation and vertically polarized emission, and I_{HH} as horizontally polarized excitation and horizontally polarized emission. Microviscosity ($\bar{\eta}$) was calculated with the modified Perrin's equation

$$\bar{\eta} = \frac{2.4r}{0.362 - r}$$

which has been shown to be applicable to phospholipid-based emulsions (Zhang and Kirsch, 2003).

3. Results

3.1. Kinetics of droplet growth

The mean droplet diameter for each emulsion was determined by triplicate PCS measurements and used to calculate volume-based mean radius. Under thermal stress (121 °C), the volume-based mean radius linearly increased as function of time with the squared correlation coefficient value greater than 0.90 (Table 1). The droplet growth rates for pH 4.0 emulsions were typically two- to five-fold greater than corresponding emulsions prepared at pH 7.4. The rate of droplet growth decreased in the presence of parabens (Table 1) although the magnitude of the effect in pH 7.4 emulsions was much less pronounced than in pH 4.0 emulsions.

The effect of paraben concentration on growth rate suppression was similar for both propyl and heptyl pararben. The maximum droplet growth rate suppres-

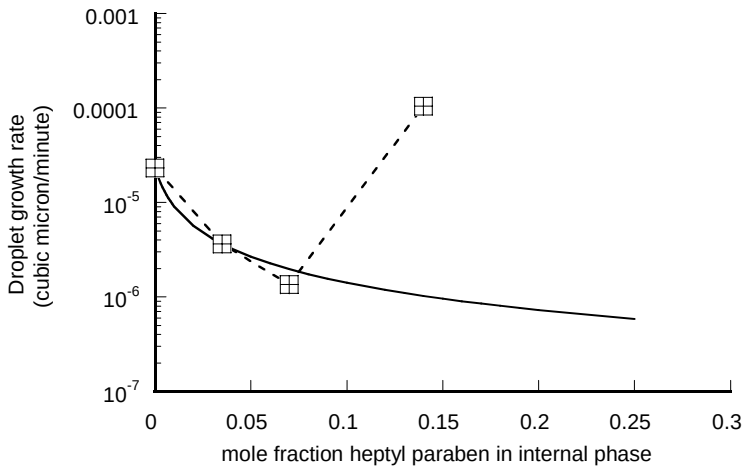

Fig. 1. Droplet growth rate as a function of the mole fraction of heptyl paraben in internal phase. Curve was generated from Eq. (1) wherein ω_1 was determined using an emulsion prepared without heptyl paraben and ω_2 was assigned a value equal to $\omega_1/155$ to allow the predicted growth rate values to be in reasonable agreement with the observed growth rates at the lowest molar fractions of paraben.

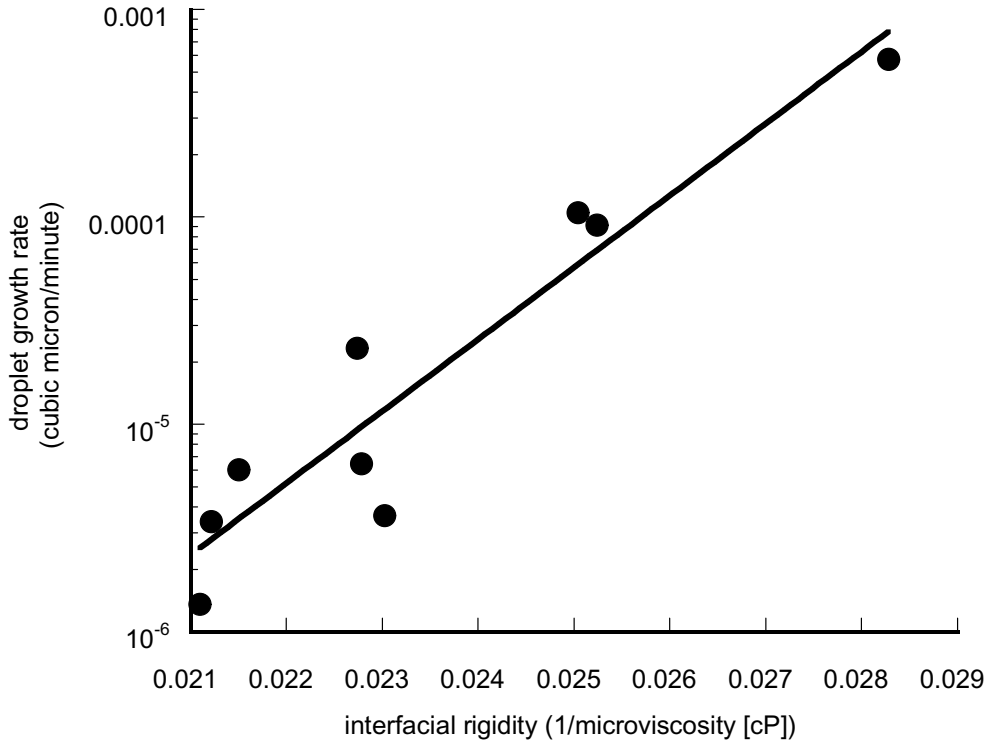

Fig. 2. Correlation between the droplet growth rate at 121 °C and the inverse microviscosity (or rigidity) of various PL-based emulsions prepared using 20% (w/w) MCT dispersed in 2.21% (w/w) glycerin solutions emulsified with 1.2% (w/w) phospholipids and pH adjusted to 4.0. A correlation coefficient of 0.992 was obtained by linear regression.

sion occurred at 0.4 M. The growth rate was greater at lower and higher concentrations for both propyl and heptyl paraben (Fig. 1).

The microviscosities of pH 4.0 and 7.4 emulsions were measured by fluorescence polarization. All of the measured values fell within the range of 48–36 cP and the relative standard deviations for triplicate measurements were <3% in all cases. The highest viscosity values were obtained from emulsions containing 0.4 M parabens (Table 1). The logarithm of the droplet growth rate was linearly correlated to the interfacial rigidity (inverse viscosity) for all emulsions (Fig. 2).

4. Discussion

The objectives of the studies presented herein are to test the hypothesis that droplet growth in emulsions containing model drugs occurs primarily by a molecular diffusion. In addition, the apparent microviscosity of these emulsions were determined to investigate the effects of incorporating drugs on the interface rigidity. Model drugs used were methyl, propyl and heptyl paraben.

The central hypothesis of the studies reported herein is that the addition of lipophilic drugs to the internal phase of the model emulsion should stabilize its physical stability in a manner consistent with a molecular diffusion mechanism. As described by Higuchi and Misra (1962), the second component in the internal phase will decrease the droplet growth as predicted by Raoult's law whereby the mass transfer of the major component from small to large droplets caused by the difference in the capillary pressure increases the second component concentration in the small droplet and decreases it in the large ones. This results in a compensation of the chemical potential drop between the

droplets which according to Eq. (1), predicts that the effect of adding a second component is to decrease the droplet growth rate as a function of increasing second component concentration until a minimum which is associated with the growth rate of droplets composed solely of the second component.

The relationship obtained in our studies (Fig. 1) did decrease with increasing paraben concentrations up to 0.4 M. However at paraben concentrations above 0.4 M growth rates increased in contradiction to theoretical predictions suggesting that additional effects of the second component on the properties of the emulsion may be important.

Another possibility is that the primary effect of drugs added to the internal phase is to modify the interfacial properties of the phospholipids. We observed that emulsions loaded with 0.4 M parabens in the MCT oil phase were more stable than paraben-free emulsions at both initial pH values of 4.0 and 7.4. The low droplet growth rate of the paraben-loaded emulsions may be due to the effects of added drugs on the rigidity of the phospholipid layer as reflected in the correlation depicted in Fig. 2. Drug interactions with biomembranes are well known. For example, the presence of methyl or propyl paraben in dipalmitoyl phosphatidylcholine (DPPC) bilayers (0.1 M ratio of drug to lipid) broadened the chain-melting transition and inhibits the pretransition as measured using DSC (Deniz et al., 1996). This observation indicated that paraben interacted with the phospholipid bilayers, changing their gel phase structure. The phenolic hydroxyl group in paraben has been shown to hydrogen bond to the phospholipid head group as determined using ^{1}H NMR (Deniz et al., 1996). The interaction of the aromatic protons with the glycerol moiety of DPPC molecule was indicated by a broadening of two aromatic proton resonances. The alkyl groups could be embedded in the hydrophobic (acyl chain) region of the phospholipid layer. In the current study, significant increases ($P < 0.05$ in paired t-test) in the microviscosity of 0.4 M paraben-loaded versus drug-free emulsions (pH 4.0) were observed. This increase in apparent rigidity of the phospholipid layer may be due to the interaction between phospholipids and paraben.

The influence of incorporated compounds on emulsion microviscosity is dependent on their concentration and structure. The addition of some compounds may decrease the microviscosity of lipid systems, which is concentration-dependent. It was found that the microviscosity of the lipid bilayer of dipalmitoylphosphatidylcholine (DPPC) decreased from 129 cP for pure DPPC liposomes to 119 cP for liposomes with 0.5 mM of lidocaine HCl at 25 °C. The microviscosity further diminished to 110, 101, 93, and 88 cP as the concentration of lidocaine HCl increased from 0.7 to 1.0, 2.0, and 3.0 mM (Han et al., 1990). In this case, the drug molecules may be located in the region close to the hydrophilic head of the phospholipids because the unshared pair of electrons of the nitrogen on the alkylamine of lidocaine HCl can form a hydrogen bond with water. The highly concentrated drug influences the lateral distance and fluid property of the phospholipid layer, resulting in a decrease in the microviscosity of the phospholipid layer. It was also observed that *n*-alkanols have fluidizing effects on the lipid bilayer of liposome made of DPPC due to formation of hydrogen bonds (Han et al., 1990). Consequently, the microviscosity decreased from 129 cP for pure DPPC liposome to 112 cP for the mixture of liposome–methanol at 300 mM. The microviscosity was also lowered to 106 cP for a mixture of liposome–*n*-propanol at 200 mM and to 91 cP for the mixture of liposome–*n*-heptanol at 3.0 mM (25 °C). Parabens in certain concentration ranges may have similar effects on the microviscosity of the emulsions.

References

Buscall, R., Davis, S.S., Potts, D.C., 1979. The effect of long-chain alkanes on the stability of oil-in-water emulsions. The significance of Ostwald ripening. Colloid Polym. Sci. 257, 636–644.

Davis, S.S., Round, H.P., Purewal, T.S., 1981. Ostwald ripening and the stability of emulsion systems: an explanation for the effect of an added third component. J. Colloid Interface Sci. 80, 508–511.

Deniz, K.U., Panicker, L., Parvathanathan, P.S., Datta, F., Vivekanandan, S., Ramanathan, K.V., Khetrapal, C.L., 1996. Drug-lipid interaction in model membrane DPPC-water: a DSC and proton NMR study. Mol. Cryst. Liq. Cryst. 288, 237–247.

Dickinson, E., Murray, B.S., Stainsby, G., 1988. Coalescence stability of emulsion-sized droplets at a planar oil-water interface and the relationship to protein film surface rheology. J. Chem. Soc., Faraday Trans. 1 84, 871–883.

Fridberg, S.E., Quencer, L.F., Hilton, M.L., 1996. Theory of emulsions. In: Lieberman, H.A., Rieger, M.M., Banker, G.S.,

(Eds.), Pharmaceutical Dosage Forms: Disperse Systems, 2nd ed. vol. 1. Marcel Dekker Inc., New York, pp. 53–90.

Han, S.K., Kim, J.S., Lee, Y.S., Kim, M., 1990. Effect of drug substances on the microviscosity of lipid bilayer of liposomal membranes. Arch. Pharm. Res. 13, 192–197.

Higuchi, W.I., Misra, J., 1962. Physical degradation of emulsions via the molecular diffusion route and the possible prevention thereof. J. Pharm. Sci. 51, 459–466.

Ivanov, I.B., 1980. Effect of surface mobility on the dynamic behavior of thin liquid films. Pure Appl. Chem. 52, 1241–1262.

Ivanov, I.B., Jain, R.K., Somasundara, P., Traykov, T.T., 1979. The role of surfactants on the coalescences of emulsion droplets. In: Mittal, K.L. (Ed.), Chemistry of Surfactants, vol. 2. Plenum Press, New York, pp. 817–840.

Jones, A.F., Wilson, S.D.R., 1978. The film dranage problem in droplet coalescence. J. Fluid Mech. 87, 263–288.

Kabalnov, A.S., Shchukin, E.D., 1992. Ostwald ripening theory: application to fluorocarbon emulsion stability. Adv. Colloid Interface Sci. 38, 69–97.

Kabalnov, A.S., Pertzov, A.V., Shchukin, E.D., 1987a. Ostwald ripening in emulsions 1. Direct observations of Ostwald ripening in emulsions. J. Colloid Interface Sci. 118, 590–597.

Kabalnov, A.S., Pertzov, A.V., Shchukin, E.D., 1987b. Ostwald ripening in two-component disperse phase systems: application to emulsion stability. Colloids Surf. 24, 19–32.

Kabalnov, A.S., Makarov, K.N., Pertzov, A.V., Shcherbakova, O.V., 1990. Solubility of fluorocarbons in water as a key parameter determining fluorocarbon emulsion stability. J. Fluorine Chem. 50, 271–284.

Lawrence, A.S.C., Mills, O.S., 1954. Kinetics of the coagulation of emulsions. Faraday Discuss. Chem. Soc. 18, 98–104.

Lee, G.W.J., Tadros, T.F., 1982a. Formation and stability of emulsions produced by dilution of emulsifiable concentrates. Part II. The influence of surfactant concentration on stability of oil-in-water emulsions. Colloid Surf. 5, 117–127.

Lee, G.W.J., Tadros, T.F., 1982b. Formation and stability of emulsions produced by dilution of emulsifiable concentrates. Part III. The coalescence of oil droplets at a planar oil-water interface. Colloid Surf. 5, 129–135.

Levy, M.Y., Benita, S., 1989. Design and characterization of submicronized o/w emulsion of diazepam for parenteral use. Int. J. Pharm. 54, 103–112.

Levy, M.Y., Benita, S., 1991. Short- and long-term stability assessment of a new injectable diazepam submicron emulsion. J. Parenter. Sci. Technol. 45, 101–107.

Lifshitz, I.M., Slyozov, V.V., 1961. The kinetics of precipitation from supersaturated solid solutions. J. Phys. Chem. Solids. 19, 35–50.

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

Simamora, P., Dannenfelser, R.M., Tabibi, S.E., Yalkowsky, S.H., 1998. Emulsion formulations for intravenous administration of paclitaxell. PDA J. Pharm. Sci. Tech. 52, 170–172.

Stella, V.J., Umprayn, K., Waugh, W.N., 1988. Development of parenteral formulations of experimental cytotoxic agents. I. Rhizoxin (NSC-332598). Int. J. Pharm. 43, 191–199.

Tadros, T.F., Vincent, B., 1983. Emulsion stability. In: Becher, P. (Ed.), Encyclopedia of Emulsion Technology. Marcel Dekker, New York, vol. 1. pp. 130–278.

Wagner, C., 1961. Theorie der alterrung von niederschlagen durch umlosen. Zeit. Elektrochem 65, 581–591.

Walstra, P, 1996. Emulsion stability. In: Becher, P. (Ed.), Encyclopedia of Emulsion Technology, vol. 4. Marcel Dekker, New York, pp. 1–61.

Yamaguchi, T., Nishizaki, K., Itai, S., Hayashi, H., Ohshima, H., 1995a. Physicochemical characterization of parenteral lipid emulsion: determination of Hamaker constants and activation energy of coalescence. Pharm. Res. 12, 342–347.

Yamaguchi, T., Nishizaki, K., Itai, S., Hayashi, H., Ohshima, H., 1995b. Physicochemical characterization of parenteral lipid emulsion: influence of cosurfactants on flocculation and coalescence. Pharm. Res. 12, 1273–1278.

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

Zhang, X., Kirsch, L.E., 2003. An Assessment of techniques for evaluating the physical stability of parenteral emulsions. PDA J. Pharm. Sci. Tech. 57, 83–92.

Zhang, X., Kirsch, L.E., 2003. Correlation of the thermal stability of phopholipid-based emulsions and microviscosity measurements using fluorescence polarization. Pharm. Dev. Tech., in press.