Effect of Nonionic Surfactant on Transport of Surface-Active and Non-Surface-Active Model Drugs and Emulsion Stability in Triphasic Systems

Submitted: June 13, 2000; Accepted: August 17, 2000; Published: September 20, 2000.

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ABSTRACT The effect of surfactant concentration on transport kinetics in emulsions using surface-active (phenobarbital, barbitol) and non- surface-active (phenylazoaniline, benzocaine) model drugs is determined. Mineral oil was chosen as the oil phase and the nonionic surfactant polyoxyethylene-10-oleyl-ether (Brij 97) was chosen as the emulsifier. Model drug transport in the triphasic systems was investigated using side-by-side diffusion cells mounted with hydrophilic dialysis membranes (molecular weight cutoffs 1 kd and 50 kd) and a novel bulk equilibrium reverse dialysis bag technique. Emulsion stability was determined by droplet size analysis as a function of time, temperature, and the presence of model drugs, using photon correlation spectroscopy. Mineral oil/water (O/W) partition coefficients and aqueous solubilities were determined in the presence of surfactant. The transport rates of model drugs in emulsions increased with an increase in Brij 97 micellar concentrations up to 1.0% wt/vol and then decreased at higher surfactant concentrations. The transport profiles of the model drugs appeared to be governed by model drug O/W partition coefficient values and by micellar shape changes at higher surfactant concentrations.

Total transport rates of phenobarbital and barbitol were faster than those of phenylazoaniline and benzocaine. Excess surfactant affected the transport rates of the model drugs in the emulsions depending on drug surface activity and lipophilicity.

INTRODUCTION

An emulsion is a thermodynamically unstable system consisting of at least 2 immiscible liquid phases, one of which is dispersed as droplets in the other. The thermodynamic instability of emulsion systems is a consequence of the high interfacial free energy that exists between the 2 phases. This free energy is the driving force for droplet coalescence and eventual phase separation. Surfactants are added to improve emulsion stability by decreasing interfacial free energy and providing a mechanical barrier to droplet coalescence and Ostwald ripening (1). Collision of dispersed phase droplets with each other or with the walls of the container can lead to thinning and rupture of the surfactant interfacial film. Consequently, droplet coalescence and eventual phase separation will occur. This effect can be overcome by the presence of excess surfactant in the bulk, which replenishes the interface when the film ruptures or thins (2). Therefore, excess surfactant is usually present in emulsion systems and may be in the form of monomers, micelles, and liquid crystals. This excess surfactant may affect drug transport in emulsion systems through micellar solubilization and change in emulsion droplet interfacial film characteristics.

Drugs may possess surface-active characteristics and associate at the mineral oil/water (O/W) interface. Surface-active drugs will reduce interfacial tension and consequently may aid emulsion stability. In the presence of surfactants, surface-active drugs may increase or decrease surface tension, depending on the nature of the interaction between the drug and the surfactant. A favorable drug/surfactant interaction will result in a reduction in interfacial tension, and an unfavorable interaction will result in an increase in interfacial tension (3). Therefore, in the presence of surfactant, surface-active drugs may enhance or reduce emulsion stability. To form micelles, surface-

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active moieties should possess a minimum of 8 carbon atoms in the lipophilic part of the molecule. Although surface-active drugs may not meet this requirement, they may form mixed micelles with the surfactant. Hence, surfactant concentration may affect the transport of surface-active drugs in emulsion systems.

The effect of concentration of a nonionic surfactant on surface-active and non- surface-active model drug transport rates and emulsion stability in triphasic (oil, water, and micellar) systems was investigated. Mineral oil was selected as the oil phase because it does not contain any surface-active components. The nonionic surfactant, polyoxyethylene-10-oley-ether (Brij 97), was selected because it forms relatively stable emulsions of mineral oil in water (3). Phenylazoaniline (PAA) and benzocaine (BZ) were selected as non- surface-active model drugs for 3 reasons: their molecular weights are similar; each contains a benzene moiety; and to enable us to compare our data with those of Yoon and Burgess (3). Phenobarbital (PB) and barbital (B) were selected as surface-active model drugs because they have molecular weights comparable to those of PAA and BZ (Figure 1). All the model drugs have different lipophilicities. PAA has a molecular weight (MW) of 197.2 dalton and is slightly soluble in water. The approximate solubility of PAA is 29 mg/L, and its pKa (negative logarithmic values of ionization constant) value is 4.4. BZ, the ethyl ester of p-aminobenzoic acid, has a molecular weight of 165.2 dalton. The pKa value of BZ is 2.5, and therefore it exists in the nonionized form at pH 7.0. The approximate solubility of BZ is 0.4 g/L in water at 25° C and pH 7.0. PB has a molecular weight of 232.2 dalton and is a weak acid with a pKa value of 7.5. B has a molecular weight of 184.2 dalton and a pKa value of 7.8. The solubilities of PB and B are 1.0 and 7.5 mg/mL in water at 25° C respectively.

MATERIALS AND METHODS

Materials

Mineral oil, sodium chloride, sodium phosphate monobasic, and hydrophilic Spectrapor® 7 dialysis membranes and dialysis bags (MW cutoffs 1 kd and

Figure 1. Chemical structures of phenobarbital, barbital, phenylazoaniline, and benzocaine.

50 kd) were purchased from Fischer Scientific (Springfield, NJ). Brij 97 was a gift from ICI (Rochester, NY). PAA was purchased from Aldrich Chemical Company, Inc (Milwaukee, WI). BZ, PB, and B were purchased from Sigma (St Louis, MO). All chemicals were used as received without further purification. Deionized water, obtained from a NANO-pure ultrapure water system (D4700, Barnstead, Dubuque, IA), was used for all experiments.

Preparation of Buffers

A 0.05 mol/L, pH 7.0 phosphate buffer system was used in all the studies. The ionic strength of the buffer was adjusted to 0.2 mol/L using sodium chloride. After preparation, the phosphate buffer was filtered through 0.22 μ m filters to remove any impurities.

Emulsion Preparation

Emulsions were prepared in 100 mL batches at room temperature. A desired mass of surfactant was added to 80 mL of pH 7.0 phosphate buffer and gently mixed. The concentrations of surfactant chosen were in the range of 1% to 6.2% wt/vol for the critical micelle concentration (CMC) and stability studies. In all other studies an initial surfactant concentration of 6.2% wt/vol was used. A known amount of model drug (PAA: 65.7 mg, BZ: 40.0 mg, PB: 60.0 mg, and B: 41.0 mg) was dissolved in 20 mL of mineral oil.

The model drug concentrations selected corresponded to their maximum solubilities in mineral oil at 37° C.

The 2 phases (80 mL of aqueous phase and 20 mL of oil phase) were mixed at low speed using a magnetic stirrer to form a coarse emulsion and introduced into the reservoir of the microfluidizer (Model 110T, Microfluidic, Newton, MA). The emulsion was passed through the microfluidizer pneumatically by compressed air at 80 psi. The microfluidizer is fitted with a 5 µ m filter to remove any impurities. Emulsions were collected after 5 passes and immediately used in the stability and transport studies. Surfactant concentration was varied by addition of extra surfactant dissolved in buffer following emulsification, resulting in a 1:1 dilution. Emulsion systems, where no excess surfactant was added, were diluted 1:1 with buffer only. Consequently, all final emulsions contained 10% vol/vol oil phase.

CMC Determination

Surface tension measurements were conducted using a microbalance surface tensiometer (K12, Kruss USA, Charlotte, NC) in the Wilhelmy plate mode. The tensiometer was equipped with a Dosimat (automatic burette; Model 665, Metrohm, Switzerland) for CMC determination. The Wilhelmy plate was rinsed with warm NANO-pure distilled water and with acetone. The plate was annealed to red-hot with a Bunsen burner. The annealing process removed impurities, which cannot be removed by rinsing, from the platinum surface. Surface tension values were determined from the measured force as follows (4):

$$F = \gamma PC \cos \theta \quad (1)$$

where γ is the surface tension, F is the measured force, P is the wetted length of the plate, and θ is the contact angle. CMC values of Brij 97 in the presence and absence of O/W emulsion systems were determined by a membrane equilibrium technique and surface tension measurement (3).

Surface Activity Determination

The effect of model drugs on interfacial tension was

determined using surface tension measurements. Surface tension measurements were conducted using a microbalance surface tensiometer (K12, Kruss USA) in the Wilhelmy plate mode. Surface tension was measured for model drugs in surfactant solutions (below the CMC of Brij 97).

Emulsion Stability Determination

Emulsion samples (0.5 mL) were sealed in 1 mL ampules and placed in temperature-controlled water baths $\pm 0.5^\circ \text{C}$ at 5° , 25° , 37° , and 60°C . Emulsion mean droplet diameters and size distributions were determined using an Accusizer Optical Particle Sizer (Model 770, Particle Sizing Systems, Inc, Santa Barbara, CA) and a Nicomp Submicron Particle Sizer (Model 370, Particle Sizing Systems, Inc). The Accusizer Optical Particle Sizer operates on the light blockage principle that detects particles in the size range of 1 µ m to 500 µ m. The Nicomp Submicron Particle Sizer is a photon correlation spectrophotometer and detects particles in the size range of 0.01 µ m to 1 µ m. These instruments were used in series to cover the entire particle size range of the emulsion systems with a single sample. All emulsions were prepared in triplicate, measurements were repeated 3 times per sample, and mean values and standard deviations were calculated.

Model Drug Solubility

Model drug solubilities were measured in phosphate buffer (0.05 mol/L, ionic strength 0.2, pH 7.0) at 37° C. Brij 97 was added to the buffer in concentrations of 0% to 2% wt/vol to determine the effect of the micellar phase on solubility. The model drug (PAA and BZ)/surfactant buffer suspensions were equilibrated at 37° C for 48 hours, filtered, and analyzed spectrophotometrically using a Spectronic 3000 Array (Milton Roy, Rochester, NY). Buffer solution and Brij 97 buffer solutions were used as reference solutions in the absence and presence of Brij 97, respectively. The absorbance peak values of PAA and BZ occurred at 377 nm and 286 nm respectively (in the absence of Brij 97 solution) and at 398 nm and 294 nm, respectively (in the presence of Brij 97 solution). A High-Performance Liquid Chromatography (Model 440, Waters Assoc, Milford, MA) equipped with an ultraviolet (UV)

detector (Model 441, Waters Assoc) and a reverse phase column (μ Bondapak – C₁₈, 10 mm, 30 cm x 3.9 mm I.D.; Waters Assoc) was used for PB and B analysis because their absorbance peaks overlapped with that of Brij 97. The mobile phase, a mixture of ultrapure deionized water, methanol, and trifluoroacetic acid (3:1:0.04, vol/vol), was operated at a flow rate of 1.3 mL/min. The column eluent was monitored at 247 nm with a sensitivity of 0.005 AUFS (sensitivity unit used for spectrophotometers). Peak areas were obtained using Perkin–Elmer programs (Omega 2, Norwalk, CT). Mean values and standard deviations were calculated from 3 sample determinations.

Oil/Buffer Partition Coefficient Determination

Two mL of oil containing model drug was kept in contact with 2 mL of pH 7.0 phosphate buffer solution at 37° C $\pm$ 0.1°C for 48 hours to allow equilibration. Preliminary experiments were conducted to determine the time to reach equilibrium. Samples were analyzed at 24 hours, 48 hours, 72 hours, and 168 hours, and it was determined that equilibrium was achieved within 48 hours. After reaching equilibrium, the 2 phases were separated, collected, and analyzed for model drug content. Aqueous samples were assayed for drug content using UV and high-performance liquid chromatography (HPLC). These experiments were repeated 3 times. Mean values and standard deviations were calculated.

Interfacial Rheology Measurement

Interfacial elasticity (mN/m) was determined using an oscillating ring interfacial rheometer (CIR Limited, UK). The platinum duNuoy ring was placed at the interface. The ring oscillates and a proximity probe transducer measures the amplitude of motion. Dynamic surface rigidity and surface viscosity moduli were generated concurrently. Temperature was controlled to 37° C $\pm$ 0.1. Solutions were prepared in phosphate buffer, pH 7.0, over a range of surfactant (1% to 6%) and model drug concentrations and surfactant:drug ratios (1:10 – 10:1). Measurements were taken over a 2-hour period, in triplicate, using freshly prepared samples for each determination. Average values and standard

deviations were calculated.

Model Drug Transport

Model drug transport rates in emulsion systems were investigated using the bulk equilibrium reverse dialysis bag and the side-by-side diffusion cell technique, and the data were compared. These methods have been described in detail previously (5).

Side-by-Side Diffusion Cell Technique

Briefly, water-jacketed side-by-side diffusion cells (glass chambers with a 4 mL volume and an 11-mm-diameter circular opening available for diffusion) mounted with cellulosic dialysis membranes (MW cutoffs: 1 kd or 50 kd) were used for kinetic studies of model drug release from emulsions (5). Samples were withdrawn from the receiver cells (2 mL) and analyzed spectrophotometrically at predetermined time intervals (Brij 97 solution—PAA: 398 nm, BZ: 294 nm; Buffer solution—PAA: 377 nm, BZ: 286 nm). PB and B were analyzed by HPLC (Waters) equipped with a UV detector (Waters Assoc) and a reverse phase column (μ Bondapak, Waters Assoc). The same volume of buffer or surfactant solution as was withdrawn for each sample was replaced into the receiver cells to maintain volume and sink conditions.

Control Studies

(i) *Transport study of model drugs from buffer solution to buffer solution*—Model drugs in buffer solution were placed in the donor cells and buffer solutions placed in the receiver cells. This study allows determination of the permeability coefficients of model drugs through the dialysis membranes.

(ii) *Transport study of model drugs from surfactant solution to surfactant solution*—Model drugs in surfactant solution were placed in the donor cells and surfactant solutions placed in the receiver cells. This study allows determination of the effect of the micellar phase on permeability coefficients of model drugs through the dialysis membranes.

Both control experiments were repeated 3 times, and mean values and standard deviations were calculated.

Bulk Equilibrium Reverse Dialysis Bag Technique

Briefly, dialysis bags (cellulosic membranes with MW cutoffs of 1 kd or 50 kd) containing the continuous phase (receiver phase) alone are suspended in a vessel containing the donor phase (diluted emulsion), and the system is stirred. At predetermined time intervals, each dialysis bag is removed and the contents are analyzed for released drug. The model drug submicron-sized emulsions (5 mL) were directly placed into 500 mL of a stirred sink solution in which numerous dialysis sacs containing 2 mL of the same sink solution were previously immersed. The dialysis sacs were equilibrated with the sink solutions for about 30 minutes prior to experimentation. At predetermined time intervals, dialysis bags were withdrawn and the contents assayed spectrophotometrically for model drug concentration. The release studies were performed at a fixed temperature of $37^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ under constant stirring. Measurements were conducted 3 times per sample, and mean values and standard deviations were calculated.

RESULTS

CMC Determination

The CMC value of Brij 97 in O/W emulsion systems could not be measured directly because the oil phase would interfere with the various methods available for CMC analysis, such as surface tension, conductivity, and osmotic pressure determination. The CMC of Brij 97 in O/W emulsion systems was measured using the method of Yoon and Burgess (3), which was a membrane equilibrium technique in combination with surface tension measurement (surfactant concentrations well above the CMC were dialyzed from the donor to the receiver chamber using the side-by-side diffusion cell). The CMC values of Brij 97 in buffer and in 10% vol/vol O/W emulsion were 0.00154% wt/vol and 3.1% wt/vol, respectively (Figure 2).

Surface Activity Determination

The surface tension values of PAA, BZ, PB, and B were measured as a function of time at the air/water interface (Table 1). The surface tension of pure water is 71.32 mN/m, which decreased in the presence of

Figure 2. Determination of the critical micelle concentration of polyoxyethylene-10-oleyl-ether (Brij 97) in buffer and in 10% vol/vol O/W emulsion systems (pH 7.0, $I = 0.2$, 37°C , mean values of three determinations); the error bars are within the symbols.

Table 1. The solubilities, log P (log values of O/W partition coefficients), and surface tension (γ) values (mN/M) of model drugs in 0.05 mol/L phosphate buffer (pH 7.0, $I = 0.2$, 37°C , mean values of three determinations).

Model Drugs	Solubility ($\mu\text{g/mL}$)	Log P	γ (mN/M) $\pm$ SD
Barbital	7560 ± 121	0.6 ± 0.002	41.6 ± 1.1
Phenobarbital	1002 ± 38	1.33 ± 0.03	46.8 ± 2.8
Benzocaine	1190 ± 45	1.80 ± 0.05	69.2 ± 0.4
Phenylazoaniline	29 ± 0.8	3.19 ± 0.09	67.2 ± 0.5

model drugs (0.0001 mol/L, Table 1). PB and B had the greatest effect, reducing the surface tension to 46.8 ± 2.8 mN/m and 41.6 ± 1.1 mN/m, respectively.

Micellar Solubilization and Partition Coefficient Studies of Model Drugs

Model drug lipophilicity as determined by oil/buffer partition coefficient and solubility studies are ranked in the order of PAA, BZ, PB, and B (Table 1). The solubilities of PAA and BZ in buffer (pH 7.0) increased with increasing Brij 97 concentration (Figure 3). There

Figure 3. Effect of polyoxyethylene-10-oleyl-ether (Brij 97) concentration on the solubilities of phenylazobenzene (PAA) and benzocaine (BZ) in 0.05 mol/L phosphate buffer (pH 7.0, $I = 0.2$, 37° C, mean values of three determinations); the error bars are within the symbols. Inserts are blown up for a magnified view at the Brij 97 critical micelle concentration region.

is an apparent change in the slope of the PAA solubility versus the Brij 97 concentration plot at 0.0015% wt/vol Brij 97. Similar trends were observed for PB and B. The solubilities of PB and B in buffer did not change in the presence of Brij 97. The model drug partition coefficients between oil and surfactant/buffer solutions (K_S) were dependent on surfactant concentration (Figure 4). K_S values decreased sharply initially with increase in surfactant concentration up to 1% wt/vol Brij 97 and then decreased with a shallow slope upon further increase in surfactant concentration.

Interfacial Rheology Determination

The interfacial elasticities of model drugs, Brij 97, and model drug/Brij 97 mixtures at the mineral oil/buffer interface were investigated as a function of time (Figure 5). Interfacial elasticity values of the model drugs and Brij 97 increased slightly initially and then remained constant over the 120-minute study period. The interfacial elasticity values of model drug/Brij 97 mixtures increased gradually initially and then reached a value of 27 mN/m for PAA, 24 mN/m for BZ, 42 mN/m for PB, and 48 mN/m for B after 120 minutes. However, the model drugs and Brij 97 alone reached values of 10 mN/m for PAA, 12 mN/m for BZ, 20 mN/m for PB, 36 mN/m for B, and 8 mN/m for Brij 97, during the 120-minute study.

Figure 4. Effect of polyoxyethylene-10-oleyl-ether (Brij 97) concentration on oil/surfactant phosphate buffer solution partition coefficient of model drugs (pH 7.0, $I = 0.2$, 37° C).

Figure 5. Effect of time on interfacial rheology of A – PAA, B – BZ, C – PB, and D – B, Brij 97, and model drug/Brij 97 mixture at mineral oil/buffer interface (pH 7.0, $I = 0.2$, 25° C, mean values of 3 determinations).

Emulsion Stability Determination

Emulsion stability was evaluated as a function of storage time, temperature, and dilution using the mean droplet diameters and droplet size distributions obtained from Nicomp analysis (Table 2). The effect of dilution was investigated because all emulsions were diluted prior to the transport studies. Brij 97 emulsions stored at 5° C, 25° C, and 37° C were stable over the 15-day study period because the mean droplet sizes, measured at different times during the study period, did not vary with normal size distribution. However, PAA and BZ samples stored

Table 2. Effect of storage time, temperature, and model drug on emulsion droplet size (polyoxyethylene-10-oleyl-ether [Brij 97] emulsions, pH = 7.4, I = 0.2). Temp = temperature.

Mean volume diameter (nm) of the emulsion droplets $\pm$ SD									
Temp (°C)	Phenylazoaniline		Benzocaine		Phenobarbital		barbital		
5	0 day	15 days	0 day	15 days	0 day	15 days	0 day	15 days	
25	111 $\pm$ 1	110 $\pm$ 1	111 $\pm$ 1	112 $\pm$ 2	103 $\pm$ 2	105 $\pm$ 1	92 $\pm$ 1	94 $\pm$ 2	
37	109 $\pm$ 1	109 $\pm$ 1	112 $\pm$ 1	112 $\pm$ 2	103 $\pm$ 1	104 $\pm$ 2	95 $\pm$ 2	96 $\pm$ 2	
60	110 $\pm$ 1	111 $\pm$ 1	111 $\pm$ 1	112 $\pm$ 1	104 $\pm$ 1	105 $\pm$ 2	94 $\pm$ 1	96 $\pm$ 2	
	110 $\pm$ 1	116 $\pm$ 2	114 $\pm$ 2	118 $\pm$ 2	105 $\pm$ 2	105 $\pm$ 2	95 $\pm$ 2	97 $\pm$ 3	

Table 3. The effect of polyoxyethylene-10-oleyl-ether (Brij 97) micellar concentration on the effective permeability coefficient of phenylazoaniline (PAA) in 10% vol/vol O/W emulsion systems through dialysis (MW cutoffs 1 kd and 50 kd) membranes at 37° C (n = 3).

Brij 97 (% w/v)	Effective permeability coefficient (cm/h) of PAA using different membranes	
	1 KD ($\times 10^2$)	50 KD ($\times 10^2$)
0	0.030 $\pm$ 0.0014	0.032 $\pm$ 0.0017
0.5	0.062 $\pm$ 0.0016	0.087 $\pm$ 0.0012
1	0.082 $\pm$ 0.001	0.102 $\pm$ 0.0028
1.5	0.069 $\pm$ 0.003	0.088 $\pm$ 0.0018
2	0.051 $\pm$ 0.002	0.076 $\pm$ 0.0022

Table 4. The effect of polyoxyethylene-10-oleyl-ether (Brij 97) micellar concentration on the effective permeability coefficients of benzocaine, phenobarbital, and barbital in 10% vol/vol mineral oil/water (O/W) emulsion systems through dialysis (MW cutoffs 1 kd and 50 kd) membranes at 37° C (n = 3).

97 (%) wt/vol)	Effective permeability coefficients (cm/h) of model drugs using different membranes					
	Benzocaine		Phenobarbital		Barbital	
	1 kd ($\times 10^2$)	50 kd ($\times 10^2$)	1 kd ($\times 10^2$)	50 kd ($\times 10^2$)	1 kd ($\times 10^2$)	50 kd ($\times 10^2$)
0	1.72 $\pm$ 0.05	2.14 $\pm$ 0.08	5.21 $\pm$ 0.15	5.41 $\pm$ 0.17	6.74 $\pm$ 0.41	6.81 $\pm$ 0.39
1	1.91 $\pm$ 0.12	2.41 $\pm$ 0.01	5.14 $\pm$ 0.19	5.36 $\pm$ 0.28	6.68 $\pm$ 0.38	6.78 $\pm$ 0.45
2	1.46 $\pm$ 0.05	1.94 $\pm$ 0.11	5.12 $\pm$ 0.38	5.34 $\pm$ 0.16	6.66 $\pm$ 0.40	6.72 $\pm$ 0.35

Increase in droplet size above 1 μ m was confirmed by Accusizer analysis. Dilution of emulsion samples with surfactant/buffer solution (1:1 dilution) did not affect mean droplet size over the 2-week study period.

Transport Studies of Model Drugs in Brij 97 Solutions

The effective drug permeability coefficients of the dialysis membranes (MW cutoffs 1 kd and 50 kd) under quasi-steady-state conditions were calculated. These values are calculated from the slope of a plot of $\ln Q_d$ (logarithmic value of drug concentration in the donor compartment) (determined using side-by-side diffusion cell technique) versus time for each model drug using Fick's first law of diffusion equation (Tables 3 and 4). The effective permeability coefficients of the model drugs in buffer solution decreased as Brij 97 concentration increased. The effective permeability coefficients of PB and B in buffer systems (pH 7.0) decreased slightly compared with PAA and BZ when Brij 97 concentration increased.

Effect of Brij 97 Concentration on Transport of Model Drugs in Triphasic Systems

The presence of the micellar phase increased the total transport rates of PAA, BZ, PB, and B in emulsion systems (pH 7.0) at all concentrations studied up to 1% wt/vol Brij 97 and then decreased with further increase in the micellar phase (Tables 3 and 4). The effective permeability coefficients of PAA, BZ, PB, and B increased with increase in Brij 97 concentration using either the side-by-side diffusion cell or the reverse dialysis bag techniques up to 1% wt/vol Brij 97 and then decreased with further increase in Brij 97 concentration (Tables 4 and 5). Total transport rates of PB and B were faster than those of PAA and BZ at all the Brij 97 concentrations investigated. The release patterns of the model drugs using the side-by-side diffusion cell technique were linear with time, and the total amounts of the model drugs released were less than those obtained using the reverse dialysis bag technique. The release patterns of the model drugs using the reverse dialysis bag technique were nonlinear with time.

at 60° C deteriorated within 9 to 15 days, as was evident from an increase and then a decrease in the mean droplet sizes determined using the Nicomp.

DISCUSSION

Effect of Brij 97 (Nonionic Surfactant) on Model Drug Emulsion Stability

The CMC values of Brij 97 in phosphate buffer and in the presence of emulsions (10% vol/vol O/W) were calculated from the surface tension data (Figure 2). The CMC value of Brij 97 increased from 0.00154% wt/vol in phosphate buffer to 3.1% wt/vol in the emulsion systems (10% vol/vol O/W) as a consequence of the large interfacial area of submicron-sized emulsions. Therefore, it is assumed that 3.1% wt/vol Brij 97 was required to form a surfactant monolayer at the emulsion (10% vol/vol O/W) interface. Consequently, surfactant in excess of that amount (added after emulsion formation) would be present in the continuous phase as monomers and micelles (6,7). This excess surfactant may aid emulsion stability (8) and may affect model drug transport. All emulsions were prepared with 3.1% Brij 97 initially to ensure monolayer coverage, and excess surfactant was added later by dilution.

The surface activity of the model drugs was determined from their capacity to decrease the surface tension value of water. Among the model drugs investigated, B is the most surface active, followed by PB. PAA and BZ are not considered surface active because they did not cause a significant reduction in the surface tension of water. PAA was the least soluble and had the highest log P value. PB and BZ have similar solubilities; however, the log P value of BZ was slightly higher than that of PB. The lower log P value of PB may be a result of the surface-active nature of PB. B was the most soluble and had the lowest log P value as a consequence of the increased hydrophilicity of this molecule and its greater surface activity.

The solubilities of PAA and BZ in buffer (pH 7.0) increased with increasing Brij 97 concentration due to micellar solubilization of these hydrophobic model drugs (Figure 3). There is an apparent change in the slope of PAA and BZ solubility versus Brij 97 concentration plot at 0.0015% wt/vol Brij 97. This correlates with the CMC value of Brij 97 as determined from surface tension measurements (0.00154% wt/vol, Figure 2). A constant slope was observed for the plot of all model drugs' solubility

versus Brij 97 concentration above the CMC value of Brij 97 for all model drugs investigated (Figures 3 and 4). In addition, the UV absorbance peaks of PAA and BZ shifted bathochromically in surfactant/buffer solutions, probably because of micellar solubilization. No significant change was seen in the solubilities of PB and B in pH 7.0 buffer in the presence of Brij 97, suggesting that these molecules were not solubilized in the micellar phase. The PB and B UV absorbance peaks were not shifted in surfactant/buffer solutions, suggesting that they do not form a complex with the surfactant. Therefore, the solubilities and consequently the transport of PB and B are unlikely to be affected by the presence of Brij 97 micellar phase.

Amidon et al. (9) described the micellar solubilized drug– free drug equilibrium distribution coefficient for 1:1 stoichiometry, K_m as follows:

$$K_m = \frac{[C_m]}{[C_w][SAA]} \quad (3)$$

$$[C_T] = [C_w](1 + K_m[SAA]) \quad (4)$$

where C_w is the drug in the aqueous phase, C_m is the drug in the micellar phase, and SAA is the micellar phase; in other words, $[SAA]$ = total Brij 97 concentration – CMC of Brij 97, and $[C_T]$ is the total drug solubility. In the case of PAA, $[SAA]$ = total Brij 97 concentration – intercept of X-axis in the solubility plot (Figure 3).

The model drug equilibrium distribution coefficient between the buffer and surfactant phase (K_m) was determined from the ratio of the slope of a plot of solubility versus surfactant concentration of model drug buffer solubility. The slopes of the solubility versus surfactant concentration plots for PAA and BZ at pH 7.0 (Figure 3) are 645 and 315, respectively. The K_m values for PAA and BZ are 58 and 0.25, respectively, indicating that PAA is more lipophilic. These results are in agreement with the partition coefficient data of the model drugs between mineral oil and buffer. It was not possible to

calculate precise K_m values for PB and B because their solubilities did not change significantly with surfactant concentration. The partition coefficient values of PAA and BZ between oil and surfactant/buffer solution (K_S) were calculated using the following equation:

$$K_S = \frac{K_0}{1 + K_m[SAA]} \quad (5)$$

where K_0 is the O/W partition coefficient. O/W partition coefficient values decreased sharply with increase in surfactant concentration up to 1% wt/vol Brij 97 and decreased with a shallow slope, with further increase in surfactant concentration (Figure 5). This change in the partition coefficient value with surfactant concentration is caused by change in the solubilizing capacity of the micelles in the aqueous phase; the change in capacity is a result of change in micellar shape from spheres to rods. Micellar shape changes were apparent from a nonlinear increase in intrinsic viscosity of surfactant solution with an increase in concentration of the model compounds. The viscosity changes associated with micellar shape change may also contribute to the change in the partition coefficient value. Micellar shape changes usually occur at higher surfactant concentrations. However, in the presence of a second compound that interacts with the micellar phase (such as the model drugs containing a benzene moiety), shape changes occur at the lower surfactant concentrations (6). This is due to stereochemical hindrance, which does not allow accommodation of the benzene ring of the model drugs in spherical micelles (7).

Although PAA and BZ are not considered surface active, the PAA/Brij 97 and BZ/Brij 97 mixtures had high interfacial elasticity compared with the individual moieties. This is due to complex formation, which is in agreement with the bathochromic shifts observed in the UV absorbance studies. The high interfacial elasticity values of the model drug/surfactant mixtures suggest that these mixtures may aid emulsion stability by forming a strong interfacial barrier for droplet coalescence. Complex formation of surfactants with the model

drug may also be responsible for a reduction in model drug transport rate. PB and B do not form complexes with Brij 97; therefore, no bathochromic shifts were observed in the UV analysis. However, their interfacial elasticity values when mixed with Brij 97 differ significantly from those of the individual moieties, which is probably due to the surface-active nature of these model drugs (Figure 6). These data imply that all the model drugs investigated tend to associate at the interface either through their surface-active nature and/or by forming complexes with the surfactant.

Emulsions were prepared with the same initial surfactant concentration (6.2% wt/vol) to avoid variation in emulsion mean droplet size and size distribution with surfactant concentration. Droplet size variation would affect interfacial area, emulsion stability, and drug release, making data interpretation difficult. A narrow droplet size distribution is necessary to achieve relative stability in emulsion systems (10). Emulsions stored at 5° C, 25° C, and 37° C for a 15-day study period were considered stable because the emulsion droplet size did not change significantly. However, samples stored at 60° C deteriorated within 9 to 15 days, as was evident from an increase and then decrease in mean droplet size, caused by droplet coalescence with time. An increase in the number of large droplets outside the measurable size range of the Nicomp (10 nm to 1000 nm) occurs, and consequently the measured size decreases. The presence of large droplets caused by emulsion deterioration was confirmed by Accusizer analysis. These results are in agreement with the work of Burgess and Yoon (11) on perfluorocarbon emulsion stability. Dilution of emulsion samples with surfactant/buffer solution (1:1 dilution) did not affect the mean droplet size or polydispersity over the 2-week study period. Variation in interfacial area resulting from instability would have affected the excess micellar concentration available in the transport studies and consequently the transport kinetics.

Effect of Brij 97 (Nonionic Surfactant) on Model Drug Transport in Emulsion Systems

The effective drug permeability coefficients

Figure 6: Effect of time on interfacial rheology of A – PAA, B – BZ, C – PB, and D – B, Brij 97 and model drug/Brij 97 mixture at mineral oil/buffer interface (pH 7.0, $I = 0.2$, 25°C, mean values of three determinations)

(reciprocal of diffusional resistance) of the dialysis (MW cutoffs 1 kd and 50 kd) membranes under quasi– steady-state conditions were calculated from the slope of the plot of $\ln Q_d$ versus time for each model drug using Fick's first law of diffusion equation as follows:

$$\ln Q_d = \ln Q_0 - A_m P_{\text{eff}} t \quad (6)$$

where Q_d is the amount of the model drug in the donor cell, Q_0 is the initial amount of model drug in the donor cell, A_m is the area of membrane available

for diffusion, P_{eff} is the effective permeability coefficient of the drug, and t is the diffusion time. The decrease in the effective permeability coefficient of BZ and PAA (pH 7.0) with increase in Brij 97 concentration may be a result of reduced free drug concentration in the aqueous phase as a consequence of micellar solubilization (Tables 3, 4, and 5). This is in agreement with the partition coefficient (Figure 5) and micellar solubilization studies (Figures 3 and 4). The effective permeability coefficients of PB and B in buffer systems (pH 7.0) decreased slightly compared with those of PAA and BZ with increase in Brij 97 concentration. PB and B are relatively hydrophilic compared with the other model drugs and are not solubilized by the micellar phase (Figure 4). This is in agreement with the UV absorbance, solubility (Figure

4), and partition coefficient studies (Figure 5). The effective permeability coefficients of PAA, BZ, PB, and B through 50 kd MW cutoff membranes were higher than those through MW cutoff 1 kd membranes as a consequence of the larger pore size of the MW cutoff 50 kd membrane. A 50 kd MW cutoff membrane can allow the passage of micelles because of their larger pore size, whereas a 1 kd MW cutoff membrane restricts the passage of the micelle because of their smaller pore size (3). Model drug complexed/solubilized within the micelles can pass through the 50 kd MW cutoff membrane, leading to higher permeability coefficients.

The release rates of the model drugs from the emulsion systems using the reverse dialysis bag technique are faster than those obtained using the side-by-side diffusion cell technique. In the side-by-side diffusion cell technique, the surface area of the cellulosic membranes available for diffusion was 3.80 cm² and the interfacial area of the dispersed droplets was 1,234,000 cm² (calculation based on mean droplet diameter). The enormous surface area of the oil droplets compared with that of the cellulosic membrane leads to saturation of the continuous phase in the donor chamber, and consequently the measured release rates are slow. Saturation of the continuous phase in the donor chamber is also possible due to the limited volume available for solubilizing released drug. Consequently, the linear release profile of the model drugs using the side-by-side diffusion cell technique appears to be due to violation of sink conditions in the donor chamber. In the reverse dialysis bag technique, the emulsion sample is diluted approximately 100 times compared with twice in the side-by-side diffusion cell method, and therefore sink conditions are maintained in the donor phase. The initial fast rate from the reverse dialysis bag method is considered to be the result of the transport of free and micellar solubilized drug from the continuous media of the donor to the receiver chambers. The slow rate may be the result of the release of drug from the oil droplets across the interfacial barrier to the receiver phase through the continuous phase of the donor chamber. This release step is slow because it is governed by the partitioning rate between the oil and aqueous phases.

The initial increase in the effective permeability coefficient of model drugs (up to 1% wt/vol micellar phase) indicates that the partitioning rate of the model drugs from the oil droplets to the continuous phase is enhanced by increase in concentration of Brij 97. The decrease in the effective permeability coefficient of model drugs at micellar concentration above 1% wt/vol Brij 97 is in agreement with the decrease in rate of decrease of the partition coefficient values with surfactant concentration for model drugs between the oil and surfactant solutions (Figure 5). This change in the permeability coefficient with surfactant concentration is probably caused by the micellar shape change discussed above.

The transport rates of PB and B from emulsion systems at pH 7.0 were fast compared with those of PAA and BZ (Table 5). These faster rates are attributed to their surface-active nature. These molecules associate at emulsion droplet interfaces rather than within the oil droplets, resulting in a smaller diffusion path length and therefore a faster transport rate. In addition, PB and B act as surfactants and reduce the initial oil droplet sizes below those of PAA and BZ, leading to an increase in the exposed interfacial area available for release and hence faster release rates.

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