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

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ABSTRACT The effect of surfactant concentration on transport kinetics in emulsions using surface-active (phenobarbital, barbital) 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 barbital 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.

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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-

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-oleyl-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 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.

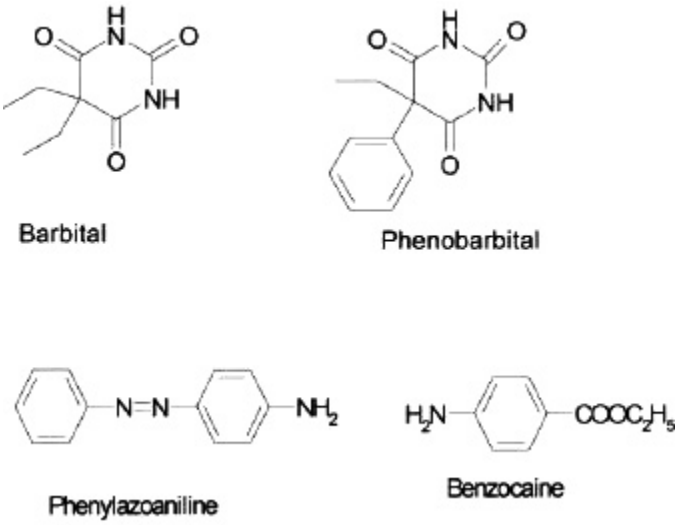

Figure 1. Chemical structures of phenobarbital, barbital, phenylazoaniline, and benzocaine.

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):

$$F = \gamma P \cos\theta \qquad (1)$$

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 ± 0.5° 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 – C_{18}, 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 ± 0.1^0C 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 ± 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° C ± 0.1^0C 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

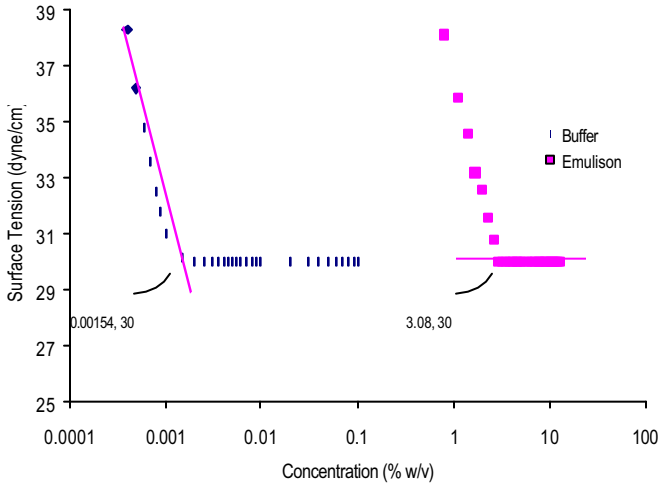

Figure 2. Determination of the critical micelle concentration of polyoxyethylene-10-oleyl-ether (Brij 97) in buffer and in 10% vol/vol O/W emulsion systems (pH 7.0, I = 0.2, 37° C, mean values of three determinations); the error bars are within the symbols.

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 (μ g/mL)	Log P	γ (mN/M) ± 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

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