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Effect of a second solubilizate on the partition coefficient of drugs in micellar solution and their permeation rate across an artificial membrane

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The distribution of a solubilizate between micelles and the aqueous phase does not obey a simple partition law when a second solubilizate species is present. Alterations of the apparent partition coefficient cannot be explained in terms of a simple displacement mechanism, following the interaction of both solubilizates with the same site of the micelle. A non linear increase in solubilizate association to micelles following an increase in surfactant concentration is observed in the presence of a second solubilizate. A depression in the cloud point temperature follows the addition of a second species and is such that cannot be interpreted as a simple additive effect. Alteration of the apparent partition coefficient in a micellar solution has an effect on the permeation rate of the solubilizate across an artificial membrane. Biopharmaceutical implications are discussed.

Drug absorption is a dynamic process which might be influenced by an effect of surfactants on the dosage form, the dissolution rate of the drug (Fincher, 1968; Gibaldi & Feldman, 1970) and the permeability of biological membranes (Levy, Miller & Reuning, 1966; Anello & Levy, 1969; Alhaique, Marchetti & others, 1975a; Alhaique, Giacchetti & others, 1975b).

When absorption is limited by dissolution rate and the surfactant concentration is above the critical micelle concentration, the total solubility of the drug increases with its dissolution rate (Higuchi, 1964; Gibaldi, Feldman & others, 1968). Thus, the absorption rate is enhanced provided that the drug concentration in solution approaches or exceeds its solubility in pure solvent and its partitioning from micellar phase to non micellar phase is rapid.

While micellar solubilization of a single species has been studied extensively (Nakagawa, 1967), there are fewer data (Tokiwa, 1970) and more problems when a second solubilizate is taken up by micelles.

It is generally accepted that a drug solubilized in micelles is not available for absorption (Levy & Reuning, 1964) and passive diffusion through biomembranes is governed by the thermodynamic activity of the drug in the aqueous phase, as determined by its partitioning between water and micelles.

When a surface-active agent is included in a formulation to modify drug absorption by micellar solubilization, it may happen that sparingly soluble compounds present, other than drug, compete for the micellar phase, thus interfering with solubilization and ultimately with the absorption rate.

We now describe an example of a second solubilizate, partitioning to a micellar solution of a drug, altering the activity of the free drug and affecting its transfer rate across biological membranes.

MATERIALS AND METHODS

Materials. The surfactant used was polysorbate 80 (Honeywill-Atlas Ltd, Carshalton, Surrey, U.K., and used as received). Chloramphenicol was the generous gift of the Carlo Erba Co., Milan, Italy. Nalidixic acid was a Società Italiana Chimici, Rome, Italy, sample. Both were purified by repeated crystallization. Analytical reagent grade benzoic acid, 2-hydroxy and 4-hydroxy derivatives were used.

Medical grade poly-dimethylsiloxane sheeting (Silastic non reinforced, lot HH 1308, Dow Corning, Midland, Mich., U.S.A.) in a labelled thickness of 5×10^{-3} inch (0·127 mm) was used in both dialysis and permeation measurements.

Assay. Chloramphenicol, nalidixic and benzoic acids were assayed by ultraviolet absorption spectroscopy using quartz cells of 1 mm path length. Surfactants, at the concentrations in the diluted solutions, did not interfere with the assay. Appropriate blanks were used. The Beer-Lambert law applied to solubilizates.

Solubility. An excess of substance was added to 25 ml volumes of aqueous solutions containing different concentrations of surfactant. The pH of the solutions was adjusted to 2·0 with 1·0 N hydrochloric acid in a measured amount thereby permitting correction of

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surfactant concentration due to dilution. The pH was maintained by the addition of 1 N HCl or 1 N NaOH under control of a pH-stat (Radiometer, Copenhagen). During the initial pH adjustment, solutions were stirred in a water jacketed vessel at 25° ± 0·1°. Changes in the ionic strength following pH adjustment had no effect on the solubility of the compounds. After pH adjustment, the solutions were placed in 50 ml Quickfit Ehrlenmeyer flasks and then shaken for 48 h in a water bath (25° ± 0·1°; 120 ± 2 strokes min^{-1}; stroke length of 4·0 cm). The time required for equilibration was established by a repetitive sampling technique. After equilibration, samples were filtered through 0·45 μm filters (Metricel GA-6, Gelman Instrument, Milan, Italy), diluted appropriately and assayed for solubilizate content.

Density. Apparent densities of surfactants in 1 and 5% w/v aqueous solutions were determined at 25° ± 0·1°, using 50 ml density bottles.

Equilibrium dialysis studies. The diffusion cell previously described (Alhaique, Marchetti & others, 1972) was used with some modifications. The outer compartments of the original cell were assembled and the polysiloxane membrane placed in between. The artificial barrier was not permeable to polysorbate 80, nalidixic acid and chloramphenicol over 5 days. The area available for diffusion was 5·92 cm^2 and each compartment occupied about 8 ml. The contents of both compartments of the cell were stirred at the same speed. The cell was initially equilibrated overnight in a bath at 25° ± 0·1° with diluted hydrochloric acid (pH = 2·0) in both sections. The acid was removed by suction; 8 ml of a micellar solution of the solubilizate was added to one compartment, and an equal volume of an aqueous solution of the same solubilizate was pipetted into the other compartment. In this way the surfactant was present only in one of the two sections of the cell. The starting solubilizate concentration and pH in both solutions were the same ($3·2 \times 10^{-4}$ M and pH = 2·0). All solutions were warmed to 25° before being placed into the cell. Equilibrium was reached within 48 h. Subsequently, the ultraviolet absorbance of the solution containing no surfactant was measured and the partition coefficient of the substance calculated (Patel & Kostenbauder, 1958).

Permeation studies. The above described diffusion cell was used. One of the compartments was filled with diluted hydrochloric acid (pH = 2·0) and the other with a 10^{-3} M sodium hydroxide solution. The cell was then equilibrated overnight at 25° ± 0·1° and, both solutions replaced by a micellar solution of the solubilizates at pH = 2·0 and a fresh sodium hydroxide solution respectively, previously warmed to the same temperature. Quantitative neutralization of the permeated species, i.e., benzoic acids, assured that the concentrations of the permeable form of the test compound was maintained at a zero value in the receptor compartment; thus 'sink' conditions apply. Constant stirring was maintained throughout the experiment and sampling from the receptor compartment was made at given times. Since the relative effect of interacting species was the aim, no volume correction following sampling was attempted.

Cloud points. Cloud point temperatures were determined following the usual procedure*, by heating a solution until it clouds and then measuring the temperature at which clearing occurs on cooling. No significant difference was observed between cloud points taken on rising or decreasing the temperature, at rates from 1°/2 min to 1°/5 min. In fact, cloud point values obtained from duplicate heating and cooling cycles agreed within 0·5°. The concentrations of polysorbate 80 ranged from 1 to 3% w/v. When two solubilizates were present, equimolecular amounts of both substances were used.

RESULTS AND DISCUSSION

The solubilities of benzoic acids in solutions containing different concentrations of polysorbate 80 are shown in Fig. 1. As expected, solubility increases

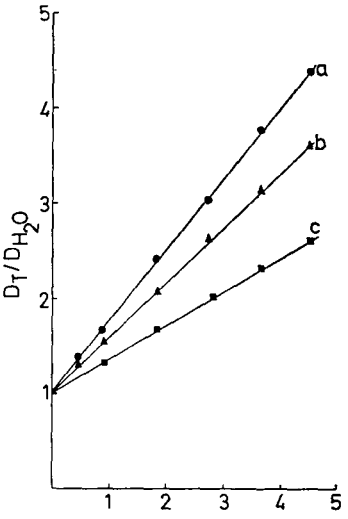

FIG. 1. Solubility of benzoic acids in different concentrations of polysorbate 80 at pH 2·0. D_T is the solubility of benzoic acids in surfactant solutions and D_{H_2O} is the solubility of benzoic acids in water. ●, *o*-hydroxybenzoic acid; ▲, benzoic acid; ■, *p*-hydroxybenzoic acid. Temperature was 25° ± 0·1°.

* ASTM D 2024–62T, ASTM STANDARDS 1964, Part 22, 351.

In early with surfactant concentration in agreement with other reports (Donbrow, Azaz & Hamburger, 1970; Mukerjee, 1971; Collett & Withington, 1972). As the pH value of sample solution is far below the pKa of the corresponding acids, ionization of the solubilizate is suppressed. The slope of the solubility ratio *vs* surfactant concentration plots is reduced with increasing solubilizate polarity (Mukerjee, 1971; Collett & Withington, 1972), showing a minimum for the most polar *p*-hydroxybenzoic acid.

If it is considered that solubilization is a partition phenomenon (McBain & Hutchinson, 1955), the distribution coefficient of benzoic acids between micelles and water can be calculated (Collett & Koo, 1975) from the plots of Fig. 1 or determined, more accurately, by means of equilibrium dialysis methods (Patel & Kostenbauder, 1958; De Luca & Kostenbauder, 1960; Bahal & Kostenbauder, 1964; Patel & Foss, 1964; Blaug & Rich, 1965).

To analyse the micelle/water distribution of a solubilizate following the addition of a second species, a number of dialysis experiments were made at different levels of additive molarity. As shown in Fig. 2 (plot a), the addition of a second species, i.e., benzoic acid, to the micellar solution of a compound,

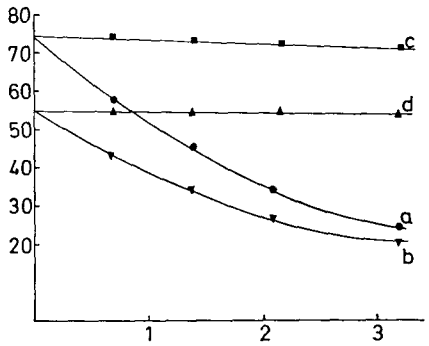

FIG. 2. Changes in micelle/water apparent partition coefficient of a solubilizate after progressive addition of a second species. Polysorbate 80 concentrations range from 1 to 3% w/v. Each plot refers to a constant concentration of the surfactant. *o*-Hydroxybenzoic acid partition coefficients by addition of benzoic acid (● 1% and ■ 3% w/v polysorbate 80). Benzoic acid partition coefficients by addition of *o*-hydroxybenzoic acid (▲ 1% and ▲ 3% w/v polysorbate 80). Temperature was 25° ± 0·1°, pH = 2·0, and the initial solubilizate concentration $3{\cdot}2 \times 10^{-4}$M. Ordinate—Micelle/water apparent partition coefficient. Abscissa—Added species (M × 10^4).

i.e., *o*- or *p*-hydroxybenzoic acid, alters the distribution of the first solubilizate. The partitioning of benzoic acid from water to the micellar phase decreases the interaction of the substituted acid with the solubilizer, resulting in an increase of its concentration in the aqueous phase.

The same effect is observed when *o*-hydroxy- (plot b) or *p*-hydroxybenzoic acids are added to a micellar solution of benzoic acid.

Alteration of the apparent partition coefficient is dependent on surfactant concentration. In fact, water/micelle distribution of both benzoic acid and its *o*-hydroxy derivative is not altered by progressive amounts of the additive at 3% w/v polysorbate 80 concentration (plots c and d).

If micelle/water apparent partition coefficients, at an equimolecular level of both solubilizate and added species ($3{\cdot}2 \times 10^{-4}$ M), are plotted *vs* surfactant concentration, a sigmoid curve is obtained (Fig. 3). The non-linear increase in solubilizate association to micelles following an increase in surfactant concentration, indicates that a sharp change in the degree of interation occurs within a critical range of polysorbate 80 concentration.

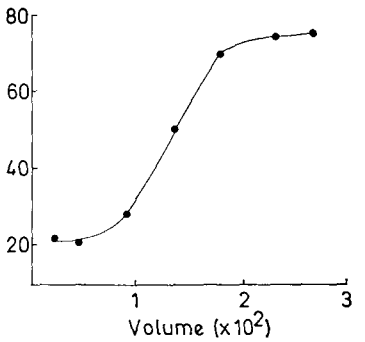

FIG. 3. Effect of surfactant concentration on micelle/water apparent partition coefficient of *o*-hydroxybenzoic acid in presence of an equimolecular amount of benzoic acid ($3{\cdot}2 \times 10^{-4}$M), at 25° ± 0·1° and pH = 2·0. Ordinate—Micelle/water apparent partition coefficient.

These findings suggest that the distribution of a solubilizate between micelles and the aqueous phase does not obey a simple partition law when a second species is present. However, deviations from the simple partition law have already been observed for a single solubilizate distributing to micelles (Mitchell & Brown, 1966; Donbrow, Molyneux & Rhodes 1967; Shimamoto & Ogawa, 1975).

In our case, alteration of the apparent partition coefficients of a solubilizate by addition of a second species cannot be explained in terms of a simple displacement mechanism, following the interaction of both solubilizates with the same site of the micelle; i.e., the polyoxyethylene mantle or the hydrocarbon core. In fact, benzoic acids do not distribute in the

same way between the core and the mantle of a micelle. Benzoic and *o*-hydroxybenzoic acids solubilize mainly in the hydrocarbon core, while *p*-hydroxybenzoic acid binds in preference to polyoxyethylene mantle (Mukerjee, 1971).

It is well known that micellar solubilization proceeds with reorganization of the micelle until saturation concentration is reached. These structural transitions are reflected by changes in cloud point temperature; the magnitude of this effect depends on species and amount of solubilizate (Nakagawa, 1967).

In Fig. 4A the effect of added solubilizates on the cloud point of 1% w/v polysorbate 80 solutions is illustrated. All benzoic acids depress the cloud point but to a different extent (plots a, b and c). The addition of equimolecular amounts of a second solubilizate to the micellar solution of one of the

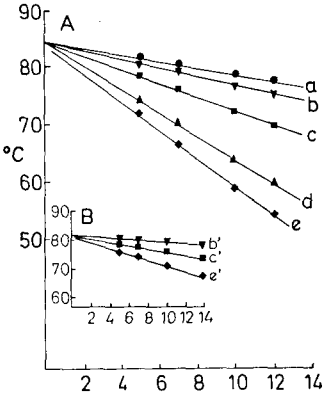

FIG. 4. (A). Effect of solubilizates and added species on the cloud point temperature of 1% w/v polysorbate 80 solution at pH = 2·0. ●, *p*-hydroxybenzoic acid; ▼, benzoic acid; ■, *o*-hydroxybenzoic acid; ▲, equimolecular *o*-hydroxybenzoic and *p*-hydroxybenzoic acids; ◆, equimolecular benzoic and *o*-hydroxybenzoic acids. Abscissa—Solubilizate and/or added species (M × 10^3). (B). Effect of solubilizates and added species on the cloud point temperature of 3% w/v polysorbate 80 solution at pH = 2·0. ▼, benzoic acid; ■, *o*-hydroxybenzoic acid; ◆, equimolecular benzoic acid and *o*-hydroxybenzoic acid.

benzoic acids causes a further depression of the cloud point temperature (Fig. 4A, plots d and e). A closer examination of the slopes of the plots in Fig. 4A indicates that the temperature depression in presence of a second solubilizate is such that cannot be interpreted in terms of a simple additive effect.

As surfactant concentration increases from 1 to 3% w/v, a depression of the cloud point temperature can still be observed, but the effect of the solubilizates is markedly reduced (Fig. 4B). Following that a decrease of the cloud point is often referred to as evidence of micelle rearrangements (Nakagawa, 1967) due to salting out of the surfactant, the observed variations in the apparent partition coefficients might be related to structural transitions.

In an attempt to find out if non parent compounds behave similarly, nalidixic acid and chloramphenicol were tested in the same experimental conditions.

The solubility of both the compounds is increased by the presence of polysorbate 80 and both substances partition to the micellar phase much to the same extent (Fig. 5). Chloramphenicol behaves similarly to benzoic acids in altering the apparent partition coefficient (Fig. 6, plots a and b) and in depressing the cloud point (Fig. 7, plot b).

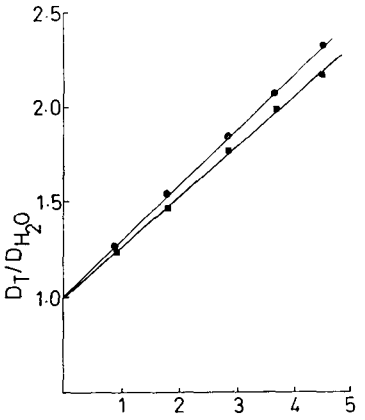

FIG. 5. Solubility of chloramphenicol and nalidixic acid in different concentrations of polysorbate 80 at pH = 2·0. D_T is the solubility in surfactant solutions and D_{H_2O} is the solubility in water. ●, chloramphenicol; ■, nalidixic acid. Temperature was 25° ± 0·1°.

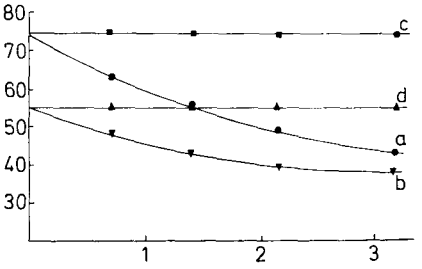

FIG. 6. Changes in micelle/water apparent partition coefficient of benzoic acids following progressive addition of chloramphenicol or nalidixic acid. In all cases, polysorbate 80 concentration was 1% w/v. *o*-Hydroxybenzoic acid partition coefficients by addition of ● chloramphenicol, ■ nalidixic acid. Benzoic acid partition coefficients by addition of ▼ chloramphenicol, ▲ nalidixic acid. Temperature was 25° ± 0·1°, pH = 2·0, initial benzoic acids concentration 3·2 × 10^{-4}M. Ordinate—Micelle/water apparent partition coefficient. Abscissa—Added species (M × 10^4).

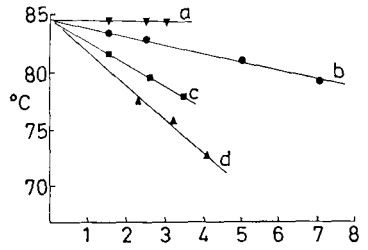

FIG. 7. Effect of solubilizates and added species on the cloud point temperature of 1% w/v polysorbate 80 solution at pH = 2·0. ▼, nalidixic acid; ●, chloramphenicol; ■, equimolecular *o*-hydroxybenzoic acid and nalidixic acid; ▲, equimolecular *o*-hydroxybenzoic acid and chloramphenicol. Abscissa—Solubilizate and/or added species (M × 10^3).

In contrast, nalidixic acid has no detectable effect either on the apparent partition coefficient (Fig. 6, plots c and d) or the cloud point (Fig. 7, plot a).

Applying Mukerjee's rationalism (1971), the distribution of nalidixic acid between core and mantle of the micelle was determined and found to be similar to that of benzoic and *o*-hydroxybenzoic acids. In fact, 98% of the total amount of nalidixic acid in the micelle is located in the hydrocarbon core. Nevertheless the acid has no significant effect on the apparent partition coefficient when added to the micellar solution of one of the tested solubilizates.

These findings add further support to the findings that alterations of the apparent partition coefficient of a solubilizate, following the addition of a second species, are related to structural transitions of the micellar phase. If the added compound does not induce a significant rearrangement in micelle structure, i.e., does not alter the cloud point temperature, no change in the apparent partition coefficient will be observed. Alteration of the partition coefficient in a micellar solution might have an effect on the permeation rate of the solubilizate across a membrane.

Fluxes of *o*-hydroxybenzoic acid through an artificial barrier in 'sink' conditions are given in Table 1.

Table 1. *Effect of an added species on o-hydroxy benzoic acid permeation through an artificial membrane in 'sink' conditions, in a model system at 25° ± 0·1°. o-Hydroxybenzoic acid and added species are equimolecular (3·2 × 10^{-4} M).*

Added species ($3·2 \times 10^{-4}$ M)	Permeation rate mol cm^{-2} s^{-1} × 10^{10}		
	H_2O	1% polysorbate 80	3% polysorbate 80
None	10·21	5·61	2·42
p-Hydroxybenzoic acid	9·24	6·12	2·42
Benzoic acid	9·26	6·50	2·43
Chloramphenicol	9·26	6·03	2·42
Nalidixic acid	9·25	5·58	2·41

As predicted, its permeation rate is increased by the addition of any one of the test compounds except nalidixic acid which had no significant effect on the partition coefficient of salicylic acid, as shown previously (Fig. 6, plot c). The increase in permeation rate is dependent on surfactant concentration, as is the effect on partition coefficient, and is no longer observed for high concentrations of surfactant.

The present results suggest that the negative effect on drug bioavailability due to micellar inclusion, can be reduced by the presence of a second compound partitioning to micelles, rearranging their structures and thereby altering the distribution coefficient.

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