Micellar Solubilization of Clofazimine Analogues in Aqueous Solutions of Ionic and Nonionic Surfactants

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INTRODUCTION

The N^2-substituted phenazine antileprotic agent clofazimine is poorly soluble in water. Despite this poor solubility, the drug was found to be well absorbed, particularly when coadministered with vegetable oils and surfactant solutions (1). Clofazimine absorption was also found to increase in the presence of naturally occurring surfactants (2). Given the importance of solubility in determining drug absorption and the ability of surfactants to increase both drug solubility and absorption, we have examined the influence of surfactant concentration on the solubility of clofazimine and a number of new substituted iminophenazines with polar functional groups in the N^2-side chain (Table I). The hydrophobic micellar moiety of both nonionic and anionic surfactants solubilizes unionized solutes of low aqueous solubility, the polarity of the solubilizate molecules being a major factor determining the degree of solubilization (3,4). A relationship between the lipophilicity of the solubilisate, expressed as its partition coefficient, and its saturation distribution between micelles and aqueous phase has been reported for substituted barbituric acids solubilized by polyoxyethylene stearates and for several steroids between water polyoxyethylene nonionic surfactant micelles (6). The molar volume of the solubilisate has also been considered to be a significant determinant of the extent of solubilization (4).

In this work the solubilization of seven N^2-substituted iminophenazines, which have been tested for their antimicrobial activities (7,8), was investigated.

MATERIALS AND METHODS

Chemicals. The seven iminophenazines used in this study (Table I) were synthesized in the Medical Research Council Laboratories, Trinity College, Dublin. The surfactants used were Triton X-100 (kindly supplied by Rohm & Haas, UK, Ltd.) and sodium lauryl sulfate (BDH—analar grade). The solvents *n*-octanol and methanol were both BDH—analar grade. The Sorenson phosphate buffer (pH 5.15) was prepared using monopotassium phosphate (BDH) and disodium phosphate (BDH). Tris buffers were used in pK_a determinations.

Solubility Measurements. The method used was a modification of that previously reported (9), solubilities being determined by stirring an excess of each compound in aqueous solutions having different surfactant concentrations. The solutions were shaken for 48 hr in a shaker water bath (37 ± 0.5°C). Equilibration was established by repetitive sampling. Samples, filtered through Gelman GA-6 filters (pore size, 0.22 μm), were suitably diluted and assayed spectrophotometrically (SP8-100 Pye-Unicam ultraviolet spectrophotometer).

Ionization Constant Determination. The pK_a values were determined using the spectrometric method (10). A pH-meter 26 (Radiometer, Copenhagen) was used for the determination of the pH values.

Partition Coefficient Determination. The partition behavior of the iminophenazines was determined at 37°C using phosphate buffer (pH 5.15) and *n*-octanol, 6 hr being sufficient to produce equilibration. The absorbance of the organic solvent layer was determined spectrophotometrically after appropriate dilution. The partition coefficients were calculated by applying the ionization correction factor to the apparent values (11).

RESULTS AND DISCUSSION

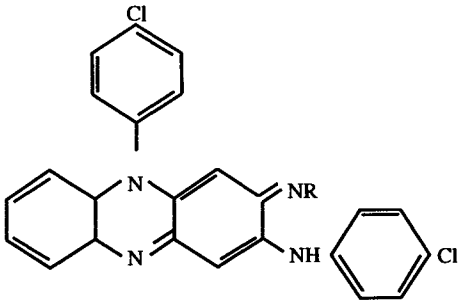
Influence of Surfactant on Iminophenazine Aqueous Solubility

The pH of surfactant solutions, particularly sodium lauryl sulfate, was found to increase as the surfactant concentration was increased; consequently solubility profiles tended to be nonlinear due to suppression of ionization. The

Table I. The N^2-Substituted Phenazines

Compound	*R*
B628	H
B663 (clofazimine)	$CHMe_2$
B826	$(CH_2)_3NEt_2$
B3779	$CH(Me)(CH_2)_3NEt_2$
B3785	$(CH_2)_3N(CH_2)_3CH_2$
B3770	$(CH_2)_3N(CH_2)_4CH_2$
B3640	$CH_2CH(CH_2)_2NHCH_2CH_2$

Clofazimine



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pH of the solution was determined at each surfactant concentration and used with the pK_a values (12) to correct for the variation in the solubility due to the pH changes. The following equations were used:

$$S = S_o + S_i + S_m \quad (1)$$

$$S = S_o (1 + 10^{pK_a - pH}) + K \cdot [M] \quad (2)$$

where S is the total amount of the drug in solution, S_o is the intrinsic solubility, S_i is the ionized drug in the aqueous phase, S_m is the quantity of the drug in the micelle, $[M]$ is the surfactant concentration, and K is the solubilizing capacity. Thus a plot of $S\text{-}S_o * 10^{pK_a - pH}$ versus M will be linear, having a slope of K. Solubility increased in proportion to surfactant concentration as a consequence of the increased number of micelles in solution (13). The values of the CMC (0.24 mM for Triton X-100 and 8.2 mM for sodium lauryl sulfate) (14) were relatively small compared to the surfactant concentration used in the study. The corrected solubility curves showed that the solubility of the iminophenazines increased linearly with increasing micelle concentration. Similar relationships have been reported using other drugs (6,15–17). The solubilizing capacity estimates indicate that Triton X-100 was more effective in solubilizing the iminophenazines than sodium lauryl sulfate. The micellar size of sodium lauryl sulfate (hydrodynamic radius, 2.5×10^{-9} m in 0.15 M NaCl; aggregation number, 62) is reportedly smaller than that of Triton X-100 (hydrodynamic radius, 4.8×10^{-9} m; aggregation number, 140) (17). However, the latter authors reported that sodium lauryl sulfate was able to solubilize more steroid than Triton X-100. In relation to the solubilizing power of ionic and nonionic surfactants for solubilizates which are located in the micellar interior, it has been observed that solubilizing capacity generally increases in the order anionic < cationic < nonionic. This effect has been attributed to a corresponding increase in the area per head group in the series, leading to "looser" micelles with less dense hydrocarbon cores which can accommodate more

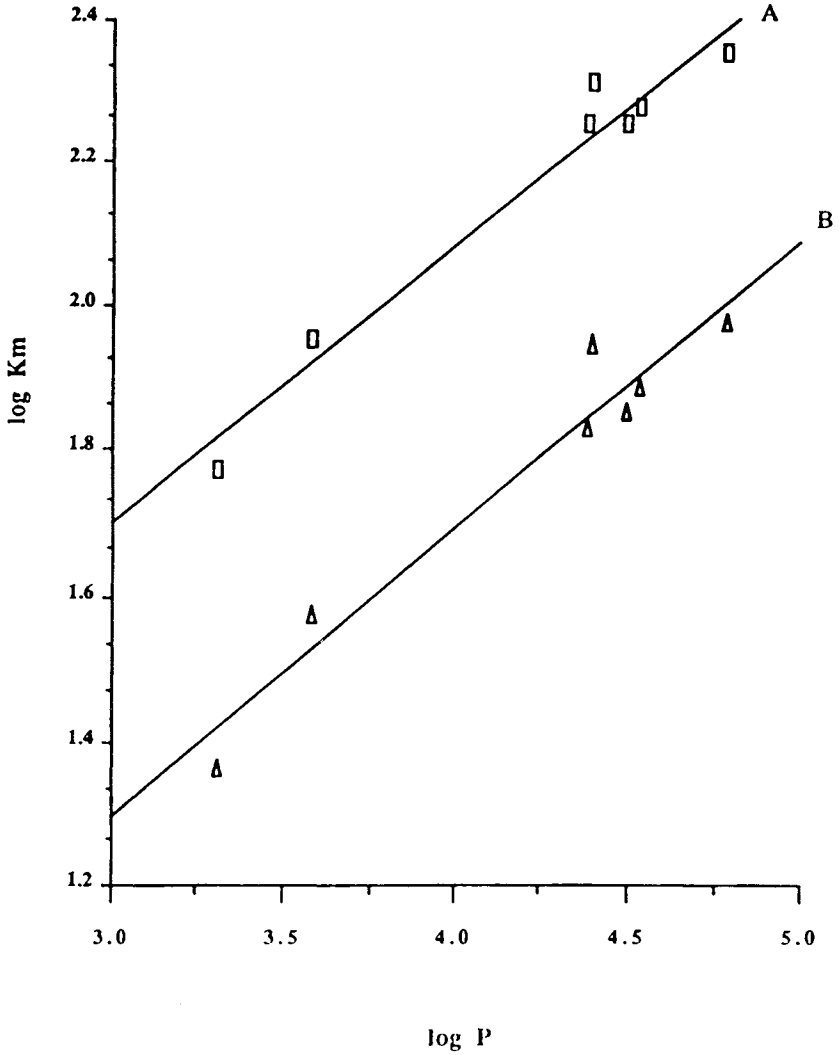

Fig. 1. Relationship between the micellar/water distribution coefficient ($\log K_m$) and the n-octanol/water partition coefficient ($\log P$) of the iminophenazines. (A) Triton X-100: $y = 0.5336 + 0.3883x$, $r = 0.98$. (B) Sodium lauryl sulfate: $y = 0.0903 + 0.4007x$, $r = 0.97$.

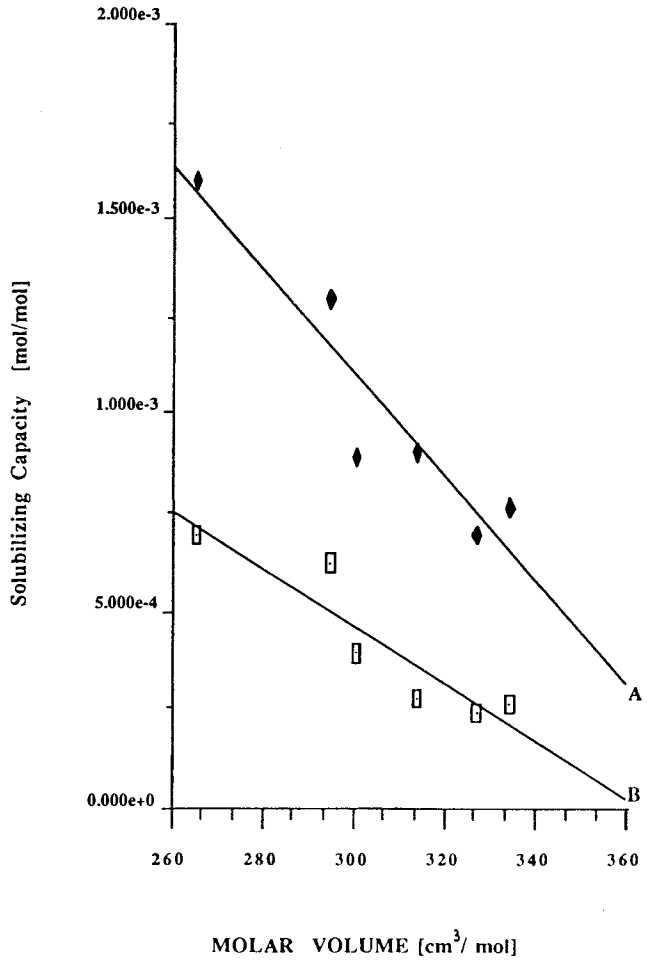

Fig. 2. Relationship between the solubilizing capacity (mol/mol) and the molar volume of the N^2-substituted iminophenazines in the micellar solution of Triton X-100 and sodium lauryl sulfate (SLS). (A) Triton X-100: $y = 0.005 - 1.312e\text{-}5x$, $r = 0.94$. (B) SLS: $y = 0.0026 - 7.301e\text{-}6x$, $r = 0.92$.

solubilizate (18). Our results are consistent with this observation.

Influence of Iminophenazine Lipophilicity

It has been reported (19) that benzoic acid derivatives show a linear relationship between their micellar/water partition coefficient and their octanol/water partition data. With simple compounds such as substituted benzoic acids it is relatively easy to discern such relationships, but with more complex drug molecules the interactions of lipophilicity, size, and shape become complex (3).

Solubilization of drugs can be treated in terms of an association equilibrium between the solutes and the micelles in a micellar solution (4,20). Thus if

$$D_w + M \overset{K_m}{\rightleftharpoons} D_m$$

where D_m and D_w are the drug solubilized in the micellar and aqueous phases, respectively, M is the micellar concentration, and K_m is the distribution coefficient between the micellar and the aqueous phases, then

$$S_m = K_m S_o M \quad (3)$$

Accordingly, Eq. (2) can be rewritten as

$$S = S_o + K_m S_o M + S_o\, 10^{pK_a - pH} \quad (4)$$

where S_m and S_o are, respectively, the nonionized solubilities in the micellar and water phases, allowing calculation of the distribution coefficients (K_m). The relationships between lipophilicity, expressed as log$P$, and logK_m are shown in Fig. 1. A reasonable linear fit for the data is reflected by the correlation coefficients of 0.98 and 0.97 for Triton X-100 and sodium lauryl sulfate, respectively. It can be seen from Fig. 1 that an increased lipophilicity of the iminophenazines resulted in an increased tendency for solubilization. The relationship between log$P$ (octanol/water) and logP_m (micelle/water) for a number of benzoic acid derivatives has been reported (21). In the latter report three parallel lines were required to represent logP_m versus log$P$ data adequately, although most derivatives could be represented by one of these lines. The additional lines were required for the nitro and cyano substitutents and the carboxylic groups. The slopes of the lines were not significantly different, ranging between 0.881 and 0.968. The authors suggested that the magnitude of the intercepts (in the range between 0.118 and 0.600) is a reflection of the site of solubilization and this difference indicated that the solubilization of these derivatives occurs in different regions of the micelle. For the iminophenazines in this study, five of the six compounds have substituted amino groups and lie on a reasonably linear line (Fig. 1). However, B663 ($R = CHMe_2$) was above the trend line with both surfactants. These results may suggest that B663 is not solubilized in the same region of the micelle as the other compounds. The less hydrophobic derivatives of benzoic acid in the nonionic surfactant are solubilized primarily in the oxyethylene mantle, while the hydrophobic derivatives are located in the micellar core (22). The linear regression equations obtained for the data show that the slopes of the two lines were similar, indicating that for both surfactants the increased tendency for solubilization of the iminophenazines was similar over the lipophilicity range. The intercepts of the two lines, however, are markedly different, sodium lauryl sulfate having the smaller value.

Influence of Solute Molar Volume

The volume of various hydrocarbons (23) solubilized by potassium laurate was found to be inversely related to the molar volume of the hydrocarbons. Likewise the amount of naphthalene, anthracene, pyrene, and dibenzoanthracene solubilized by dodecylpentaglycol ether was found to be inversely related to the molecular size of these solubilizates (24).

The relationship obtained between solubilizing capacity and iminophenazine molar volume (25,26) in Fig. 2 shows that the solubilizing capacity is inversely related to the molar volume; a reasonable fit for the data is reflected by the correlation coefficients of 0.92 and 0.94 for sodium lauryl sulfate and Triton X-100, respectively. It is evident that Triton, which has the greater solubilizing power, is also more sensitive to solute molar volume. The results suggest that derivatives having molar volumes greater than 380 cm^3/mol will

be too large to be solubilized. The inverse relationship is to be expected if solubilization is a consequence of incorporation of drug in the micelles. In addition to volume, polarity and shape are contributory factory.

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