DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 19(11), 1265-1282 (1993)



# ION-PAIRING OF DYCLONINE WITH DYES

Chun-Ren Chen[1], Joel L. Zatz[1] and Eugene Reilly[2,3]

[1]Rutgers College of Pharmacy, Piscataway, N.J. 08855-0789

[2] SmithKline Beecham, Parsippany, NJ

[3] Present Address : Chelsea Laboratories, W. Hempstead, NY

## ABSTRACT

This potential for ion-pairing between dyclonine, a local anesthetic, and pharmaceutical dyes commonly used in liquid pharmaceutical formulations was examined. Occurrence of ion-pairing at a pH of 3 was confirmed by shift of the absorption maximum of all five sulfonate-containing dyes, and by measurements of octanol/water partition coefficient and surface tension. There was also an increase in the uptake of dyclonine by phosphatidylcholine liposomes in the presence of a dye (tartrazine) at pH 3 but not at pH 7, where the uncharged anesthetic is dominant.

## INTRODUCTION

Dyclonine hydrochloride forms ion-pairs with inorganic salts and alkanesulfonates[1,2]. The effect of various inorganic ions on partitioning of dyclonine ion-pairs into phosphatidylcholine (PC) liposomes correlated better with surface tension reduction than partitioning into octanol[1]. Negatively charged organic ions (alkanesulfonates) lower surface tension at much lower concentrations than inorganic ions. Both octanol partitioning and liposome uptake were increased with increasing chain length of the alkanesulfonates[2].

The alkanesulfonates were chosen for investigation as models for pharmaceutical dyes that contain sulfonate groups. They are available as relatively pure compounds of known chemical structure whereas most dyes contain small amounts of salts, reactants and related compounds as impurities. The results described above suggest that combinations of such dyes with dyclonine may also form ion-pairs.

Previous studies have revealed interactions between various drugs and dyes in which ion-pairing probably played a role. The spectrum of 4-phenylazo-1-naphthol-2,4'-disulfonate was altered by exposure to dodecyl and hexadecyltrimethylammonium Br, at concentrations below their critical micelle concentration[3]. Other studies described interaction of cationic surfactants with Orange II[4] and amines with aromatic sulfonic acid dyes[5].

The purpose of this study is to evaluate ion-pair formation between dyclonine and a series of commercially available FD&C dyes using spectral, partitioning and surface tension measurements.



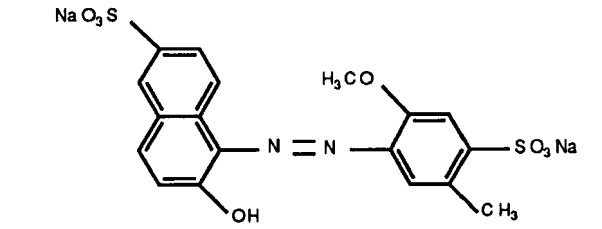
## MATERIALS

Dyclonine HCl was supplied by SmithKline Beecham. Tartrazine, FD&C red #40, fast green FCF, brilliant blue FCF, and sunset yellow FCF were obtained from Warner-Jenkinson (St. Louis, MO). Their assigned structures are shown in Figure 1[6]. L-α–Egg yolk phosphatidylcholine and bovine brain L-α–phosphatidyl-L-serine were obtained from Sigma Chemicals (St. Louis, MO). Sodium chloride, monobasic potassium phosphate, dibasic sodium phosphate, 1-octanol, hydrochloric acid, reagent grade, were purchased from Fisher Scientific (Springfield, NJ). Ultra pure pyrogen-free water for surface tension measurements was obtained from a Barnstead NANOpure II system.

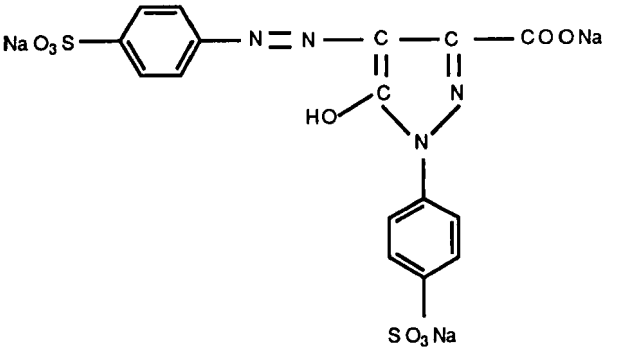
## METHODS

### Partition between Octanol and Aqueous Phase

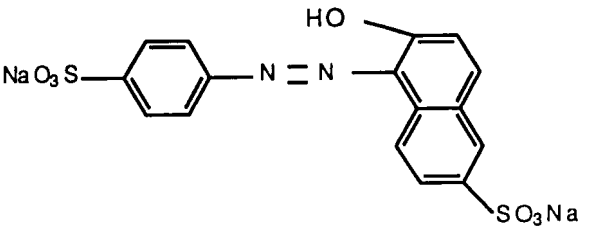
The procedure was the same as that previously described[1]. Octanol and aqueous phases were presaturated with each other. 0.1% w/v. of dyclonine hydrochloride and dye (if present) were dissolved in the aqueous phase. An equal volume of octanol was




(A)



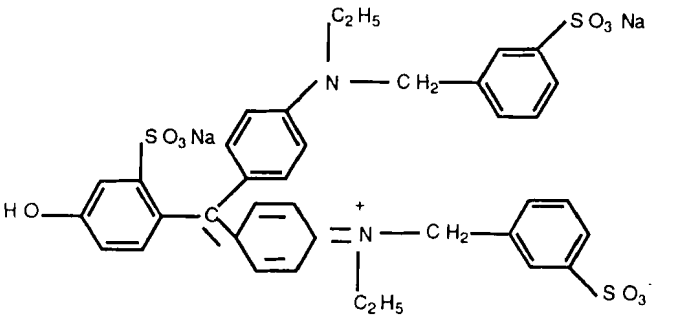

(B)



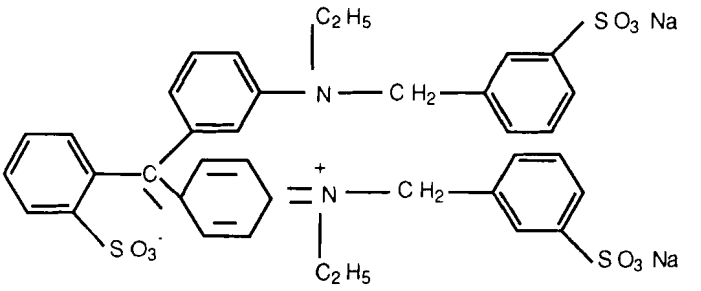

(C)

FIGURE 1

Structures assigned to dyes. (A) FD&C red #40;
(B) tartrazine; (C) sunset yellow FCF; (D) fast green FCF;
(E) brilliant blue FCF.




(D)




(E)

FIGURE 1 Continued

added to the above solution. The mixture was then shaken vigorously for 30 minutes and equilibrated at 25° C in a water bath shaker for 2 hours. The aqueous and octanol phases were separated by centrifuging the mixture at 2000 rpm for 5 minutes. Drug concentrations in the aqueous phase before and after partitioning were assayed. In the partition study at a pH

value of 5, the sample was adjusted to a pH value of 3 with diluted HCl solution to stabilize the dyclonine hydrochloride prior to analysis. All experiments were performed in triplicate.



Partition between Liposomes and Phosphate Buffer

A known amount of phosphatidylcholine (PC) solution in ethanol or equimolar mixture of phosphatidylcholine in ethanol/phosphatidylserine in chloroform (PC-PS) was put in a rotary round bottom flask under vacuum to evaporate the. organic solvent. A suitable amount of known concentration of dyclonine hydrochloride in phosphate buffer was added to the dried phospholipid. The suspension was sonicated till clear in a water bath at 25° C.

Suspensions containing 0.2 % liposomes were equilibrated with a known concentration of tartrazine solution at 25° C. The free drug was separated from liposomes by ultrafiltration using Centron® 30 membranes (Amicon, Danvers, MA). Free drug concentration in the filtrate and initial drug concentration before equilibrium were assayed. All experiments were performed in triplicate.

The partition coefficient, $P_L$ was calculated using Eq. 1[7].

$$P_L = \frac{(C_L/W_L)}{(C_{aq}/W_{aq})} \qquad \text{Eq. 1}$$

where $C_L$ and $C_{aq}$ represent the dyclonine concentrations in the liposome and aqueous phase, respectively. The former is calculated as the difference between initial and final dyclonine concentrations in the aqueous phase. $W_L$ and $W_{aq}$ are the weights of lipid and aqueous phase, respectively.

Surface Tension Measurement

Surface tension was obtained by a Rosano™ surface tensiometer at 25° C using the Wilhelmy plate method[8]. The platinum plate was roughened prior to use. Water purity was checked by electrical resistance and surface tension. All experiments were performed at least in duplicate.

Spectrophotometric Measurement

For spectrophotometric measurement, a suitable amount of dye was dissolved in pH 3 HCl solution or 0.2 M pH 3.5 sodium bitartrate buffer solution. A spectrophotometric scan covering wavelengths from 700 to 400 nm was run. In the interaction studies, a suitable amount of dyclonine hydrochloride or salt was added to the above dye solution and the scan rerun.

To prepare dye solutions in sodium bitartrate buffer, a suitable amount of dye was dissolved in about 150 ml of deionized water containing 10 g of sodium bitartrate in a 250 ml volumetric flask and diluted to volume. 25 ml of this solution was then diluted to 1000 ml in a volumetric flask containing 10 g of sodium bitartrate.

## RESULTS AND DISCUSSION

Spectrophotometric Studies

Addition of dyclonine to 3.63 $x10^{-5}$ M FD&C red #40 in the pH 3.5 sodium bitartrate buffer caused a shift of the absorption maximum to a longer wavelength and a decrease in absorbance

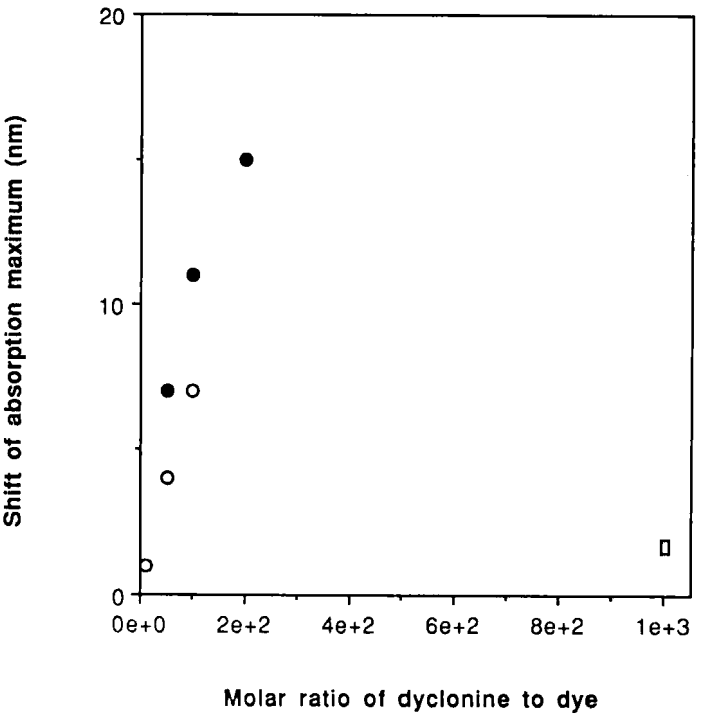



FIGURE 2

Effect of dyclonine to dyes molar ratio on the shift of absorption maximum of visible spectrum of dyes in pH 3.5 sodium bitartrate buffer □, brilliant blue FCF; ○, tartrazine; ●, FD&C red #40.

(Fig. 2 and 3). As the concentration of dyclonine increased, the absorption maximum shifted to longer wave lengths. For the brilliant blue FCF, a much higher concentration of dyclonine was required to shift the absorption maximum than for FD&C red #40 and tartrazine. When the ratio of dyclonine to brilliant blue FCF reaches a thousandfold the absorption maximum is only slightly shifted. These changes were not due to an increase in ionic strength as addition of 0.1 M sodium chloride did not

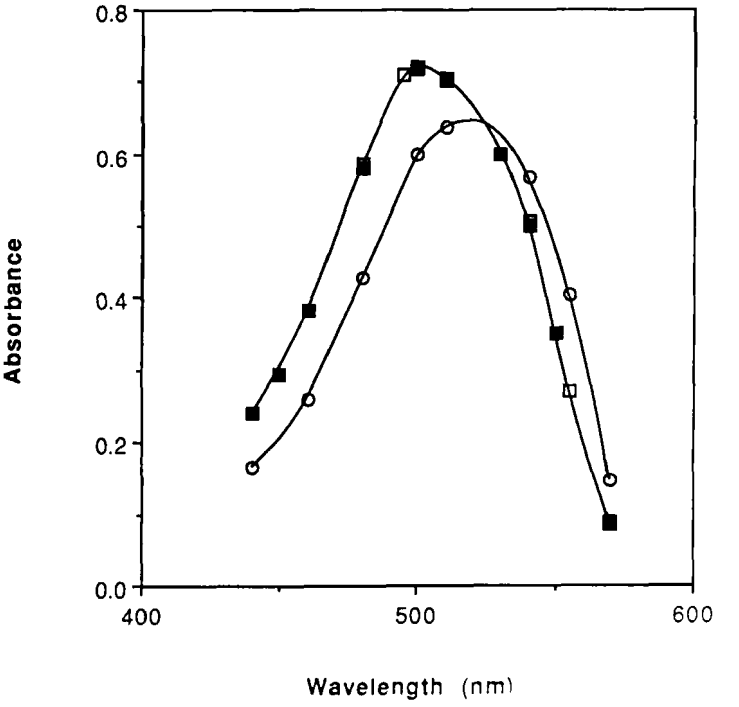



FIGURE 3

Effect of 3.1x $10^{-3}$ M dyclonine and 0.1 M NaCl on the visible spectrum of 3.1 x $10^{-5}$ M FD&C red #40 in 0.001N HCl solution □, FD&C red #40 only; ■, FD&C red #40 and 0.1 M NaCl; ○, FD&C red #40 and dyclonine.

affect the spectrum of the dye (Fig 3). Dyclonine itself does not absorb in the visible region. The bathochromic effect confirmed the microenvironment of dye was changed to more nonpolar due to the aggregation of dyclonine and dye molecules through ion-pairing[3,9]. FD&C red #40 and tartrazine have only two sulfonate groups and brilliant blue FCF has three, so it is more hydrophilic. It is thus more difficult for brilliant blue FCF to form ion-pairs with dyclonine than FD&C red #40 and tartrazine.

This finding is consistent with that of concentration of dyclonine required to partition brilliant blue FCF and FD&C red #40 from an aqueous to chloroform phase. The dyclonine to brilliant blue FCF ratio was much greater than dyclonine to FD&C red #40 for equivalent partitioning.

## Partition Studies

The dyes studied have sulfonate functional groups and are usually negatively charged in the pH range at which pharmaceutical solutions are formulated. The dye molecules used in this study also contain a hydrophobic portion. It is possible that the dyes would affect the partition property of dyclonine hydrochloride. A small concentration of dye was used to study the effect because in high concentration, they coprecipitated with dyclonine hydrochloride. Also, dyes are used in low concentration in pharmaceutical products.

Figure 4 shows that apparent partition coefficients increased as the brilliant blue concentration was increased. The partition coefficient without any dyes added at pH 5 is higher than at pH 3 because of the larger contribution of dyclonine base (the pKa of dyclonine HCl is 7.3). However, the apparent partition coefficient at pH 3 can be higher than that at pH 5 if dye is added at the lower pH. The slope of the straight line obtained by plotting partition coefficient versus dye concentration at pH 3 for tartrazine (1308) is greater than that of brilliant blue FCF (1013) or fast green FCF (1011). With the

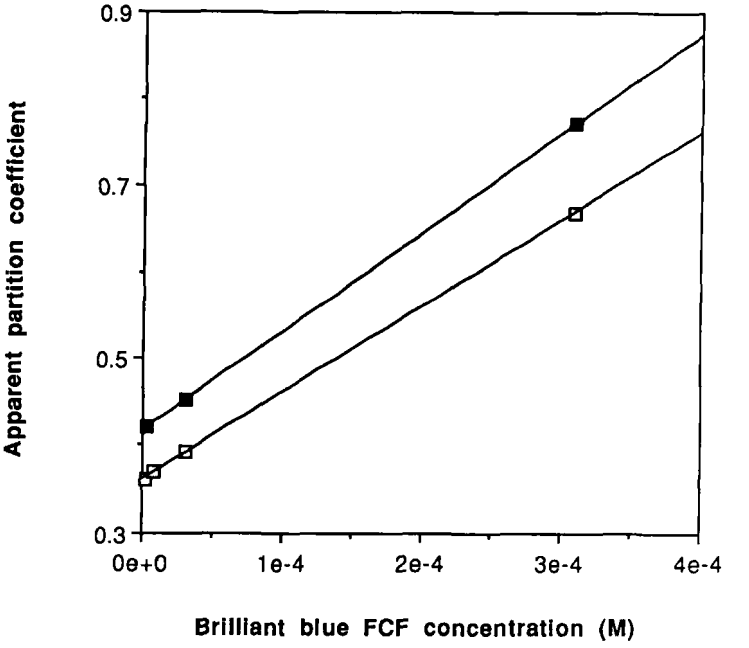



FIGURE 4

Effect of brilliant blue FCF concentration on partition of dyclonine HCl between octanol and 0.001 N HCl solution □, pH 3; ■, pH 5.

same mechanism discussed above, tartrazine containing two sulfonate groups has a higher partition coefficient than those of brilliant blue FCF and fast green FCF containing three sulfonate groups.

## Surface Tension Studies

None of the dyes (tartrazine, brilliant blue FCF, sunset yellow FCF, FD&C red# 40, and fast green FCF) had any effect on surface tension of water at pH values of 3 and 5 with or without 0.1 M

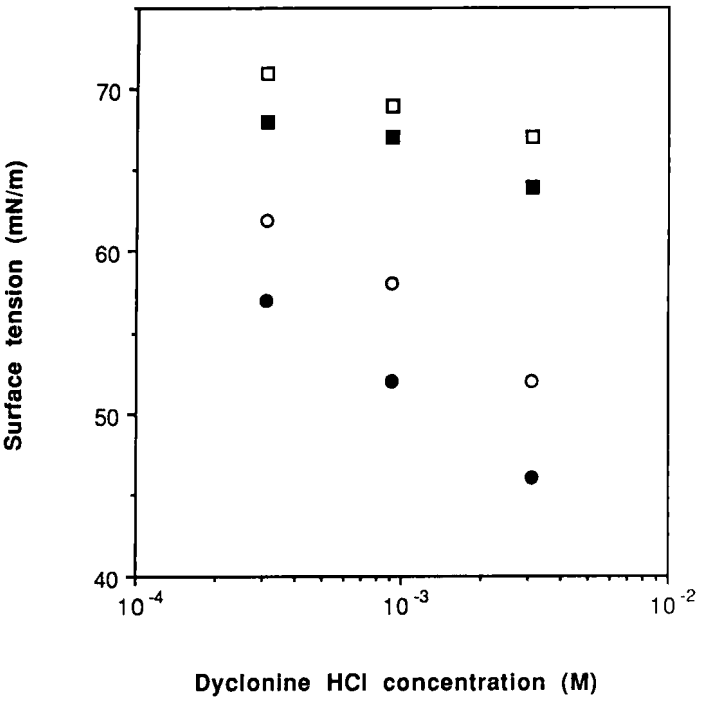



FIGURE 5

Effect of brilliant blue FCF concentration on surface tension of dyclonine HCl in 0.001 N HCl solution □, 0; ■, 3.1 x $10^{-6}$ M; ○, 3.1 x $10^{-5}$ M; ●, 3.1 x $10^{-4}$ M.

NaCl at concentrations up to 3 x $10^{-3}$ M. However, when a dye was added to dyclonine hydrochloride in 0.001 N hydrochloride solution the surface tension of dyclonine hydrochloride was decreased. A typical example appears in Figure 5. The decrease in surface tension was correlated with dye concentration.

Commercial dyes contain salts as impurities[6]. Chloride and sulfate are most commonly encountered and usually in highest concentration. Both chloride and sulfate decrease surface

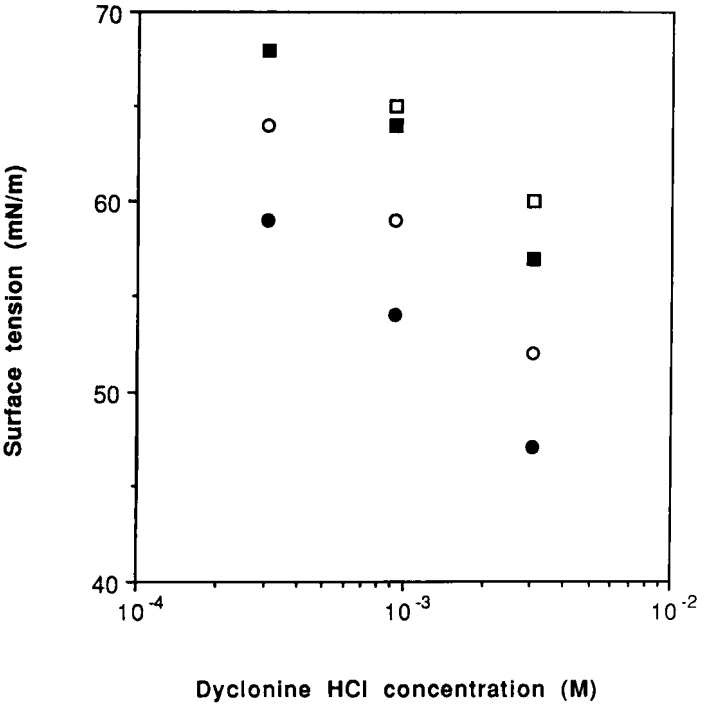



FIGURE 6

Effect of brilliant blue FCF concentration on surface tension of dyclonine HCl in 0.001 N HCl solution in the presence of 0.1 M NaCl □, 0; ■, $3.1 \times 10^{-6}$ M; ○, $3.1 \times 10^{-5}$ M; ●, $3.1 \times 10^{-4}$ M.

tension of dyclonine hydrochloride[1]. However, when the hydrochloric solution was swamped with 0.1 molar sodium chloride, the addition of brilliant blue still decreased the surface tension of dyclonine hydrochloride solutions at different concentrations as shown in Figure 6. This shows that the decrease in surface tension of dyclonine hydrochloride was not due to ionic impurities. Rather, it suggests the formation of ion-

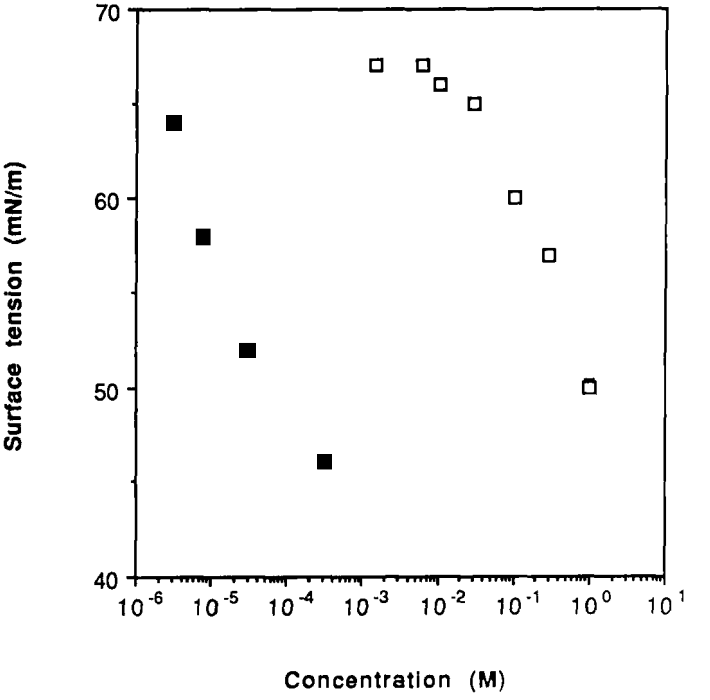



FIGURE 7

Effect of anion concentration on surface tension of 0.1 % dyclonine HCl in 0.001 N HCl solution □, NaCl; ■, brilliant blue FCF.

pairs between the positively ionized form of dyclonine hydrochloride and negatively charged dyes, because all the dyes have sulfonate groups and they would dissociate at the very low pH. Since dyclonine hydrochloride does not form micelles[1] and the dyes are not surface active in the concentration studied, ion-pairing provides the only explanation.

It is known the concentration of the dyclonine hydrochloride, pH of the solution, ionic strength of the solution,

and property of counterions affect the surface tension of dyclonine hydrochloride. For a meaningful comparison of one factor all other factors should be fixed. So the concentration of counterions needed to decrease the surface tension to the same level was compared.

The effect of inorganic sodium chloride and organic brilliant blue on the surface tension of dyclonine hydrochloride was compared at the same pH and dyclonine hydrochloride concentration. From Figure 7 it is seen that brilliant blue had a much greater effect (by 3 orders of magnitude) in reducing the surface tension of 0.1 % dyclonine hydrochloride than sodium chloride. This is because dyes have a large hydrophobic portion. The tendency to form ion-pairs is much higher for a large organic ion than inorganic salts. When two anions have the same charge, their effects in decreasing surface tension of dyclonine hydrochloride is dependent on the size of the hydrophobic portion. However, the effect of structures among different dyes is not so apparent as dyes have different sizes of hydrophobic portion.

Liposome Study

Tartrazine was chosen as a representative compound to study the effect of dyes on the uptake of dyclonine into liposomes. $P_L$ data in Figures 8 are the ratio of measured $P_L$ to that of a reference, the appropriate dyclonine system containing no additives. Figure 8 shows that $3.1 \times 10^{-4}$ molar tartrazine

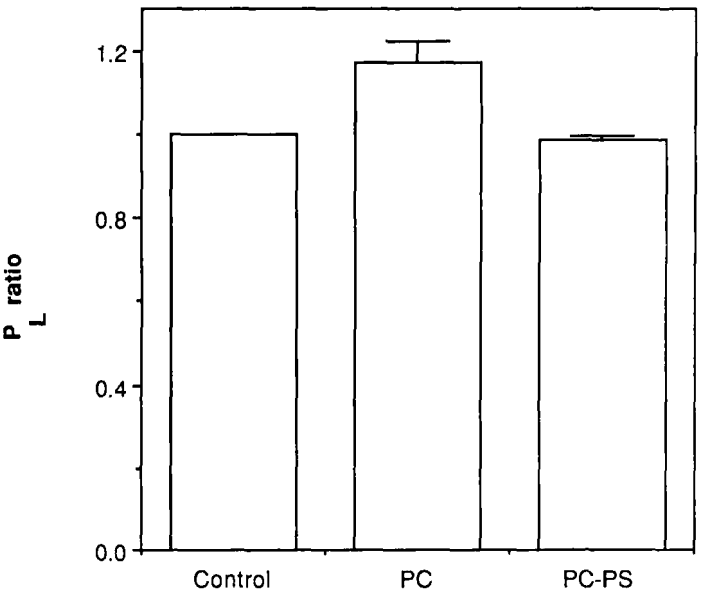



FIGURE 8

Effect of 3.1x $10^{-4}$ M tartrazine on partition of dyclonine HCl into liposomes from pH 3.2 phosphate buffer solution

increased the partition of dyclonine HCl into PC liposomes from pH 3.2 phosphate buffer. There is no significant effect of this dye at pH 7 or on the uptake of dyclonine into PC-PS liposomes at either pH.

In conclusions, we found that there was interaction between dyclonine HCl and several dyes attributed to ion-pair formation. Dyclonine affects the visible spectrum and partition of dyes, while dyes influence the partition, surface activity, and interaction of dyclonine HCl with neutral liposomes.

## ACKNOWLEDGEMENT

The authors acknowledge project support from SmithKline Beecham. We also thank Mr. Robert Wiegand for assistance with drug analysis.

## REFERENCES

(1) C-R Chen and J. Zatz, Effect of Electrolytes on Dyclonine Interaction with Liposomes, to be published.

(2) C-R Chen and J. Zatz, Effect of Alkanesulfonates on Dyclonine Interaction with Liposomes, to be published.

(3) R.L. Reeves, R.S. Kaiser, and H.W. Mark, "The Nature of Species Giving Spectral Changes in an Azo Dye on Interaction with Cationic Surfactants Below the Critical Micelle Concentration," *J. Colloid Interface Sci.* **45**, 396 (1973).

(4) G. Zografi, P.R. Patel and N.D. Weiner, "Interactions Between Orange II and Selected Long Chain Quaternary Ammonium Salts," *J. Pharm. Sci.* **53**, 544 (1964).

(5) R.L. Hull and L.A. Biles, "Physical Chemical Study of the Distribution of Some Amine Salts Between Immiscible Solvents. II Complexation in the Organic Phase," *J. Pharm. Sci.*, **53**, 869 (1964).

(6) "Certified Food Colors," Warner-Jenkinson Company.

(7) G.V. Betageri and J.A. Rogers, "The Liposome as a Distribution Model in QSAR Studies," *Int. J. Pharm.*, **46,** 95 (1988).

(8) A.W. Adamson, "Physical Chemistry of Surfaces" 4th ed. P. 24. (1982)

(9) R.M. Diamond, "The Aqueous Solution Behavior of Large Univalent Ion. A New Type of Ion-Pairing," *J. Phys. Chem.*, **67**, 2513 (1963).,