Interaction of Some Pharmaceuticals with Macromolecules II

Binding of Certain Benzoic Acid Derivatives by Polysorbate 80 and Cetomacrogol 1000

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The magnitude of intermolecular association occurring in aqueous solution between benzoic acid, a series of amino-, chloro-, and hydroxybenzoic acids, and two macromolecules, polysorbate 80 and cetomacrogol 1000, was determined by means of an equilibrium dialysis technique. All the benzoic acids studied were found to exhibit reversible association with the surfactants of the polyether type. The degree of interaction between the aromatic acids and the macromolecules appears to be dependent upon the type of functional group and its position in the interacting molecule. The binding affinity of polysorbate 80 for benzoic acid decreased with an increase in pH. The binding of the benzoic acids by polysorbate 80 and cetomacrogel 1000 was found to be describable by a Freundlich-type adsorption relationship.

EARLIER work on this subject (1–5) has been concerned with the interaction of *p*-hydroxybenzoic acid esters and phenols with nonionic macromolecules and the importance of the data relative to the preservative activity of the compounds, with the possible mechanism of interaction between preservatives and the macromolecules. Evidence of the complex formation between phenols and cetomacrogol 1000[1] has been presented by Hadgraft (6) as well as by Mulley and Metcalf (7). Hugo and Newton (8) reported, in their studies of iodine solubilized by cetomacrogol 1000, that it is the available iodine which is related to the antimicrobial activity. Furthermore, this type of interaction has been shown to modify the absorption characteristics of drugs. Levy and Reuning (9) demonstrated that the gastric absorption of salicylic acid was decreased rather significantly in the presence of 2% polysorbate 60.

Higuchi and Lach (10) showed the tendency of certain aromatic carboxylic acids to form molecular complexes with polyethers such as polyethylene glycols. They indicated that the complex formation between these acids and polyethylene glycols could be due to hydrogen bond formation between the hydrogen of the carboxyl group and negative centers of the polyethylene glycol molecules. It would thus be expected that benzoic acid and its derivatives might interact with polysorbate 80[2] and cetomacrogol 1000[3] molecules. It has been reported (11) that the interactions resulting from hydrogen bond formation between a weakly acidic and a weakly basic compound tend to decrease upon dissociation of the interacting acid. Thus, if the principal binding force between benzoic acid and polysorbate 80 is due to hydrogen bond formation, the increase in pH which tends to dissociate the acid should result in a decrease in the extent of binding of benzoic acid by polysorbate 80.

It is the purpose of this study to determine the degree of any intermolecular association which might occur between various benzoic acids and two representative macromolecules, polysorbate 80 and cetomacrogol 1000. The present communication is concerned with the effects of chloro, hydroxy, and amino substituents of benzoic acid on these interactions as well as with the influence of varying pH on the binding of benzoic acid by polysorbate 80.

EXPERIMENTAL

Reagents.—Recrystallized benzoic acid,[4] m.p. 122°; recrystallized *o*-hydroxybenzoic acid,[4] m.p. 158–159°; recrystallized *m*-hydroxybenzoic acid,[5] m.p. 201°; recrystallized *p*-hydroxybenzoic acid,[5] m.p. 213–214°; recrystallized *o*-chlorobenzoic acid,[5] m.p. 139–140°; recrystallized *m*-chlorobenzoic acid,[5] m.p. 155–156°; recrystallized *p*-chlorobenzoic acid,[5] m.p. 239–240°; recrystallized *o*-aminobenzoic acid,[5]

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[1] Cetomacrogol 1000 B.P.C., a polyethylene glycol 1000 monocetyl ether represented by the general formula: $CH_3\cdot(CH_2)_m\cdot[O{-}CH_2{-}CH_2]_n\cdot OH$, where m = 15 or 17, and n = 20–24.

[2] Polyoxyethylene (20) sorbitan monooleate. Marketed as Tween 80 by Atlas Chemical Industries, Canada, Ltd., Brantford, Canada.

[3] Texofor A1P. Supplied through the courtesy of Glovers (Chemicals) Ltd., Leeds, England.

[4] J. T. Baker Chemical Co.

[5] Eastman Organic Chemicals.

m.p. 145–146°; *m*-aminobenzoic acid,[5] m.p. 173–174°; *p*-aminobenzoic acid,[6] m.p. 187–188°; polysorbate 80 and cetomacrogol 1000, commercial samples.

Equilibrium Dialysis Procedure.—The experimental technique employed in the current work was essentially the same in principle as that employed by Patel and Kostenbauder (1). Dialysis membranes utilized in the studies involving polysorbate 80 and cetomacrogol 1000 were nylon membranes.[7] These membranes were found to be satisfactory, since they proved to be permeable to the benzoic acids and impermeable to cetomacrogol 1000. They were previously shown to be impermeable to polysorbate 80 (1). The general procedure is the same as that used by Patel and Foss (5) with minor modification in the design of the plastic dialysis cells.

Each dialysis cell consisted of two Plexiglas blocks, 7.6 × 7.6 × 1.9 cm., each half with a cylindrical cavity having an internal diameter of 3.4 cm. and height of 1.3 cm. with a total capacity of 12 ml. Threaded Plexiglas plugs with knurled heads provided access to the cell cavities. To assemble the cells, a semipermeable nylon membrane was clamped between the two symmetrical halves, 10 ml. of solution was pipeted into each cavity as required, and the stoppers, fitted with O-rings,[8] were screwed in tightly. The solutions of the drugs were prepared, and in order to suppress the dissociation of the aromatic acids, the pH of the solutions was adjusted to a value of 2, except in the case of *o*-hydroxy- and *o*-chlorobenzoic acids where the solutions were adjusted to pH 1 using a HCl–KCl buffer (12). Citrate buffer (12) was employed for the pH range of 3 to 5.4. In the entire investigation, the ionic strength of all the buffered solutions was adjusted to 0.1 with potassium chloride. It was observed that, especially at pH 1, the polysorbate 80 solutions were unstable upon storage for a period of 1 week, possibly due to hydrolysis of ester linkage liberating free oleic acid which was found to be titratable with standard sodium hydroxide solution. Subsequent stability studies of polysorbate 80 (13) indicated that any decomposition of polysorbate 80, within the experimental limits of time, temperature, and pH, was negligible. All the solutions employed in the present work were freshly prepared with deionized distilled water.

Utilizing the above procedure, equilibrium was established at 30° in 2 days for *o*-hydroxy, *ortho*, *meta*, and *p*-chlorobenzoic acids, 6 days for *ortho* and *p*-aminobenzoic acids, and 9 days for *m*-aminobenzoic acid. At the end of equilibrium time, 1- or 2-ml. aliquots were removed from both sides of the membrane, diluted with 0.01 *N* HCl to suppress the dissociation of acids, and the concentrations of the compounds under study were determined with a Beckman DU spectrophotometer at the following wavelengths: benzoic acid, 230 mμ; *o*-hydroxybenzoic acid, 237 mμ; *m*-hydroxybenzoic acid, 236 mμ; *p*-hydroxybenzoic acid, 255 mμ; *o*-chlorobenzoic acid, 280 mμ; *m*-chlorobenzoic acid, 231 mμ; *p*-chlorobenzoic acid, 241 mμ; *o*-aminobenzoic acid, 221 mμ; *m*-aminobenzoic acid, 225 mμ; *p*-aminobenzoic acid, 225 mμ. Any interference due to the macromolecule was eliminated by using the appropriate concentration in the reference cell. The pH of the solutions in the dialysis studies was recorded at the end of the experiment, with no appreciable change noted. All pH measurements were made with a Beckman zeromatic pH meter, model 76, using a combination electrode.

[5] Fisher Scientific Co., Ltd.
[7] Capran, polyamide film, 0.0005 in. Allied Chemical Corp., Morristown, N. J.
[8] National O-Rings, Division of Federal Mobil Service, Detroit, Mich.

RESULTS

Polysorbate 80.—Blaug and Ahsan (14) determined the solubility of *ortho*- and *p*-hydroxybenzoic acids in weakly acidic (0.005 *N* H_2SO_4) solutions containing up to 2% polysorbate 80. In the present work the extent of binding of these acids by polysorbate 80 was determined by the dialysis method and the concentration of polysorbate 80 was extended up to 8%. Data for the interaction of *ortho*, *meta*, and *p*-hydroxy as well as aminobenzoic acids are plotted in Fig. 1. The data were plotted to show the ratio, *R*, of the total/free drug as a function of concentration of the macromolecule. This ratio is fairly constant at a particular concentration of the surfactant, and it can often be employed to calculate the free or unbound drug from the known concentration of drug in solution. All the hydroxybenzoic acids interacted reversibly with

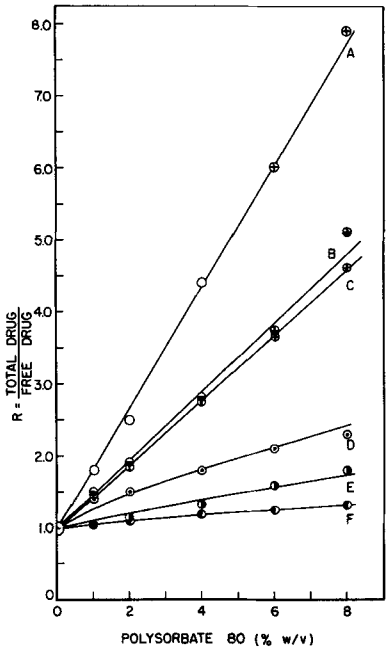

Fig. 1.—Binding of hydroxy and aminobenzoic acids by polysorbate 80 in aqueous solution at 30°. Key: A, total *o*-hydroxybenzoic acid at pH 1; ○, 8.80–11.3 × 10^{-3} *M*; ⊕, 1.75 × 10^{-2} *M*. B, total *m*-hydroxybenzoic acid at pH 2; ◒, 8.16–9.23 × 10^{-3} *M*; ◒, 1.00–1.14 × 10^{-2} *M*. C, total *p*-hydroxybenzoic acid at pH 2; ⊙, 8.26–9.62 × 10^{-3} *M*; ⊕, 1.02–1.80 × 10^{-2} *M*. D, total *o*-aminobenzoic acid at pH 2 ranged from 7.78 to 10.4 × 10^{-4} *M*. E, total *p*-aminobenzoic acid at pH 2 ranged from 7.22 to 9.35 × 10^{-4} *M*. F, total *m*-aminobenzoic acid at pH 3 was 9.25 × 10^{-4} *M*. Each point represents the average of two determinations, except in the case of curve F.

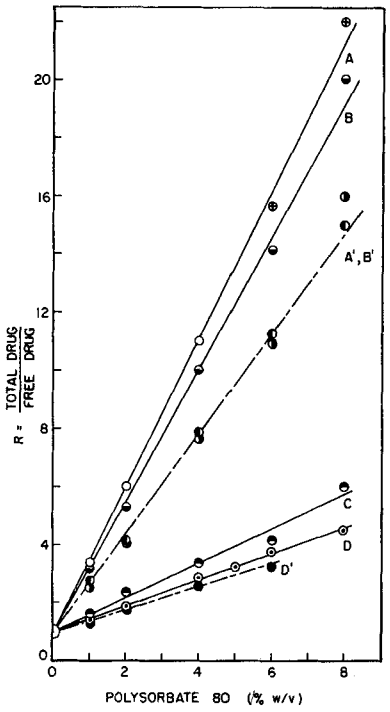

Fig. 2.—Binding of benzoic acid and chlorobenzoic acid by polysorbate 80 in aqueous solution at 30°. Key: A, total *p*-chlorobenzoic acid at pH 2; ○, 8.91×10^{-4} to 3.25×10^{-3}; ⊕, 4.02–6.81×10^{-3} *M*. A′, total *p*-chlorobenzoic acid (unbuffered, measured pH was 4.4 ± 0.1) ranged from 6.54×10^{-4} to 3.54×10^{-3} *M*. B, total *m*-chlorobenzoic acid at pH 2; ○, 3.68–9.01×10^{-3} *M*. B′, total *m*-chlorobenzoic acid (unbuffered, measured pH was 3.4 ± 0.1) ranged from 3.71×10^{-4} to 1.15×10^{-3} *M*. C, total *o*-chlorobenzoic acid at pH 1 ranged from 8.9×10^{-4} to 1.66×10^{-3} *M*. D, total benzoic acid at pH 2 ranged from 1.00 to 2.04×10^{-2} *M*. D′, total benzoic acid (unbuffered, measured pH was 3.2 ± 0.1) ranged from 8.88×10^{-3} to 1.12×10^{-2} *M*. Each point represents the average of two determinations.

polysorbate 80. Blaug and Ahsan (14) showed that there was no increase in the solubility of *m*-hydroxybenzoic acid in aqueous solutions of up to 2% of polysorbate 40. Comparison of the binding data of benzoic acid by polysorbate 80 of Fig. 1 and the solubility data (15) indicate the slope values of the plot of ratio total/free drug against concentration of polysorbate 80 are in good agreement, the slope value being 0.5 in each case. This suggests that the solubilization of benzoic acid and the binding as determined in the dialysis studies can probably be attributed to the same mechanism. Similar binding in the case of interaction between methyl *p*-hydroxybenzoate and polysorbate 80 has been reported in the previous work (1).

The magnitude of interaction of benzoic and chlorobenzoic acids with polysorbate 80 is shown in Fig. 2. The curves A′, B′, and D′ demonstrate significant difference between the binding tendencies in aqueous solutions and at pH 2 (curves A, B, and D). This is to be expected because of slightly higher pH of the aqueous solutions of polysorbate 80. It is evident from this figure that the substitution of a chlorine atom in benzoic acid increases its binding affinity for polysorbate 80.

Figure 3 illustrates adsorption isotherms for the interaction of benzoic acid with 5% polysorbate 80 at varying pH values, illustrating that the binding decreases with increase in pH. The binding of benzoic, chloro-, and hydroxybenzoic acids was describable by Freundlich-type plots as illustrated by Fig. 4. Similar plots demonstrating the interaction of *o*-hydroxybenzoic acid with polysorbate 80 and the binding of some drugs by plasma proteins have been described in the literature (9, 16).

Cetomacrogol 1000.—Data for the binding of benzoic, hydroxy, amino, and chlorobenzoic acids are presented in Figs. 5 and 6. All the compounds studied interacted with cetomacrogol in a manner identical to that described under polysorbate 80. Cetomacrogol 1000 interacted with all the benzoic acids studied to a greater extent than polysorbate 80. Chakravarty *et al.* reported the interaction of benzoic and hydroxybenzoic acids with polyoxyl 40 stearate based on the solubility determinations (17). Solubilization of some benzoic acid derivatives by polyoxyethylene stearates has been reported by Goodhart and Martin (18). Curve D of Fig. 6 demonstrates that the magnitude of binding of benzoic acid by cetomacrogol 1000 in the dialysis studies and the extent of solubilization as determined by solubility method are essentially equal, indicating that the mechanism involved in both

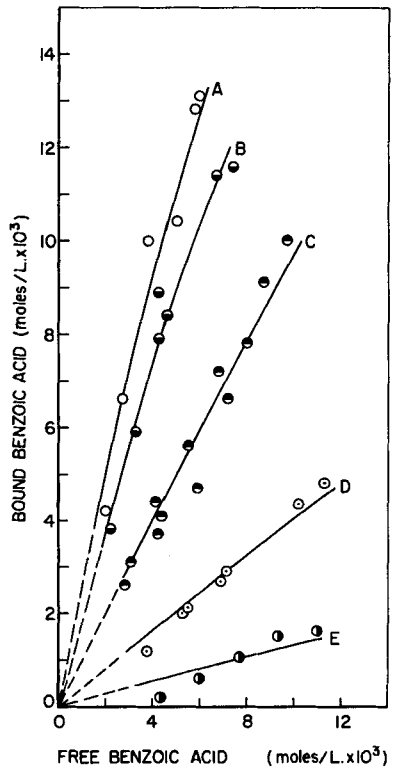

Fig. 3.—Adsorption isotherms for binding of benzoic acid by polysorbate 80 in aqueous solution at 30° and various pH's. Key: A, pH 1.9; B, pH 3.2; C, pH 4.2; D, pH 4.7; E, pH 5.2 (±0.1).

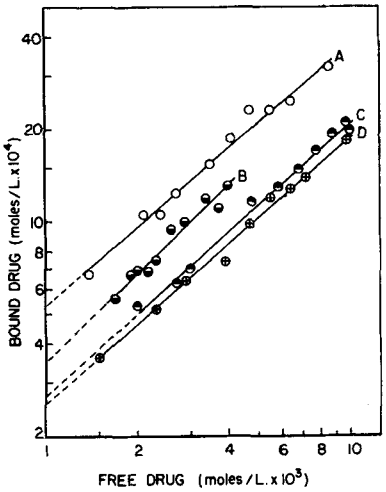

Fig. 4.—Freundlich-type plots for binding of benzoic acids by 0.5% w/v polysorbate 80 at 30°. Key: A, *o*-hydroxybenzoic acid; B, *o*-chlorobenzoic acid; C, benzoic acid; D, *p*-hydroxybenzoic acid. (Drawn by method of least-squares fit using IBM 1620.)

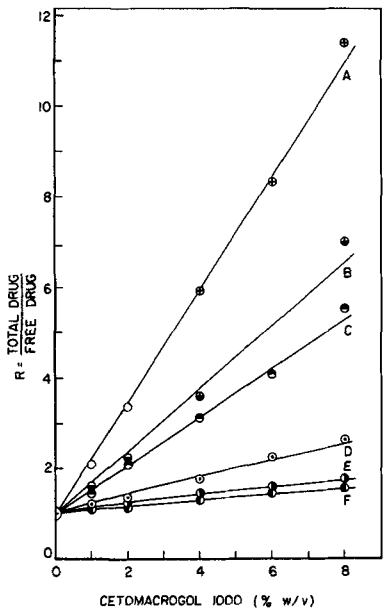

Fig. 5.—Binding of hydroxy and aminobenzoic acids by cetomacrogol 1000 in aqueous solution at 30°. Key: A, total *o*-hydroxybenzoic acid at pH 1; ○, 9.32–10.8 × 10^{-3} *M*; ⊕, 1.69–2.45 × 10^{-2} *M*. *B*, total *m*-hydroxybenzoic acid at pH 2; ◒, 8.49–9.44 × 10^{-3} *M*; ◒, 1.05–1.07 × 10^{-2} *M*. C, total *p*-hydroxybenzoic acid at pH 2 ranged from 8.68 × 10^{-3} to 2.28 × 10^{-2} *M*. D, total *o*-aminobenzoic acid at pH 2.2 ranged from 7.25 × 10^{-3} to 1.09 × 10^{-2} *M*. E, total *p*-aminobenzoic acid at pH 2.3 ranged from 6.82 to 8.06 × 10^{-3} *M*. F, total *m*-aminobenzoic acid at pH 4.3, 8.05 × 10^{-3} *M*. Each point represents the average of two determinations.

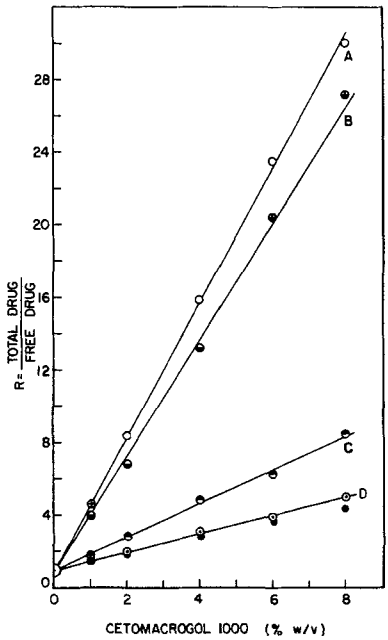

Fig. 6.—Binding of benzoic acid and chlorobenzoic acids by cetomacrogol 1000 in aqueous solution at 30°. Key: A, total *p*-chlorobenzoic acid at pH 2; ⊕, 8.86 × 10^{-4} *M*; ○, 2.09–3.00 × 10^{-3} *M*. B, total *m*-chlorobenzoic acid at pH 2; ◒, 4.06–9.88 × 10^{-3} *M*; ◒, 1.32–1.64 × 10^{-2} *M*. C, total *o*-chlorobenzoic acid at pH 1; ○, 1.22–2.01 × 10^{-3} *M*. D, total benzoic acid at pH 2; ◉, 8.52 × 10^{-3} *M*; ⊙, 1.06–2.71 × 10^{-2} *M*. The dark points below curve D indicate binding as determined by solubility study. Each point represents the average of two determinations.

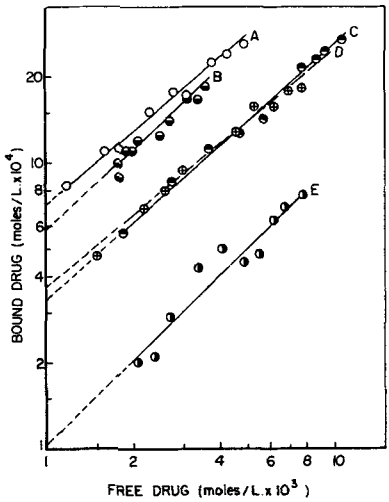

Fig. 7.—Freundlich-type plots for binding of benzoic acids by 0.5% w/v cetomacrogol 1000 at 30°. Key: A, *o*-hydroxybenzoic acid; B, *o*-chlorobenzoic acid; C, benzoic acid; D, *p*-hydroxybenzoic acid; E, *o*-aminobenzoic acid. (Drawn by method of least-squares fit using IBM 1620.)

studies might be the same. Figure 7 is a Freundlich-type plot depicting the results of the binding of benzoic, amino-, chloro-, and hydroxybenzoic acids by cetomacrogol 1000.

DISCUSSION

In the hydroxybenzoic acid series, *o*-hydroxybenzoic acid showed the greatest binding tendency for polysorbate 80, followed by *meta* and *para* isomers. Blaug and Ebersman (19) reported identical behavior of hydroxybenzoic acids toward hyprose ester. Substitution in the *ortho* position increases the proton-donating property of benzoic acid, which in turn increases its binding affinity for polysorbate 80 and cetomacrogol 1000. There was a noticeable increase in the binding tendency of *p*-hydroxybenzoic acid as compared to the *meta* isomer for both polysorbate 80 and cetomacrogol 1000. This could be probably due to the fact that the *m*-isomer is slightly more acidic than the *para* compound.

In the amino-substituted compounds, the *ortho*-substituent interacted with the macromolecules studied to a greater extent, behaving similarly to the hydroxy counterpart, followed by *para* and *m*-aminobenzoic acids. Blaug and Ebersman (19) reported identical behavior of aminobenzoic acids toward sucrose and propoxylated sucrose esters. The binding tendency of aminobenzoic acid was considerably lower than that of benzoic acid. This would be expected since the dialysis studies were carried out in relatively strong acidic media where the amino compounds were present as the protonated species. The protonated ion would not be involved in hydrogen bonding and would tend to inhibit any form of hydrophobic interactions.

The magnitude of binding of chlorobenzoic acids by the macromolecules increased from *ortho* to *meta* to *p*-chlorobenzoic acid. All the three compounds showed greater binding affinity for the macromolecules, as compared to benzoic acid. The electron-withdrawing chlorine atom in the chlorobenzoic acid increases its proton-donating power, making it more reactive with polysorbate 80 and cetomacrogol 1000. The *ortho*-substituent showed the least tendency to interact with the macromolecules. This is in direct contrast to the results observed in the case of *o*-hydroxy and *o*-aminobenzoic acids. Due to the relatively large size of the chlorine atom, *o*-chlorobenzoic acid would be sterically less favored in the hydrogen bonding with the nonionics. This might be supported further by the fact that *p*-chlorobenzoic acid, with least steric hindrance, showed the maximum binding affinity for both the macromolecules.

The effect of pH on the polysorbate 80–benzoic acid interaction shows that binding decreased with an increase in pH, indicating that the binding is primarily due to the undissociated acid. Increase in pH tends to ionize benzoic acid, making it less susceptible to hydrogen bond formation (11). Autian and Shaikh have reported a similar effect of pH on the binding of sorbic acid by nylon (20).

The exact nature of these interactions is not completely known at the present time. Micellar solubilization of the acidic compound (18, 19, 21) or the formation of molecular complexes of the type described by Higuchi and Lach (10) has been suggested as the possible mechanism of interaction between carboxylic acids and surfactants of polyether class. Hyde *et al.* (22) have reported that organic acids and phenols are solubilized in soap solution by micellar solubilization. Blaug and Ebersman (19) have postulated that aromatic acids are possibly incorporated in the palisade layer of the surfactant micelle. A micelle of a nonionic (21) might be expected to provide an ideal model for the association with aromatic acids providing for both the possibility of hydrogen bond formation and hydrophobic interactions. Regardless of the nature of these interactions, it is to be expected that the activity of the compounds might be modified due to the binding (2, 8, 9, 23). For example, benzoic acid would be partly bound and thus inactivated in the presence of polysorbate 80 and cetomacrogol 1000. Data presented in this work can be used to calculate the free concentration of the drugs according to the procedure described by Pisano and Kostenbauder (2).

SUMMARY

Equilibrium dialysis studies indicate a rather generalized binding tendency of some benzoic acid derivatives for polysorbate 80 and cetomacrogol 1000.

The magnitude of interaction between the carboxylic acids and the macromolecules increased from amino to hydroxy to chlorobenzoic acids, and it was influenced by the position of these functional groups in the interacting molecule.

The change in pH of the benzoic acid–polysorbate 80 interaction showed a decrease in association with an increase in pH, indicating that binding is primarily due to undissociated benzoic acid.

The data obtained in this work facilitate the calculation of unbound drugs under study in a system containing polysorbate 80 and cetomacrogol 1000 at a particular pH value.

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