REFERENCES

(1) Glass, G. B. J., Skeggs, H. R., Lee, D. H., Jones, E. L., and Hardy, W. W., *Nature*, **189**, 138(1961).
(2) Macek, T. J., personal communication.
(3) Smith, E. L., "Vitamin B_{12} and Intrinsic Factor," Ed. Heinrich, Stuttgart, Germany, 1957, p. 3.
(4) Carr, J. W., personal communication.
(5) Skeggs, H. R., Hanus, E. J., McCauley, A. B., and Rizzo, V. J., *Proc. Soc. Expll. Biol. Med.*, **105**, 518(1960).
(6) Skeggs, H. R., *J. Biol. Chem.*, **184**, 211(1950).
(7) Higuchi, T., and Marcus, A. D., THIS JOURNAL, **43**, 530(1954).
(8) Bender, M., *Chem. Rev.*, **60**, 50(1960).
(9) Garrett, E. R., *J. Am. Chem. Soc.*, **79**, 3401(1957).

Interaction of Some Pharmaceuticals with Macromolecules I

Effect of Temperature on the Binding of Parabens and Phenols by Polysorbate 80 and Polyethylene Glycol 4000

By N. K. PATEL and N. E. FOSS

A quantitative evaluation of the effect of temperature on the interaction of methyl, propyl, and butylparaben, phenol and *p*-chlorophenol with polysorbate 80 was obtained with an equilibrium dialysis technique utilizing a plexiglas dialysis cell and a semipermeable membrane. Interactions of parabens with polyethylene glycol 4000 have been studied by the solubility method. The binding affinity of polysorbate 80 for the parabens employed decreased with increase in temperature, whereas that of polyethylene glycol 4000 showed an increase. There was no evidence of temperature dependency of the binding of phenol and *p*-chlorophenol with polysorbate 80. Parachlorophenol showed a greater tendency to interact with polysorbate 80 than with phenol. The significance of these results relative to the possible mechanism of these interactions is considered.

IN RECENT YEARS considerable attention has been centered on the interaction of preservatives with nonionic macromolecules (1–5) and the importance of considering such interactions when determining proper preservative concentrations (2). These interactions are particularly noticeable with phenolic preservatives in the presence of surfactants which are polyoxyethylene derivatives of fatty acid esters. Several review articles have been published describing the possible mechanism of the interaction of phenolic compounds with surfactants (6–9).

Higuchi and Lach (10) and Guttman and Higuchi (11) hypothesized that the complex formation between phenols and polyethylene glycols could be due to hydrogen bond formation between the hydrogen of the phenolic hydroxyl group and basic oxygen atom of polyethylene glycol. It would thus be expected that proton donors like phenol and *p*-chlorophenol might form molecular complexes with surfactants containing polyoxyethylene groups.

Guttman and Higuchi (11) have indicated that addition of thermal energy to the phenol-PEG interaction might result in a decrease in association between phenol and PEG. Thus, if the principal binding force between a phenolic preservative and a nonionic surfactant is due to hydrogen bond formation, the increase in temperature might decrease the intermolecular association in such a system. Furthermore, nonionic surfactants usually contain a relatively large number of hydrophobic groups in the molecule and contribution of hydrophobic interactions (10) to the magnitude of binding of a phenolic preservative by the macromolecule might be of considerable importance.

In the present investigation an equilibrium dialysis technique was utilized to obtain information regarding the extent of binding of methyl, propyl, and butylparaben, phenol and *p*-chlorophenol with polysorbate 80[1] at various temperatures, for the purpose of relating these interactions to the reported possible mechanism of binding. The solubility method was employed to study the interaction of methyl and propylparaben with PEG 4000 at 20°. The selection of the phenolic compound was based on the relative proton donating power of the compound and on the length of the hydrocarbon chain of the *p*-hydroxybenzoic acid esters.

EXPERIMENTAL

Reagents.—Recrystallized methyl *p*-hydroxybenzoate,[2] m.p. 127–128°; recrystallized propyl

Received May 8, 1963, from the School of Pharmacy, Duquesne University, Pittsburgh, Pa.
Accepted for publication June 13, 1963.
This work was carried out at the School of Pharmacy, University of Maryland, Baltimore.
The authors express sincere thanks to Dr. H. B. Kostenbauder, School of Pharmacy, Temple University, Philadelphia, Pa., for his valuable suggestions.
Presented to the Pharmaceutical Sciences Section, American Association for the Advancement of Science, Philadelphia meeting, December 1962.

[1] Polyoxyethylene (20) sorbitan monooleate. Marketed as Tween 80 by the Atlas Powder, Co., Wilmington, Del.
[2] Methyl Parasept, purified; supplied through the courtesy of Heyden Newport Chemical Corp., New York, N. Y.

p-hydroxybenzoate,[3] m.p. 96–98°; recrystallized butyl *p*-hydroxybenzoate,[4] m.p. 70–72°; phenol, reagent grade (Mallinckrodt Chemical Works); *p*-chlorophenol, reagent grade (Eastman Organic Chemicals); polysorbate 80, a commercial sample; polyethylene glycol 4000.[5]

Equilibrium Dialysis Method.—The experimental technique was identical in principal to that used by Patel and Kostenbauder (1) in studying the interaction of parabens with polysorbate 80. Because the nylon membrane[6] employed during the present investigation was in the form of a thin film, a dialysis cell similar to that suggested by Kostenbauder (12) was utilized.

A typical dialysis cell is shown in Fig. 1. Each dialysis cell consists of two plexiglas[7] blocks $2^1/_2 \times 2^1/_2 \times 1^1/_{16}$ in., each having a cavity of 20-ml. capacity and four $^3/_{16}$ in. holes at the corners. The dialysis membrane was placed between the two blocks with the cavities facing each other. The blocks were held firmly together by the use of four stainless steel head bolts $3^1/_2 \times {}^3/_{16}$ in., inserted in the corner holes and secured with stainless steel wingnuts. Each block has a threaded hole entering the cavity used for the introduction of the solution or the removal of sample for analysis. After the two blocks were securely tightened, 10 ml. paraben solution was placed in the cavity on one side of the membrane and 10 ml. polysorbate 80 solution was placed on the other side of the membrane. The entire assembly was then stoppered tightly by means of plexiglas screw-plugs with thin polyethylene gaskets and agitated at a constant temperature until equilibrium was attained (1).

Nylon membranes were unsatisfactory for the dialysis studies involving phenol and *p*-chlorophenol, since aqueous solutions of these substances converted the structure of nylon from a thin membrane to a gummy, resinous mass. A thin rubber membrane[8] was found to be highly satisfactory, since it proved to be permeable to phenol and impermeable to polysorbate 80. The experimental procedure was identical to that reported previously (1).

After equilibration, aliquots were removed from both sides of the membrane and concentrations of the compounds under study were determined spectrophotometrically at a wavelength of 255 mμ for methyl, propyl, and butylparaben, 270 mμ for phenol, and 278 mμ for *p*-chlorophenol, using a Beckman DU spectrophotometer.

Solubility Method.—The solubility of methyl and propylparaben was determined in the presence of varying concentrations of polyethylene glycol 4000. The experimental procedure (3), method of assay, and treatment of the data have been described previously (1).

RESULTS

Effect of Temperature on the Binding of Parabens by Polysorbate 80.—Binding of methyl and propylparaben by polysorbate 80 at 30° has been reported previously (1). Blaug and Ahsan (5) determined the solubility of propyl and butylparaben in aqueous solutions of polysorbate 80 at 0°. In the present study the magnitude of binding of methyl, propyl, and butylparaben by polysorbate 80 at varying temperatures was determined by the dialysis method.

Figures 2–4 show that the paraben-polysorbate 80 interaction exhibits a marked temperature dependency. The slope values given in these figures are indicative of the relative magnitude of binding of parabens by polysorbate 80. It is evident from these slope values that an increase in temperature decreases the extent of binding of methyl, propyl, and butylparaben by polysorbate 80.

Figures 2–4 illustrate further that the degree of binding increases in general order of 1:6:18 from methyl to propyl to butylparaben; the corresponding slope values increase from 0.7 to 4.4 to 12.9 at 30° and 0.63 to 3.9 to 11.3 at 40°.

Effect of Temperature on the Binding of Phenols by Polysorbate 80.—The phenol-polysorbate 80 interaction shows only a minor temperature de-

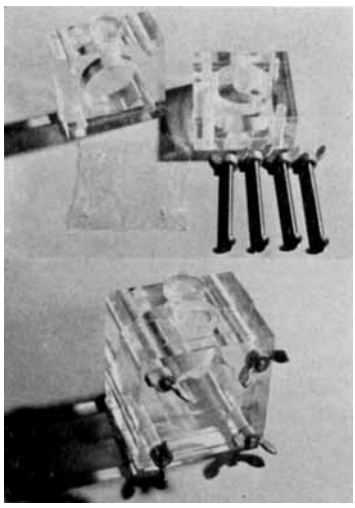

Fig. 1.—Dialysis cell.

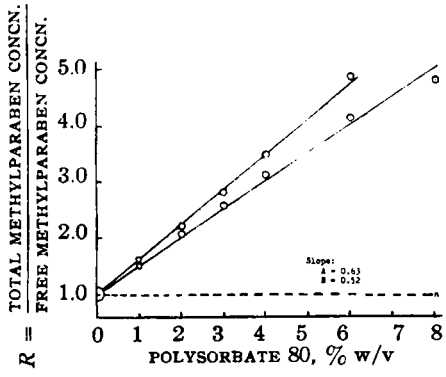

Fig. 2.—Binding of methylparaben by polysorbate 80 in aqueous solution at 40 and 50°. A, 40°; B, 50°. The broken line represents a theoretical line which would be obtained if no binding were to have taken place. Such a line will be labelled "n" in the figures of this paper.

[3]Propyl Parasept, purified; supplied through the courtesy of Heyden Newport Chemical Corp., New York, N. Y.
[4]Butyl Parasept, purified; supplied through the courtesy of Heyden Newport Chemical Corp., New York, N. Y.
[5]"Carbowax" 4000; supplied through the courtesy of Union Carbide Chemicals Co., New York, N. Y.
[6]Plaskon Nylon, 0.5 mil thickness; supplied through the courtesy of Central Research Laboratories, Allied Chemical Corp., Morristown, N. J.
[7]Methyl methacrylate. Marketed as Plexiglass by the Rohm and Haas Co., Philadelphia, Pa.
[8]Supplied through the courtesy of Julius Schmid, Inc., New York, N. Y.

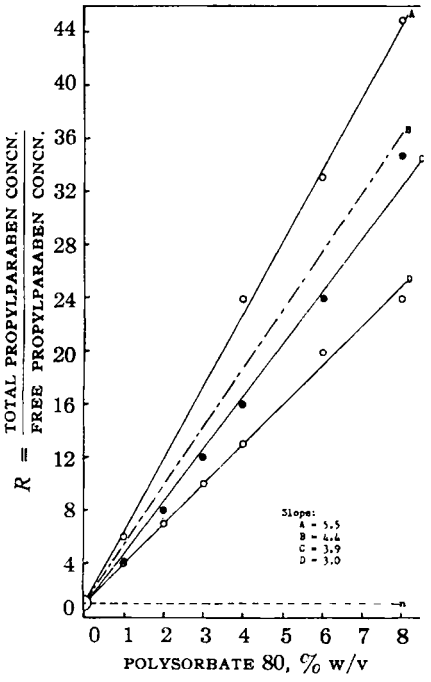

Fig. 3.—Binding of propylparaben by polysorbate 80 in aqueous solution at various temperatures. A, 20°; B, 30°. Data from Patel and Kostenbauder (1): C, 40°; D, 50°.

pendency as shown in Fig. 5. There was no indication of any change in the degree of binding of *p*-chlorophenol by polysorbate 80 at 40 and 50° (Fig. 6). Figures 5 and 6 illustrate that the binding tendency of polysorbate 80 toward phenols increases from phenol to *p*-chlorophenol. The slope value is 1.4 for *p*-chlorophenol compared to 0.25 for phenol, indicating that the magnitude of binding of *p*-chlorophenol with polysorbate 80 is in the order of approximately six times that of phenol.

Effect of Temperature on the Binding of Parabens by PEG 4000.—Solubilities of methyl and propylparaben were determined in aqueous solutions of PEG 4000 at 20° to observe the effect of this temperature on the extent of interactions compared to results previously reported at 30° (3). Curves *A* and *B* of Figs. 7 and 8 indicate that the extent of interaction of both the parabens with PEG increases with the increase in temperature.

DISCUSSION

Polysorbate 80.—Micellar solubilization of the phenolic preservative (7, 13) or the formation of molecular complexes of the type described by Higuchi and co-workers (10, 11) has been suggested as the possible mechanism of the interaction between a phenolic compound and polysorbate 80. A micelle of a nonionic surfactant (7) might be expected to provide an ideal model for the association with a phenol providing for both the possibility of hydrogen bond formation and hydrophobic interactions.

The hydrogen bonding tendency can easily be shown by correlating the magnitude of binding with proton-donating power of the phenolic compounds. The affinity of polysorbate 80 for *p*-chlorophenol is approximately six times greater than that for phenol. The substitution of a chlorine atom in the benzene ring increases the proton-donating power of phenol, making it more susceptible to hydrogen bond formation (14).

Higuchi and Lach (10) demonstrated that compounds such as phenol with acidic hydrogen would associate with electrophilic atoms such as oxygen; and they further suggested that such an interaction could be rendered favorable by "squeezing together" of the hydrophobic portions of the interacting molecules. This is a possible explanation of the experimental findings that as the hydrophobic character of the phenolic compound was increased from methyl to propyl to butylparaben, the binding affinities of these compounds for polysorbate 80 increased in the order of approximately 1:6:18.

Thermal agitation would be expected to exert two antagonistic tendencies on the binding of phenol by polysorbate 80. It would result in a decrease in association of phenol with polysorbate 80 by weakening attractive forces between them (11). On the other hand, it might be favored by the thermal desolvation of the polyoxyethylene chain. These two tendencies apparently nullify each other in the binding of phenol and *p*-chlorophenol by polysorbate 80. Furthermore, the molecular structure

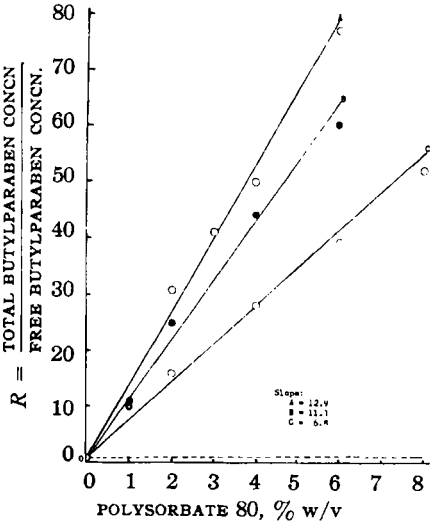

Fig. 4.—Binding of butylparaben by polysorbate 80 in aqueous solution at various temperatures. A, 30°; B, 40°; C, 50°.

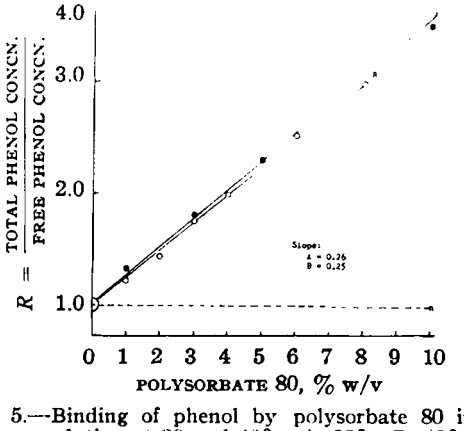

Fig. 5.—Binding of phenol by polysorbate 80 in aqueous solution at 30 and 40°. A, 30°; B, 40°.

of the interacting compounds and the steric hindrance may account for the decreased magnitude of binding of parabens by polysorbate 80 at higher temperatures. Addition of heat to such a system tends to increase rotation and vibration of the hydrocarbon moieties (7) of the ester linkages in both polysorbate 80 and paraben molecules. It seems probable that hydrogen-oxygen bonding would be sterically and statistically less favored at an elevated temperature. Phenol and *p*-chlorophenol with the absence of any ester linkage showed no temperature dependency, while butylparaben, having the largest ester linkage, showed a maximum temperature dependency.

Polyethylene Glycol 4000.—The effect of temperature on PEG-paraben interaction indicates that the binding increases with temperature. These interactions would be favored by thermal desolvation of the polymer chain, thus providing more sites for attachment for the competing interaction with paraben (11). Addition of heat might result also in a decrease in association of paraben with the polymer by weakening attraction forces between them. In addition, there is no steric hindrance due to ester linkage which is present in polysorbate 80. Thus, it is likely that desolvation of the polymer chain, allowing the competing paraben molecules to take part in the hydrogen bonding and "squeezing together" of the hydrophobic moieties (10) without any steric hindrance contribute to the stability of the complex at a higher temperature.

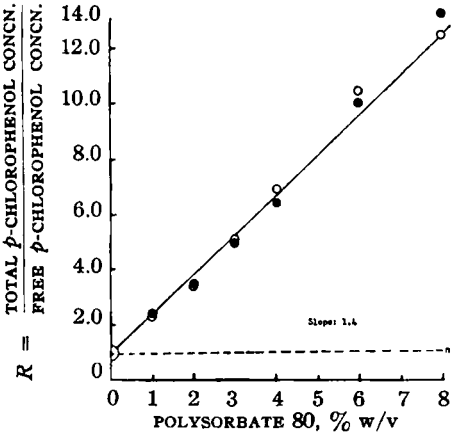

Fig. 6.—Binding of *p*-chlorophenol by polysorbate 80 in aqueous solution at 40 and 50°. O, 40°; ●, 50°.

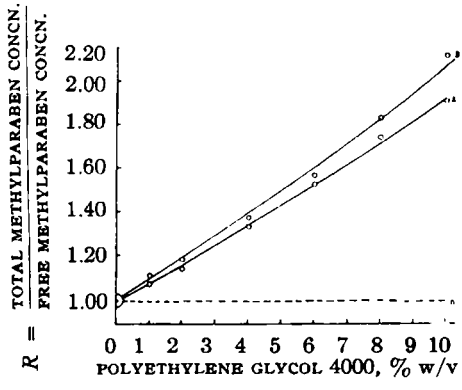

Fig. 7.—Ratio, *R*, of total methylparaben concentration to free methylparaben concentration as a function of the concentration of PEG 4000 at 20 and 30°. A, 20°; B, 30°. Data from Miyawaki, Patel, and Kostenbauder (3).

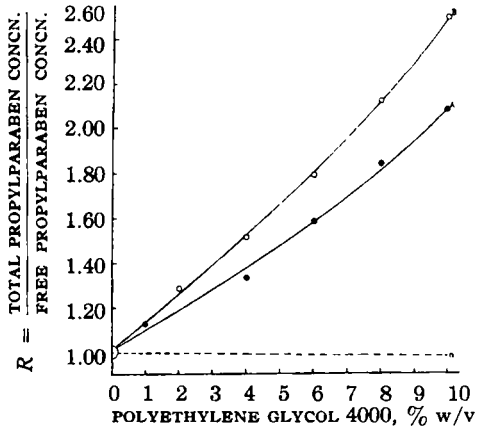

Fig. 8.—Ratio, *R*, of total propylparaben concentration to free propylparaben concentration as a function of the concentration of PEG 4000 at 20 and 30°. A, 20°; B, 30°. Data from Miyawaki, Patel, and Kostenbauder (3).

SUMMARY

The interaction of methyl, propyl, and butylparaben, phenol and *p*-chlorophenol with polysorbate 80 has been studied quantitatively by means of a dialysis technique utilizing a plexiglas dialysis cell and a semipermeable membrane.

The degree of binding of polysorbate 80 by methyl, propyl, and butylparaben increased approximately in the order of 1:6:18.

The extent of this intermolecular association was dependent upon the relative proton-donating properties of the phenolic compound.

The binding affinity of polysorbate 80 for the parabens employed in this study decreased with increase in temperature, whereas that of polyethylene glycol 4000 showed an increase. The phenol-polysorbate 80 interaction was found to be temperature independent.

Interpretation of these data regarding the possible mechanism of interaction of a phenolic compound with macromolecules of the polyoxyethylene type has been discussed.

REFERENCES

(1) Patel, N. K., and Kostenbauder, H. B., THIS JOURNAL, **47**, 289(1958).
(2) Pisano, F. D., and Kostenbauder, H. B., *ibid.*, **48**, 310(1959).
(3) Miyawaki, G. M., Patel, N. K., and Kostenbauder, H. B., *ibid.*, **48**, 315(1959).
(4) Ahsan, S. S., and Blaug, S. M., *Drug Std.*, **28**, 95 (1960).
(5) Blaug, S. M., and Ahsan, S. S., THIS JOURNAL, **50**, 441(1961).
(6) Moore, C. D., and Hardwick, R. B., *Mfg. Chemist*, **27**, 305(1956).
(7) Beckett, A. H., and Robinson, A. E., *Soap, Perfumery Cosmetics*, **31**, 454(1958).
(8) deNavarre, M. G., *Am. Perfumer Aromat.*, **73**, 31 (1959).
(9) Kostenbauder, H. B., *ibid.*, **75**, 28(1960).
(10) Higuchi, T., and Lach, J. L., THIS JOURNAL, **43**, 465 (1954).
(11) Guttman, D., and Higuchi, T., *ibid.*, **45**, 659(1956).
(12) Kostenbauder, H. B., personal communication.
(13) Aoki, M., Kamata, A., Yoshioka, I., and Matsuzaki, T., *J. Pharm. Soc. Japan*, **76**, 939(1956).
(14) Nagakura, S., *J. Am. Chem. Soc.*, **76**, 3070(1954).