(14) Patton, S., and Kurtz, G. W., *J. Dairy Sci.*, **34**, 669(1951).
(15) Dunkley, W. L., and Jennings, W. G., *ibid.*, **34**, 1064(1951).
(16) Sidwell, C. G., Salwin, H., and Benca, M., *J. Am. Oil Chemists' Soc.*, **31**, 603(1954).
(17) Yu, T. C., and Sinnhuber, R. O., *Food Technol.*, **11**, 104(1957).
(18) Keeney, M., and Bassett, R., *J. Dairy Sci.*, **42**, 945 (1959).
(19) deKoning, A. J., and Silk, M. H., *J. Am. Oil Chemists' Soc.*, **40**, 165(1963).
(20) Jacobson, G. A., Kirkpatrick, J. A., and Goff, H. E., Jr., *ibid.*, **41**, 124(1946).
(21) Brownley, C. A., Jr., and Lachman, L., *J. Pharm. Sci.*, **53**, 452(1964).
(22) Sinnhuber, R. O., Yu, T. C., and Yu, Te Change, *Food Res.*, **23**, 626(1958).
(23) Schmidt, H., *Fette Seifen Anstrichmittel*, **61**, 881(1959).
(24) Dahle, L. K., Hill, E. G., and Holman, R. T., *Arch. Biochem. Biophys.*, **98**, 253(1962).
(25) Day, E. A., *J. Dairy Sci.*, **9**, 1360(1960).
(26) Palmateer, R. E., Yu, T. C., and Sinnhuber, R. O., *Food Technol.*, **14**, 528(1960).
(27) Wishnetsky, T., and Stuckey, B. N., *ibid.*, **15**, 503 (1961).
(28) Tarladgis, B. G., *et al.*, *J. Am. Oil Chemists' Soc.*, **37**, 44(1960).
(29) Tarladgis, B. G., Pearson, A. M., and Dugan, L. R., Jr., *ibid.*, **39**, 34(1962).
(30) Yu, T. C., and Sinnhuber, R. O., *Food Technol.*, **16**, 115(1962).
(31) Sinnhuber, R. O., and Yu, T. C., *ibid.*, **12**, 9(1958).
(32) Yamada, T., *Biochem.*, **35**, 369(1963).
(33) Karkkainen, V. J., and Antila, M., *Milchwissenschaft*, **17**, 479(1962).
(34) Jaminet, F., *Il Farmaco Pavia, Prat. Ed.*, **14**, 73 (1959).
(35) Yamada, T., *Biochem.*, **35**, 309(1963).

Interaction of Parachlorometaxylenol with Macromolecules

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A quantitative evaluation of the interaction between parachlorometaxylenol and various macromolecules was obtained by solubility and dialytic procedures. The macromolecules studied were polyvinylpyrrolidone, polyethylene glycol 6000, polysorbate 80, methylcellulose, and poly(methyl vinyl ether/maleic anhydride). The binding affinity of polyvinylpyrrolidone and poly(methyl vinyl ether/maleic anhydride) for parachlorometaxylenol exhibited only a minor temperature dependency. The parachlorometaxylenol–polyethylene glycol interaction was found to be temperature independent. Polysorbate 80 interacted with parachlorometaxylenol to a greater degree than did the other macromolecules studied. Interpretation of the data relative to the possible mechanisms of these interactions is considered.

In recent years, nonionic polymeric substances have been used extensively in the formulation of pharmaceutical dosage forms. Although these polymers are usually considered to be chemically inert, many of them undergo interaction with drug molecules in aqueous solution. These interactions may result in physical incompatibilities as well as the inactivation of preservatives and antimicrobial agents. The inhibitory effect of nonionic macromolecules on phenolic preservatives has been reported (1–12).

Higuchi and Lach (13) and Guttman and Higuchi (14) have investigated the complex formation between phenols and polyethers such as polyethylene glycols. More recently, Patel and Foss (15) have studied the interaction of parabens and phenols with polysorbate 80 and polyethylene glycol 4000. They demonstrated that *p*-chlorophenol exhibited a greater tendency to interact with polysorbate 80 than did phenol.

Parachlorometaxylenol (PCMX) has been used as an antimicrobial agent for a number of years in Great Britain. The biological activity and clinical usefulness of the compound have been reported (16–18). In a study conducted by Mulley and Metcalf (19), it was demonstrated that the solubility of PCMX in aqueous solution was augmented by the presence of polyethylene glycol 1000 monocetyl ether. They attributed the increased solubility of PCMX to its incorporation into micelles.

The present investigation was undertaken to obtain a quantitative evaluation of the interaction between PCMX[1] and various macromolecules. An equilibrium dialysis technique was utilized to study the binding of polyvinylpyrrolidone[2] (PVP), polysorbate 80,[3] and methylcellulose[4] with PCMX. The solubility method was used to study the interaction of PVP, poly-

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[1] Ottasept Extra. Supplied through the courtesy of the Ottawa Chemical Co., Toledo, Ohio.
[2] Plasdone, type K 29–32. Supplied through the courtesy of Antara Chemicals, a division of General Aniline and Film Corp., New York, N. Y.
[3] Marketed as Tween 80 by Atlas Chemical Industries, Inc., Wilmington, Del.
[4] Methocel 15 cps. Supplied through the courtesy of Dow Chemical Co., Midland, Mich.

ethylene glycol 6000[5] (PEG 6000), and poly(methyl vinyl ether/maleic anhydride)[6] (PVMA-119) with PCMX at 30 and 40°.

EXPERIMENTAL

Reagents.—Recrystallized PCMX, m.p. 115–115.5°; PVP; PEG 6000; polysorbate 80; methylcellulose 15 cps.; and PVMA-119.

Apparatus.—Constant-temperature water bath, with rotating spindle; Beckman model DU spectrophotometer; 1-cm. silica cells; and 60-ml. glass-stoppered bottles.

Solubility Method.—The solubility procedure employed in this study was similar to the method previously reported by Higuchi and Lach (20). Excess quantities of PCMX were accurately weighed and placed in 60-ml. glass-stoppered bottles together with varying quantities of the macromolecule. The bottles were placed in a constant-temperature bath and equilibrated at 30 ± 0.1° or 40° for 48 hr. After equilibration, aliquot portions of the supernatant liquid were removed and the PCMX concentration determined spectrophotometrically at a wavelength of 280 mμ using a Beckman DU spectrophotometer with the slit width adjusted at 0.3 mm. A blank for each macromolecule concentration was used to compensate for any absorption by the macromolecule. The interaction of PCMX with macromolecules is evidenced by either an increase or decrease in the solubility of the phenolic molecule.

Equilibrium Dialysis Method.—The dialytic procedure used in the present study was essentially the same as that described by Higuchi and Kuramoto (21) with the exception that seamless cellulose membranes[7] were employed rather than Visking cellulose casings.[8] Dialysis bags prepared from Visking cellulose casing were initially investigated for use as dialysis membranes but were found to be unsatisfactory due to their permeability to methylcellulose and polysorbate 80. A seamless cellulose membrane was found to be an effective dialysis membrane since it proved to be impermeable to the macromolecules and permeable to the low molecular weight drug in aqueous solution. Five-milliliter samples of a 2% aqueous solution of the macromolecule were accurately pipeted into bags prepared from cellulose dialyzer tubing. The dialysis bags were tightly closed and placed in 60-ml. wide-mouth glass-stoppered bottles, each containing a different concentration of PCMX. A series of control bottles in which distilled water was used in place of the polymer solution was included to verify attainment of equilibrium and to correct for any binding of PCMX by the membrane. The bottles were placed in a constant-temperature bath and equilibrated at 30 ± 0.1° for 24 hr. After equilibration, aliquot portions of the external solutions were removed and the concentration of PCMX determined spectrophotometrically. The degree of binding of PCMX by the macromolecules was determined as the difference between the total amount of PCMX in the system (bound + unbound) and the concentration of the unbound PCMX in the external solution. If complexing did occur, the total PCMX concentration on the macromolecule side increased and the unbound drug concentration in the external solution decreased.

[5] Marketed as Carbowax 6000 by Union Carbide Chemicals Co., New York, N. Y.
[6] Gantrez AN-119. Supplied through the courtesy of Antara Chemicals, a division of General Aniline and Film Corp., New York, N. Y.
[7] Dialyzer tubing, Fisher Scientific Co.
[8] Visking dialysis tubing, Visking Co., Division of Union Carbide Corp., Chicago, Ill.

RESULTS AND DISCUSSION

Solubility Studies.—The interactions of PCMX with PVP, PEG 6000, and PVMA-119 are shown in Figs. 1–3 as a function of the macromolecule concentration. The observed decrease in solubility of PCMX at low concentrations of PVP was attributed to the formation of an insoluble complex. A

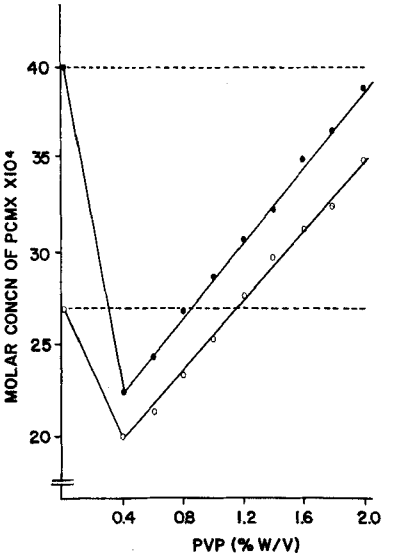

Fig. 1.—Binding of PCMX by PVP in aqueous solution at 30 and 40°. The broken line represents a theoretical line which would be obtained if no binding were to have taken place. Key: ○——○, 30°; ●——●, 40°.

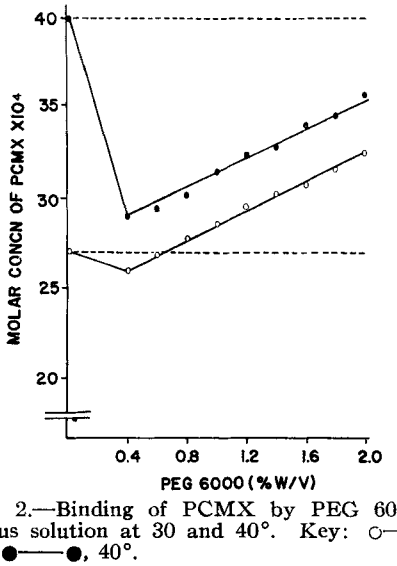

Fig. 2.—Binding of PCMX by PEG 6000 in aqueous solution at 30 and 40°. Key: ○——○, 30°; ●——●, 40°.

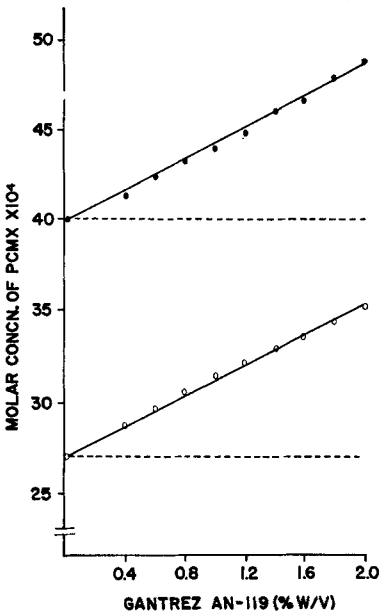

Fig. 3.—Binding of PCMX by PVMA-19 in aqueous solution at 30 and 40°. Key: o——o, 30°; ●——●, 40°.

minimum value was obtained, after which the solubility of the complex increased with an increase in PVP concentration. The linear relationship with a positive slope is indicative of the dependency of the interaction on the concentration of the complexing agent. The mechanism of binding of molecules capable of functioning as proton donors with PVP has been reported previously (21). The complexing of PCMX with PVP is similarly attributed to the affinity of the carbonyl oxygen of PVP for the acidic hydrogen of PCMX. The PCMX–PVP interaction was shown to exhibit only a minor temperature dependency as indicated in Fig. 1. The positive slopes of the interactions at 30 and 40° are 9.5×10^4 and 9.9×10^4, respectively.

A similar type of interaction was observed with PEG 6000 in that at low concentrations of the polymer the solubility of PCMX was decreased to a minimum value, after which a further increase in the concentration of the complexing agent increased the solubility of the phenolic molecule, PCMX. It can be seen that the affinity of PEG 6000 for PCMX is less than that of PVP. These interactions were found to be essentially temperature independent; the positive slopes at the two temperatures are practically the same. The interaction of PCMX with PEG 6000 is probably due to the hydrogen bonding between the acidic hydrogen of the phenol and the nucleophilic oxygen of the polymer. Mulley and Metcalf (19) have described the formation of water-insoluble complexes with monohydroxy phenols and PEG 1000 monocetyl ether. They produced evidence for the formation of hydrogen bonds between the phenolic hydroxyl group and the ether chain of nonionic surfactants.

The results of the studies on the binding of PCMX by PVMA-119 clearly indicate the degree of complexing is a function of the concentration of the macromolecule. No initial decrease in the solubility of PCMX was observed at low concentrations of the polymer. The PCMX–PVMA-119 interaction demonstrated a minor temperature dependency as seen in Fig. 3. The positive slopes of the interactions at 30 and 40° are 4×10^4 and 4.3×10^4, respectively. The PVMA-119 molecule is described as a water-soluble copolymer of methyl vinyl ether and maleic anhydride. When dissolved in aqueous media, the anhydride linkage is cleaved with the formation of the polymeric free acid. The presence of the carbonyl oxygen would suggest a dipole–dipole interaction between the negative centers and the acidic hydrogen of the PCMX molecule.

Dialysis Studies.—The binding of PCMX by 2% aqueous solutions of methylcellulose, PVP, and polysorbate 80 are presented in Figs. 4 and 5

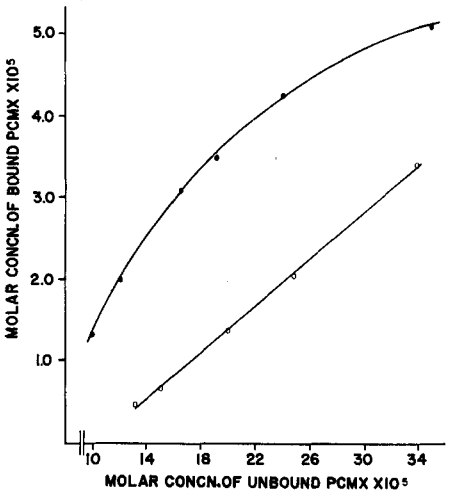

Fig. 4.—Binding of PCMX by 2% aqueous solutions of macromolecules at 30°. Key: o——o, methylcellulose 15 cps.; ●——●, PVP.

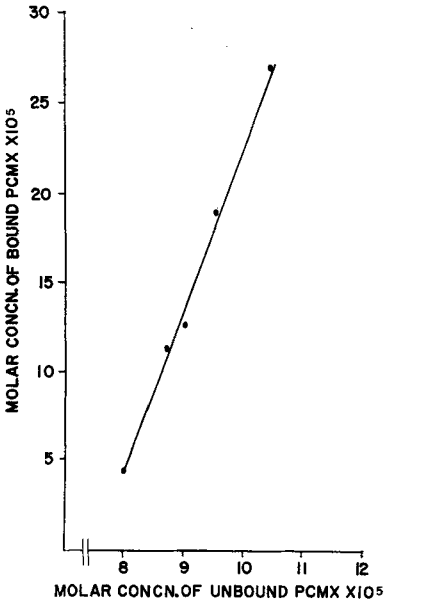

Fig. 5.—Binding of PCMX by a 2% aqueous solution of polysorbate 80 at 30°.

TABLE I.—COMPARATIVE BINDING OF PCMX BY 2% SOLUTIONS OF MACROMOLECULES

Polysorbate 80			PVP			Methylcellulose		
PCMX concn. $\times 10^{-5}$	% Bound	R^a	PCMX Concn. $\times 10^{-5}$	% Bound	R	PCMX Concn. $\times 10^{-5}$	% Bound	R
12.3	35.0	1.54	11.3	11.5	1.13	13.5	3.7	1.04
19.6	56.1	2.28	14.0	14.3	1.17	15.8	4.4	1.05
21.3	57.3	2.34	19.7	16.2	1.19	21.4	6.5	1.07
28.0	65.7	2.92	22.5	15.6	1.18	27.1	7.8	1.08
37.5	72.0	3.57	28.3	15.2	1.18	37.4	9.1	1.10
			40.1	12.7	1.15			

[a] R refers to the ratio of (total PCMX)/(unbound PCMX).

as a function of the unbound PCMX concentration. A comparison of the relative degree of binding by these macromolecules is given in Table I. From the data presented, it is evident that the degree of binding of PCMX by methylcellulose and PVP was relatively small as compared to the binding exhibited by the polyoxyethylene macromolecule, polysorbate 80.

Examination of Table I reveals the effect of drug concentration on the interaction of PCMX with three nonionic macromolecules. In the range of concentrations investigated, it was found that the percentage of total drug in the bound form was dependent upon the concentration of PCMX. The ratio of (total PCMX)/(unbound PCMX) was found to increase to a greater degree with an increase in total drug concentration in the interactions with polysorbate 80 than in the interactions with PVP and methylcellulose.

The mechanism of binding of PCMX by methylcellulose can probably be ascribed to hydrogen bonding similar to that reported by Tillman and Kuramoto (22) in their study on the interaction of methylcellulose with esters of *p*-hydroxybenzoic acid.

The binding tendency which polysorbate 80 exhibits for PCMX may be explained as an association of the acidic hydrogen of the PCMX molecule with nucleophilic atoms, such as oxygen, in the macromolecule. It is possible that both hydrogen bonding and micelle formation are involved in the solubilization of PCMX by polysorbate 80. This is in agreement with the mechanisms proposed for the formation of molecular complexes by hydrogen bonding (13, 14) and micellar solubilization (8, 23) of phenolic compounds with nonionic macromolecules.

REFERENCES

(1) Bolle, A., and Mirimanoff, A., *J. Pharm. Pharmacol.*, **2**, 685(1950).
(2) Erlandson, A. L., and Lawrence, C. A., *Science*, **118**, 274(1953).
(3) deNavarre, M. G., *J. Soc. Cosmetic Chemists*, **7**, 427(1956).
(4) *Ibid.*, **8**, 68(1957).
(5) Barr, M., and Tice, L. F., *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 445(1957).
(6) Pisano, F. D., and Kostenbauder, H. B., *ibid.*, **48**, 310(1959).
(7) Lawrence, C. A., and Erlandson, A. L., *ibid.*, **42**, 352 (1953).
(8) Aoki, M., *et al.*, *J. Pharm. Soc. Japan*, **76**, 939(1956).
(9) Bouchardy, M., and Mirimanoff, A., *Pharm. Acta Helv.*, **26**, 69(1951).
(10) Bolle, A., *ibid.*, **26**, 284(1951).
(11) Judis, J., *J. Pharm. Sci.*, **51**, 261(1962).
(12) Bennett, E. O., "Advances in Applied Microbiology," Academic Press Inc., New York, N. Y., 1959, p. 123.
(13) Higuchi, T., and Lach, J. L., *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 465(1954).
(14) Guttman, D., and Higuchi, T., *ibid.*, **45**, 659(1956).
(15) Patel, N. K., and Foss, N. E., *J. Pharm. Sci.*, **53**, 94(1964).
(16) Rapps, N. F., *J. Soc. Chem. Ind.*, **52**, 175(1933).
(17) Zondek, B., *J. Urol.*, **48**, 747(1942).
(18) Seldowitz, M., *Arch. Dermatol. Syphilol.*, **42**, 576 (1940).
(19) Mulley, B. A., and Metcalf, A. D., *J. Pharm. Pharmacol.*, **8**, 774(1956).
(20) Higuchi, T., and Lach, J. L., *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 349(1954).
(21) Higuchi, T., and Kuramoto, R., *ibid.*, **43**, 398(1954).
(22) Tillman, W. J., and Kuramoto, R., *ibid.*, **46**, 211 (1957).
(23) Beckett, A. H., and Robinson, A. E., *Soap, Perfumery Cosmetics*, **31**, 454(1958).