

Interaction of Preservatives with Macromolecules V

Binding of Chlorobutanol, Benzyl Alcohol, and Phenylethyl Alcohol by Nonionic Agents

By C. K. BAHAL and H. B. KOSTENBAUDER

Possible interaction of chlorobutanol and two aromatic alcohols, benzyl and phenylethyl, with polysorbate 80, polyvinylpyrrolidone, and methylcellulose was investigated by equilibrium dialysis studies. All these common alcoholic preservatives were found to exhibit reversible association with polysorbate 80. Only chlorobutanol was bound by polyvinylpyrrolidone, and none of the alcohols interacted with methylcellulose.

QUANTITATIVE DATA have previously been presented for the extent of association occurring between a number of common preservatives and nonionic macromolecules such as polyethylene glycols, polyvinylpyrrolidone, methylcellulose, and polysorbate 80. Data are available for the binding of preservatives such as phenols (1-6), carboxylic acids (1, 7, 8), and quaternary ammonium compounds (9). This communication is concerned with the presentation of data for additional classes of common preservatives, chlorobutanol, and the aromatic alcohols. Although there is microbiological evidence to indicate that chlorobutanol is inactivated in the presence of polysorbate 20 and it has been observed that chlorobutanol interacts with macromolecules, there has been no previous investigation of the magnitude of the interaction.

Deeb and Boenigk (10) found that a 0.5 per cent aqueous solution of chlorobutanol was a more effective antibacterial than a 0.75 per cent solution of chlorobutanol in 3 per cent polysorbate 20. They also observed that the solubility of chlorobutanol was greatly increased in the presence of polysorbate 20. Inactivation of chlorobutanol and phenylethyl alcohol in presence of polysorbate 60 has been reported by Barr and Tice (11).

Although there have been no reports of inactivation of benzyl alcohol in the presence of macromolecules, this preservative was included for comparison.

EXPERIMENTAL

Materials.—Chlorobutanol, anhydrous, U.S.P.; benzyl alcohol, reagent grade; phenylethyl alcohol, N.F.; polysorbate 80¹; polyvinylpyrrolidone²;

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¹ Polysorbate 80 is polyoxyethylene 20 sorbitan monooleate. Marketed as Tween 80 by Atlas Chemical Industries, Wilmington, Del.

² Plasdone C, Antara Chemicals, New York, N. Y.

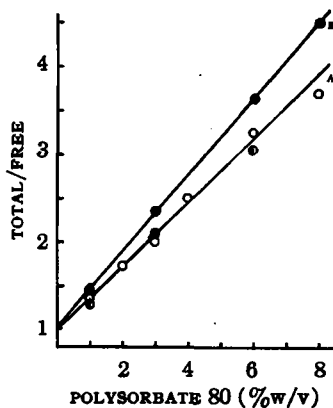


Fig. 1.—Binding of chlorobutanol by polysorbate 80. Key: A, at 30°—total chlorobutanol in solution: O, $2.87 \times 10^{-2} M$; Δ , $4.37 \times 10^{-2} M$; \bullet , $5.78 \times 10^{-2} M$. B, at 45°—total chlorobutanol in solution: \bullet , $2.91 \times 10^{-2} M$.

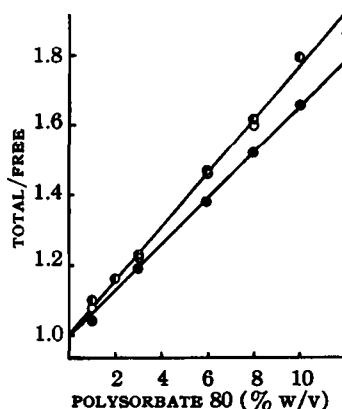


Fig. 2.—Binding of benzyl alcohol by polysorbate 80. Key: A, at 30°—total benzyl alcohol in solution: O, $9.31 \times 10^{-2} M$; \bullet , $19.43 \times 10^{-2} M$. B, at 45°—total benzyl alcohol in solution: \bullet , $19.43 \times 10^{-2} M$.

methylcellulose, 10 cps.³; and nylon⁴ and cellophane⁵ membranes were used.

Dialysis Procedure.—The technique used in the present studies was essentially the same as that employed by Miyawaki, *et al.* (4), with the exception that the films of nylon and cellophane were employed with plastic dialysis cells instead of as dialysis bags. Nylon membranes, which have been shown to be impermeable to polysorbate 80 (3), were selected for use in studies involving polysorbate 80, while cellophane was used for polyvinylpyrrolidone and methylcellulose studies.

Each dialysis cell consisted of two Plexiglas⁶

³ Methocel, 10 cps., Dow Chemical Co., Midland, Mich.

⁴ Plaskon Nylon, 0.0005 in., Allied Chemical Corp., New York, N. Y.

⁵ Visking Cellulose Casing, Visking Corp., Chicago, Ill.

⁶ Rohm & Haas Co., Philadelphia, Pa.

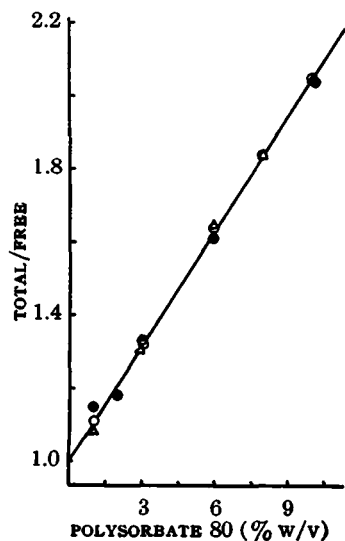


Fig. 3.—Binding of phenylethyl alcohol by polysorbate 80. Key: at 30°—total phenylethyl alcohol in solution: ●, 3.93×10^{-2} M; ○, 7.62×10^{-2} M. At 45°—total phenylethyl alcohol in solution: △, 3.93×10^{-2} M.

blocks, $6.3 \times 6.3 \times 2.6$ cm., each half with a cavity having a capacity of 20 ml. Threaded Plexiglas plugs provided access to the cell cavities. To assemble the cells, a semipermeable membrane was clamped between the two symmetrical halves, solutions were pipeted into each cavity as required and the stoppers, fitted with polyvinyl chloride washers, were screwed in tightly. The cells were then rotated at 9 r.p.m. in a constant temperature water bath maintained at the required temperature $\pm 0.2^\circ$ until equilibrium was established. Using nylon membranes, equilibrium was established at 30° in 6 days for chlorobutanol, 96 hours for phenylethyl alcohol, and 48 hours for benzyl alcohol. Changes in volume of the two cell compartments, found only with polyvinylpyrrolidone and methylcellulose, were recorded and necessary corrections were made for the macromolecule concentration.

Assay Methods.—Benzyl alcohol and phenylethyl alcohol were assayed with a Beckman DU spectro-

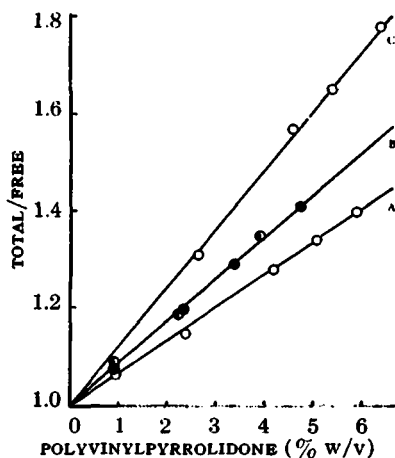


Fig. 4.—Binding of chlorobutanol by polyvinylpyrrolidone. Key: A, at 15°—total chlorobutanol in solution: ○, 2.85×10^{-2} M. B, at 30°—total chlorobutanol in solution: ●, 2.87×10^{-2} M; ○, 5.77×10^{-2} M. C, at 45°—total chlorobutanol in solution: ○, 2.88×10^{-2} M.

photometer at wavelengths of 256 $m\mu$ and 258 $m\mu$, respectively. Chlorobutanol was determined by alkaline hydrolysis followed by determination of the chloride ion by potentiometric titration with 0.01 N silver nitrate solution, using a platinum-tungsten electrode pair (12).

RESULTS

Polysorbate 80.—Data for interaction of preservatives with polysorbate 80 are plotted in Figs. 1-3. The ratio of total/free preservative was essentially constant over the range of preservative concentrations utilized. With 3 per cent polysorbate 80 at 30°, 50% chlorobutanol, 18% benzyl alcohol, and 25% phenylethyl alcohol were bound. With an increase in temperature from 30 to 45°, the magnitude of interaction increased for chlorobutanol, decreased for benzyl alcohol, and remained unchanged for phenylethyl alcohol.

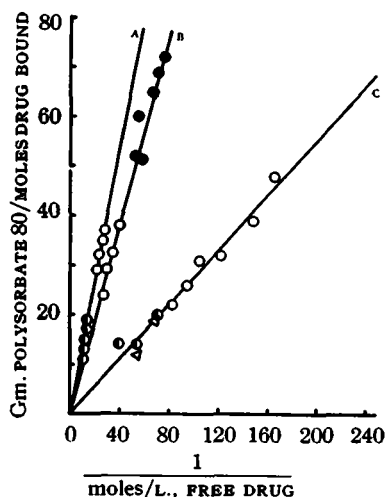


Fig. 5.—Langmuir-type plots for binding of preservatives by polysorbate 80 at 30°. Key: A, total benzyl alcohol in solution: ○, 9.31×10^{-2} M; ●, 19.43×10^{-2} M. B, total phenylethyl alcohol in solution: ●, 3.93×10^{-2} M; ○, 7.62×10^{-2} M. C, total chlorobutanol in solution: ○, 2.87×10^{-2} M; △, 4.37×10^{-2} M; ●, 5.78×10^{-2} M.

Polyvinylpyrrolidone.—The results of dialysis studies with polyvinylpyrrolidone are plotted in Fig. 4. Of the preservatives studied, only chlorobutanol interacted with polyvinylpyrrolidone and the extent of binding increased with an increase in temperature.

Methylcellulose.—None of the preservatives studied showed any interaction with methylcellulose.

DISCUSSION

Nature of Interaction.—Langmuir-type plots for chlorobutanol, benzyl alcohol, and phenylethyl alcohol (such as Fig. 5), when extrapolated to infinitely high free preservative concentration, show a line passing through the origin rather than an intercept corresponding to a limiting binding capacity. Such plots suggest a binding mechanism similar to partitioning into a separate phase, rather than adsorption on a micellar surface or to specific

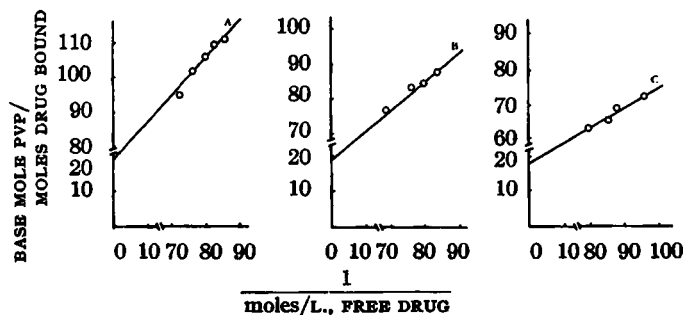


Fig. 6.—Langmuir-type plots for binding of chlorobutanol by polyvinylpyrrolidone (PVP). Key: A, at 15°—total chlorobutanol in solution: 2.85×10^{-2} M; B, at 30°—total chlorobutanol in solution: 2.87×10^{-2} M; C, at 45°—total chlorobutanol in solution: 2.88×10^{-2} M. [Drawn by method of least squares (17).]

TABLE I.—INTERACTION OF CHLOROBUTANOL WITH POLYVINYLPIRROLIDONE

Temp., °C.	Binding Constant K, L. mole ⁻¹	Enthalpy Change (ΔH) cal. mole ⁻¹	Free Energy Change (ΔG) cal. mole ⁻¹	Entropy Change (ΔS_u) cal. deg. ⁻¹ mole ⁻¹
15	17.5	4078	-1639	27.8
30	22.9	4078	-1887	27.7
45	35.4	4078	-2255	27.9

sites on the macromolecule. This behavior is in direct contrast to the interaction of *p*-hydroxybenzoic acid esters with polysorbate 80 (3).

Langmuir-type plots for interaction of chlorobutanol with polyvinylpyrrolidone, Fig. 6, indicate a limiting binding capacity (0.05 sites per base mole of the polymer). The binding sites did not change with an increase in temperature. Thermodynamic functions calculated from data for binding of chlorobutanol by polyvinylpyrrolidone are presented in Table I.

The positive (unfavorable) enthalpy and the large positive unitary entropy term for interaction of chlorobutanol with polyvinylpyrrolidone are similar to values observed for binding of nonpolar molecules such as benzene or nitrobenzene to polyvinylpyrrolidone (13). Molyneux and Frank (13) and others (14, 15) have attributed interactions of this type mainly to the large unitary entropy change accompanying the disruption of the "iceberg" structure of water molecules around hydrocarbon groups in aqueous solution (hydrophobic bonding).

Temperature dependency of interactions with polysorbate 80 are not readily interpreted. Isooctane-water partition coefficients for chlorobutanol, benzyl alcohol, and phenylethyl alcohol all show an increase in the ratio, concentration in organic phase/concentration in aqueous phase, with an increase in temperature from 30 to 45° (16). If the interaction with polysorbate 80 were simply an oil/water partitioning phenomenon, one might expect that the binding to surfactant would vary with tempera-

ture in the same way as the oil/water partition coefficient varies. Undoubtedly, the interaction with the micellar surfactant is considerably more complex.

SUMMARY

When chlorobutanol is used as a preservative in the presence of polysorbate 80 or polyvinylpyrrolidone and when benzyl alcohol and phenylethyl alcohol are used in presence of polysorbate 80, it is advisable to use concentrations of these preservatives slightly in excess of the concentrations normally employed in aqueous solutions. Data presented in this work can be used to calculate the actual concentration required according to the procedure previously described (5). Methylcellulose did not interact with any of the preservatives used in this study and polyvinylpyrrolidone showed no tendency to bind benzyl alcohol or phenylethyl alcohol.

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