any further change in their absorbance properties. The polyoxyethylene monostearates and alkyl phenoxy polyethoxy ethanol did not show any abrupt increase in absorbance at a critical point above their CMC because they produced uniformly turbid mixtures. This was due possibly to a lack of purity of the commercial products. Polyethylene glycol and PVP polymers, which do not exhibit a CMC, gave smooth curves as expected without any breaks. Their graphs are shown in Fig. 9.

Although the interaction or binding of tannin as such may not necessarily be detrimental to pharmaceutical products, the fact that tannin occurs in numerous pharmaceutical formulations of natural origin could cause frequent unexpected incompatibilities if surfactants were added or whenever these products were mixed with other preparations containing surfactants. The decrease in the concentration of the stabilizers or solubilizers through their binding with tannin will obviously decrease the effective concentration of the stabilizing agent which may lead to the ultimate breakdown of some liquid pharmaceutical products. Except by complete separation of the ingredients, the only way that this incompatability may be overcome is by the addition of excess dispersing agent (18). Allawala and Riegelman (19) have pointed out that for optimum conditions of preservative effectiveness, the ratio of phenol to surfactant should be at a minimum. Therefore, in preserved tannin-containing pharmaceuticals this incompatibility with surfactants should be considered.

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Interaction of Nonionic Hydrophilic Polymers with Phenols II

Interaction of Phenol and Hydroxyphenols with Certain Polyethylene Glycols

By B. N. KABADI* and E. ROY HAMMARLUND

The general nature of the interaction between phenol and PEG was investigated utilizing equilibrium dialysis through a cellophane membrane and NMR analysis of the insoluble complexes. Equilibrium dialysis experiments indicated that there was no correlation between the binding of phenols and the increase in acidity associated with the addition of hydroxyl groups on the phenol nucleus. An increase in temperature was found to decrease the degree of binding of phenol with PEG. NMR data indicated that for high molecular weight PEG's complexed with phenol and *p*-chlorophenol, the insoluble, oily complexes contained two ETO base moles to each cosolute molecule.

ALTHOUGH THE interactions of nonionic polymers, e.g., polyvinyl pyrrolidone (PVP) and polysorbate 80, with various phenolic derivatives have been investigated rather extensively, the analogous reactions of polyethylene glycols (PEG) with phenols have received much less attention (1–4). Higuchi and co-workers (5, 6) have studied the interaction of PEG's and barbiturates and iodine in potassium iodide solution. They demonstrated that at a high cosolute concentration a complex formed which had a stoichiometric ratio of 2 base moles of ethylene oxide units (ETO) of the glycol to each cosolute molecule.

Various attempts to determine the mechanism of such interactions and the stoichiometric relationships of the complexes formed by interaction

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of phenols and PEG derivatives have resulted in the appearance of contradictory statements in the literature (7–10). These are due largely to the many experimental difficulties encountered and the insufficiency of reproducible experimental evidence. Some of the difficulties which have prevented the determination of a definite stoichiometry of the formed complexes are: (*a*) the oily insoluble nature of the precipitate prevents the use of the conventional solubility titration method; (*b*) commercial nonionic surfactants are not pure products; (*c*) above their CMC's surfactants form micelles, and because of their solubilizing potential, these micelles would interfere with the isolation of a complex for study.

To avoid most of these difficulties this current study was limited to the PEG's because they are more homogeneous than the surfactants and they do not form micelles in water.

The object of this study was to investigate the general nature of the interaction between phenol and PEG and to attempt to determine the stoichiometry of the resulting complex by correlating equilibrium dialysis data of the reaction mixtures with NMR data obtained from the isolated oily complex. A literature search failed to reveal that NMR data had been employed previously in this type of an investigation on PEG–phenol complexes.

PEG 20,000 was chosen for this study for two reasons: (*a*) previous reports indicated that the low molecular weight PEG's did not form complexes, whereas the higher molecular weight PEG's did (5, 9, 11, 12); (*b*) PEG's with molecular weights of 6000 and less permeated cellulose dialyzing membranes, whereas PEG 20,000 did not, thus equilibrium dialysis could be used as an analytical tool (1).

EXPERIMENTAL METHODS AND RESULTS

Reagents

Phenol, *p*-chlorophenol, pyrogallol, phloroglucinol, and resorcinol (all reagent quality); PEG 300, 400, 1500, 6000, and 20,000 (commercial products).

Preparation of PEG 20,000 for Dialysis.—A 4.0% w/v commercial PEG 20,000 polymer solution was placed in Visking,[1] seamless, cellulose, dialyzing tubes which were then tied securely at both ends and dialyzed at room temperature against a large volume of distilled water. The dialysate was replaced with fresh distilled water at 4-hr. intervals, and the dialysis was continued until there was no frothing in the dialysate solution. This procedure usually took about 5 days. The dialyzed PEG 20,000 solution was dried under reduced pressure. The dried residue was powdered and this was employed in the equilibrium dialysis study. The yield was approximately 90%.

[1] Union Carbide Corp., Visking Division, Chicago, Ill.

Equilibrium Dialysis Method.—The equilibrium dialysis technique used in the present study was essentially the same as that used by Patel *et al.* and others (1, 3, 13). The phenols were estimated quantitatively by using a Beckman DU spectrophotometer. The wavelengths used for the various assays were: phenol, 270 mμ; resorcinol, 275 mμ; pyrogallol, 264 mμ; phloroglucinol, 267 mμ; and *p*-chlorophenol, 272 mμ.

Twenty milliliters of 0.5% w/v of the glycol polymer solution under study was placed in a series of cellophane bags. Each was then tightly tied and placed in a 125-ml. glass-stoppered flask containing 20 ml. of various concentrations of phenol solution ranging from quite dilute to saturated solutions. The control contained water in place of PEG solution. The flasks were agitated for 18 hr. at a constant temperature of 5°. Preliminary experiments had shown that equilibrium was reached under these conditions. The mixtures in the dialysis bags developed various degrees of cloudiness ranging from slightly turbid to extremely opaque, oily emulsions. The amount of phenol in the dialysate solutions was determined spectrophotometrically from its molar absorptivity, and the bound phenol

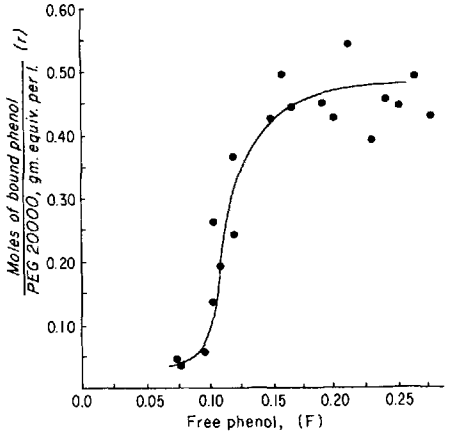

Fig. 1.—Interaction of phenol and PEG 20,000.

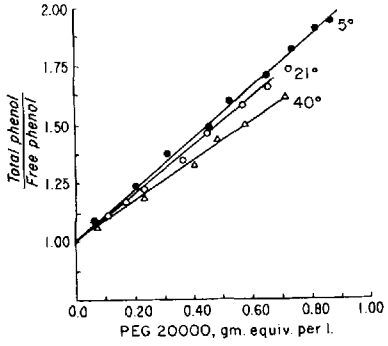

Fig. 2.—Effect of temperature on the binding of phenol and PEG 20,000.

concentration was calculated by difference from the control blank.

The data for the various concentrations of phenol bound to PEG 20,000 are plotted in the form of a Langmuir curve as shown in Fig. 1. At the lower concentrations the data were fairly reproducible; however, at the saturated concentration of phenol the points designating the amount of bound phenol were scattered. This same experiment was repeated using *p*-chlorophenol, and its Langmuir isotherm was found to be of the same shape as that of phenol in Fig. 1.

Effect of Temperature on Binding of Phenol by PEG.—The equilibrium dialysis experiment was carried out at different temperatures by keeping the phenol concentration constant and varying the concentration of the polymer. The results are plotted in Fig. 2 which shows that an increase in temperature decreased the degree of binding only slightly. This was due possibly to disruption of hydrogen bonding between the phenolic hydroxyl and the ether oxygen of the glycol.

Binding of PEG and Certain Hydroxyphenols.—The equilibrium dialysis method was used to study

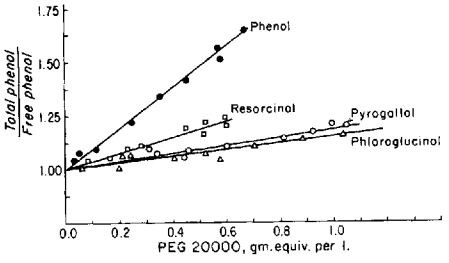

Fig. 3.—Binding of certain phenols with PEG 20,000.

the binding of certain hydroxy phenols with PEG. The data are plotted in Fig. 3. One might expect that as the number of the phenolic hydroxyl groups increased with a corresponding increase in the acidity, the phenols and PEG would interact more strongly. However, the amount of interaction in this case was found to be less, indicating that some others factors were involved. It appears likely that not all of the hydroxyl groups of the phenols are involved directly in complexation with polyglycols, and it is also possible that the degree of hydration of the various phenols may influence the complex formation. Furthermore, phloroglucinol, unlike the other phenols studied, did not precipitate PEG solution in any concentration indicating a lack of "squeezing out" action of the phloroglucinol–PEG complex from water.

Nuclear Magnetic Resonance Studies

The complexes of the various phenols and PEG 20,000 were difficult to work with because of their sticky nature and low solubility in water. The lack of light absorption of the PEG prevented the use of this analytical technique. Furthermore, the quantitative dialysis method could not be used to study the interaction of the low molecular weight

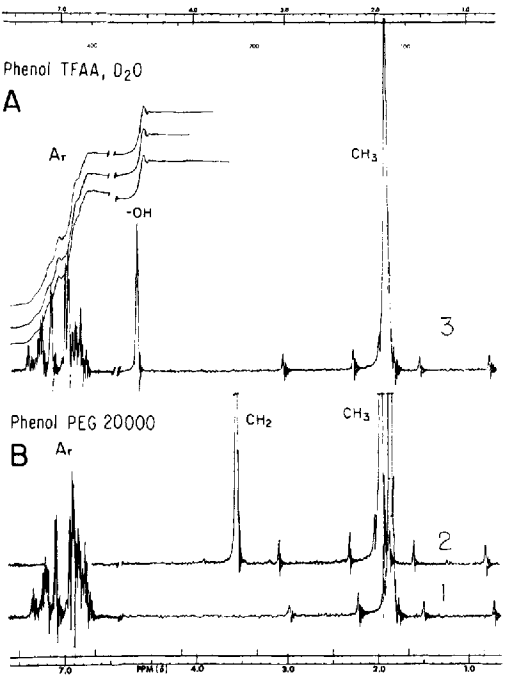

Fig. 4.—NMR spectra of phenol and PEG 20,000 in acetonitrile containing 1% TMS. Key: B1 and A3, phenol; B2, PEG 20,000.

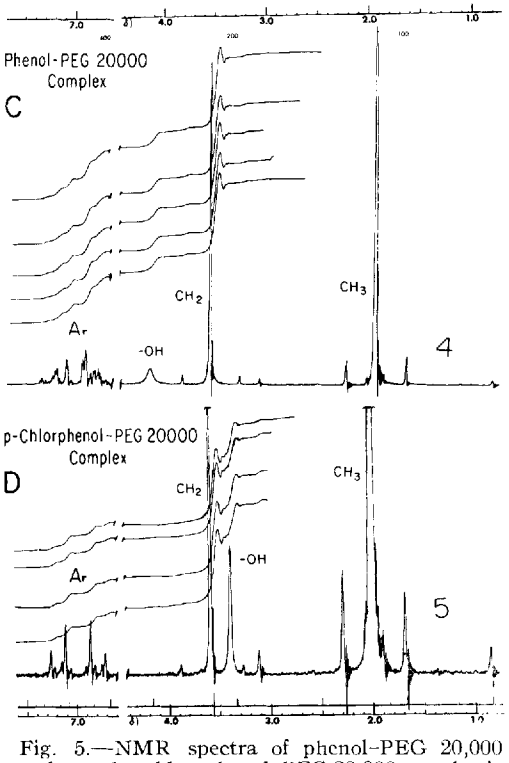

Fig. 5.—NMR spectra of phenol–PEG 20,000 complex and *p*-chlorophenol–PEG 20,000 complex in acetonitrile containing 1% TMS. Key: C4, phenol–PEG 20,000 complex; D5, *p*-chlorophenol–PEG 20,000 complex.

PEG's because they permeated the membranes. These difficulties were overcome by utilizing nuclear magnetic resonance (NMR) analyses to ascertain the ratio of PEG protons to phenol protons in the oily complex.

Preliminary Work on Pure Samples.—The NMR spectra were obtained using an analytical high resolution NMR spectrophotometer (Varian Associates A-60). Preliminary NMR spectra of pure phenol in acetonitrile containing 1% tetramethylsilane (TMS) did not indicate a hydroxyl proton, Fig. 4, B1. The addition of traces of D_2O and trifluoroacetic acid (TFAA) to the sample to catalyze the exchange of hydroxyl protons with D_2O produced the desired phenolic hydroxyl proton signal. The spectrum of this mixture is shown in Fig. 4,A. PEG 20,000 was added to this mixture and the NMR data were obtained. The signal from the phenol and PEG hydrogens did not overlap and could be integrated successfully as shown in Fig. 4,B. However, similar studies on phenol–PVP and the various phenol–polysorbate[2] mixtures did not produce conclusive results because of the complexity of their NMR spectra.

Examination of the spectra of several known ratios by weight of phenol and PEG upon integration disclosed that the ratios of the aromatic protons of phenol to the methylene protons of glycol were close to the calculated values within experimental limits. This spectrum is shown in Fig. 5,C. The lowest field signal which appeared as a multiplet centered at about $\Delta = 7$ and was due to the protons attached directly to the aromatic nucleus. The middle signal which appeared as a sharp singlet at $\Delta = 3.56$ was from the protons of the polyoxyethylene chain. The high field signal at $\Delta = 1.9$ was from the methyl protons of the solvent, acetonitrile. The position of the signal of the hydroxyl protons varied between 3 and 5 Δ values depending upon the water content of the sample.

Analyses of Oily Complexes of PEG and Phenol.—The oily complex mixtures formed by the interactions between a high concentration of phenol individually with PEG 6000 and PEG 20,000 each were collected from within the cellophane dialysis bags which had been agitated slowly for about 18 hr. at 5° in a shaker bath. The NMR spectrum of the oily portion was obtained in acetonitrile containing traces of TMS, D_2O, and TFAA, and was found to be identical in all respects to the spectrum shown in Fig. 5,C. Therefore, in this investigation the NMR spectrometry was found to be applicable as an analytical tool for determining the ratio of bound phenol to polymer. The actual structure of the phenol–polymer complex or any changes in its structure in the elected solvent, acetonitrile, have not been investigated. The integrated ratios of the aromatic protons of phenol to the methylene protons of PEG in the oily complex obtained from the different concentrations of phenol were not constant. However, following a thorough washing of the oily complex with distilled water, fairly reproducible ratios were obtained and are shown in Table I. The ratio of the methylene protons to the aromatic protons was found to be between 1.5 and 1.6.

[2] Marketed as Tweens by Atlas Chemical Industries, Inc., Wilmington, Del.

In a study of the low molecular weight PEG's, 1% w/v solutions of the PEG's were added to equal volumes of 7% phenol solutions and kept overnight in a separator. Oily complexes formed in the lower layer in each funnel and the NMR spectra of the complexes were obtained as previously described both before and after washing with water. It was found that the ratio for PEG 1500 before washing varied considerably from its predicted value (Table I). Likewise, the values for PEG 300 and 400 after washing were not as expected. The remaining ratios came out close to the predicted values. In a similar manner PEG 6000 and 20,000 were interacted with *p*-chlorophenol, and its oily complex was washed with water and gave proton ratios close to the predicted value as seen in Table I.

The water content of the insoluble material varied considerably from sample to sample. The integrated ratio of the water protons was always less than 3 moles considering the ratio of protons of 1 mole of phenol or 2 ETO units of glycol. However, the presence of any water associated with the insoluble complex was not investigated extensively.

TABLE I.—INTEGRATED RATIOS OF METHYLENE PROTON TO AROMATIC PROTON NMR PEAK AREAS FOR PHENOL– AND *p*-CHLOROPHENOL–PEG COMPLEXES

	Methylene Protons/Aromatic Protons (8/5)		
PEG Complexed with Phenol	Before Washing	After Washing	Predicted[a]
200,00	1.509	1.592	1.6
6000	1.470	1.524	1.6
1500	1.24	1.558	1.6
600	...	1.558	1.6
400	...	1.118	1.6
300	...	0.780	1.6
PEG Complexed with *p*-Chlorophenol			(8/4)
20,000	...	2.2	2.0
6000	...	1.7	2.0

[a] Assuming that there were 2 base mole units of ethylene glycol (8 protons) to 1 mole of phenol (5 protons) or *p*-chlorophenol (4 protons).

DISCUSSION

The Langmuir isotherm in Fig. 1 indicated that at low concentrations of free phenol the binding tendency for phenol is rather small. At somewhat higher concentrations of free phenol, the amount of binding depended upon the phenol concentration, and this portion of the curve shows a rapidly increasing slope. At still higher phenol concentrations, the results were not satisfactory because the points were highly scattered. The binding of phenol and PEG as shown by the dialysis data was similar to the binding of phenol by PVP (1) or cetylpyridinium chloride by methylcellulose and polysorbate 80 (14). Deluca and Kostenbauder (14) considered that isotherms such as these sometimes resulted when adsorption of a critical quantity of cosolute caused changes in the configuration of

the tightly coiled polymer molecules resulting in the availability of additional binding sites.

Although the stoichiometric relationship between the ETO of the PEG and phenol was not clearly defined in the dialysis experiments, the behavior of the polymers in the presence of increasing concentrations of phenol were meaningful. Since phenols have a greater tendency for hydrogen bonding than do water molecules (15), the addition of a small amount of phenol to the PEG would lead to the displacement of a few water molecules per polymer. At these low concentrations of phenol, the binding tendency of phenols seems to be comparatively small, and the complex remains in solution. As the ratio of phenol to glycol is increased, phenol would be expected to alter the orientation of the polyphenol complex, and some hydrogen bonded water molecules will be displaced. At the same time the water solubility of the phenol also is reduced by the interaction of the phenolic hydroxyl group with the ETO chains because the hydroxyl group is no longer available for hydrogen bonding with water molecules. This combination of the reduction in solubility of the phenol and the disorganization of the hydrated polymer could explain the precipitation of a hydrated phenol–polymer complex at a critical point. This was found to occur at a very low concentration of phenol.

At the point where the precipitation began to occur, the formation of this precipitate would change convoluted linear polymer chains into a more compressed configuration (16). This rearrangement apparently was initiated by the binding of relatively few phenol molecules which sterically made available many more binding sites for the attachment of additional phenol because this change in state was accompanied by a significant increase in phenol binding, *i.e.*, there was binding of phenol to PEG beyond the point of initial precipitation. Thus, the tendency for an individual phenol molecule to attach itself to a polyether molecule was relatively small, but when several phenol molecules combined with an ether chain, a favorable environment for additional binding was created. As a result more phenol molecules were bound and were held on the complex in the same fashion as with the nonionic surfactant micelles. This phenomenon was seen in the very high phenol region of the dialysis experiment. In this region the oily complex which separated out of the aqueous phase might possibly bind more and more phenol molecules, or the oily colloidal phase may act as a new separate phase which has a considerable attraction to the phenol molecules, and there will be partitioning of phenol between this complex and the aqueous phase. An alternative explanation would be that the oily complex possibly interfered in the equilibrium process of dialysis which caused the points on the graph in Fig. 1 to be scattered over a wider region (17).

Kato (18) has shown the presence of a "micelle-like" structure in simple aqueous solutions of a number of glycols. Thus, the attachment of the aromatic moiety to the water-soluble polyglycol molecules appears to have conferred hydrophobic characteristics to the association complex in proportion to the amount of phenol complexed (19). The solubility of the complex thus would be altered due to binding of phenol to a polymer and the insoluble complex would increase the turbidity which ultimately resulted in the separation of an oily phase when the phenol concentration was high.

Although the commonly used equilibrium dialysis method could not be applied effectively to determine the stoichiometric composition of the complex, a relatively new experimental procedure to this field—the NMR spectrometric method—has been utilized.

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