Investigation of a Cellulosic Polymer–Polyhydroxyl Polymer Interacted Polyelectrolyte System II

Polyhydroxyl Compound Binding and Preservative (Paraben Ester) Solubilization in CMHEC 43L–Polyethylene Glycol Systems

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A dialysis study of carboxymethylhydroxyethyl cellulose and polyethylene glycol 200 and 400 in aqueous solutions with subsequent assay of the polyethylene glycol, showed substantial binding of the polyethylene glycol to the CMHEC, which increased with increasing concentrations of both components. A large increase in the solubility of the paraben esters determined by a solubility method and spectrophotometric assay was noted with CMHEC and polyethylene glycol 200, 400, and 4000. A bacteriological study of CMHEC and polyethylene glycol 200 and 400 with *Aspergillis niger* and *Rhizopus nigricans* showed that the increases in the solubility of the paraben esters affected the bacteriostatic properties of the systems studied. Adequate preservation of these systems with the paraben esters was obtained readily with concentrations of the paraben esters slightly above the total solubility in distilled water at 25° and much below the total solubility in the combination systems.

REPORTS OF the interaction of the paraben esters with polyethylene glycols (1–4) and surfactants (5–10) have been made; the methods of investigating these interactions have included equilibrium dialysis with spectrophotometric determinations (1–4, 10) and bacteriological plate studies (5–9). The bacteriological plate studies indicate that the extent of this interaction is comparable to the bacteriostatic inactivation of the paraben esters. Actual potentiation of the bacteriostatic activity of the paraben esters has been shown with propylene glycol (11). Some workers have stated that the primary activity of the preservatives (paraben esters) in systems is due to the uncomplexed preservative remaining outside of the micelle (12). The application of equilibrium dialysis to the investigation of polymeric system interaction has been used widely (13–19). A dialysis study of paraben esters with carboxymethylcellulose and various other polymers showed that with carboxymethylcellulose essentially no binding occurred (20).

The primary objectives of this study were (*a*) to investigate the extent of polyethylene glycol binding to CMHEC 43L by equilibrium dialysis and chemical assay for the free polyethylene glycol and (*b*) to determine the extent of preservative binding in the cellulose polymer–polyhydroxyl compound interacted system.

Received June 3, 1963, from the School of Pharmacy, Purdue University, Lafayette, Ind.
Accepted for publication August 10, 1964.
Presented to the Scientific Section, A.PH.A., Miami Beach meeting, May 1963.
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EXPERIMENTAL

Materials.—CMHEC 43L[1] and polyethylene glycol[2] 200, 400, and 4000 were used in the study. Methylparaben U.S.P. recrystallized (m.p. 128–129°)[3] and propylparaben U.S.P. recrystallized (m.p. 97–98°)[4] were used in the paraben solubility study. The cultures used in the bacteriological aspect of the study were *Aspergillis niger* ATCC 6277 malt and *Rhizopus nigricans* ATCC 6227a malt.[5] Ceric ammonium hexanitrate purified grade[6] was used in the assay procedure for the polyethylene glycols.

Equipment.—The dialysis and solubility studies were conducted at 25 ± 1° with a rotating-bottle apparatus in a constant-temperature water bath (21) with an additional cooling coil added for better temperature stability, as previously described (22, 23).

The Beckman model DU spectrophotometer was used in the paraben solubility study at 255 mμ with 1-cm. matched silica cells. The Bausch & Lomb spectronic 20 colorimeter-spectrophotometer was used at 450 mμ with matched 0.5-in. test tubes in the polyethylene glycol assay method. An autoclave was used for the sterilization of the culture media and samples prior to inoculation; the cycle used was 15 lbs. pressure for 15 minutes.

Procedures

Dialysis Study.—The dialysis study was conducted in 90-ml. Boston round bottles with aluminum foil cap liners. The caps of the bottles were sealed with waterproof (Scotch Brand No. 33)

[1] Hercules Powder Co., Wilmington, Del.
[2] Carbowax 200 and 400, products of Carbon and Carbide Chemicals Co., New York, N. Y., and polyglycols 4000 were supplied as generous samples through the courtesy of the Dow Chemical Co., Midland, Mich.
[3] Aseptoform, Fries Brothers, Carlstadt, N. J.
[4] Propyl parasept, Heyden Chemical Co., New York, N. Y.
[5] Obtained from Dr. D. Dunham, Purdue University, Lilly Hall of Life Sciences.
[6] Catalog No. C-248, Fisher Scientific Co., Fairlawn, N. J.

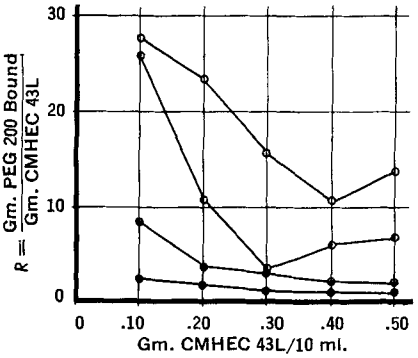

Fig. 1.—The ratio, *R*, of grams of PEG 200 bound per gram of CMHEC 43L as a function of CMHEC 43L concentration, at 25°C. Key: ●, 1% PEG 200; ◒, 3% PEG 200; ◐, 5% PEG 200; ○, 10% PEG 200.

electricians tape. The fluid volumes used were 70 ml. distilled water outside of the cellulose casing and 20 ml. inside of the cellulose casing. The dialysis was run for 48 hours at 25 ± 1° in the rotating-bottle apparatus in a constant-temperature water bath (21). The CMHEC 43L polymer together with the polyethylene glycol 200 or 400 was placed in the 20 ml. inside of the casing, which was knotted securely at both ends to prevent leakage. Visking No-Jax casing 30/32 was used for the study. The required amount of polyethylene glycol was added to the inside volume to provide 1, 3, 5, and 10% of the respective polyethylene glycol calculated on the total volume of 90 ml. The outside volume of the dialysis solution was measured after the 48-hour period of rotation, and an aliquot was taken for the assay of the polyethylene glycol content.

Polyethylene Glycol Assay.—The assay procedure used was based on the quantitative reaction of ceric ammonium hexanitrate with the hydroxyl groups of the polyethylene glycol. The method used in the present study is a modification of the method originally proposed by Hillenbrand (24). The reaction may be written as $(NH_4)_2Ce(NO_3)_6 + ROH \rightarrow (NH_4)_2Ce(NO_3)_5OR + HNO_3$.

A stock solution of the ceric ammonium hexanitrate purified grade reagent was prepared by dissolving 6.250 Gm. of the reagent in sufficient 1 *M* HNO_3 to make 100 ml. The following general procedure was used.

A 1-ml. sample of the dialyzed fluid or a 1-ml. dilution of the dialyzed fluid was diluted to 7 ml. with distilled water, and 3 ml. of the reagent stock solution was added. The absorbance of this solution was determined at 450 mμ after the solution was gently mixed for 1 minute and before 2 minutes had elapsed. Standard curves were prepared with known quantities of polyethylene glycol 200 and 400 in distilled water. Blanks (corresponding concentrations of CMHEC 43L) were run concurrently with the dialysis samples. The contents of the dialysis casing were not assayed due to the complex nature of the system.

Solubility Method.—The solubility method was used in the paraben ester binding study. The solubility involved the addition of excess paraben (methyl or propyl) to known quantities of CMHEC 43L and polyethylene glycol contained in 50 ml. These samples were also placed in 90-ml. Boston round bottles, the bottles were sealed as previously described, then rotated in the bath for 48 hours. At the end of the 48-hour period, the samples were filtered, aliquots taken, and dilutions prepared which could be determined with the Beckman model DU spectrophotometer at 255 mμ. The concentrations of the respective paraben esters determined represented the maximum solubility of the methyl or propyl paraben in the respective interacted CMHEC 43L–polyethylene glycol systems. Blanks without paraben were run concurrently with the samples and prepared in dilutions the same as the samples. At least two sample dilutions were prepared of each CMHEC–PEG–paraben system, and the maximum solubilities of the respective paraben esters assayed were averaged. The systems evaluated consisted of CMHEC 43L (1, 2, and 3%) and PEG 200 or PEG 4000 and CMHEC 43L (1 and 2%) and PEG 400. The concentrations of polyethylene glycol used were 5, 10, 20, and 30%.

Bacteriological Study.—The particular systems described immediately above were studied in the bacteriological series since they showed the greater increases in the solubility of the paraben esters. The organism investigated in the polyethylene glycol 200 series was (with all concentrations of CMHEC 43L used) *R. nigricans*. In the polyethylene glycol 400 series, the following organisms were used: 1% CMHEC 43L, *A. niger* and *R. nigricans;* 2% CMHEC 43L, *R. nigricans;* and 3% CMHEC 43L, *R. nigricans*. The concentrations of methyl and propyl paraben used were slightly above the total solubility in distilled water at 25° and much below the total solubility at 25° in the presence of CMHEC 43L and polyethylene glycol. (See Tables II and III.)

The following method was used in the sample preparation of the bacteriological study. Eight milliliters contained the requisite amount of paraben ester and polyethylene glycol. The cellulose polymer was added as the dry powder, then 2 ml. of the culture nutrient media (consisting of 4% glucose and 1% peptone) was added. The entire sample

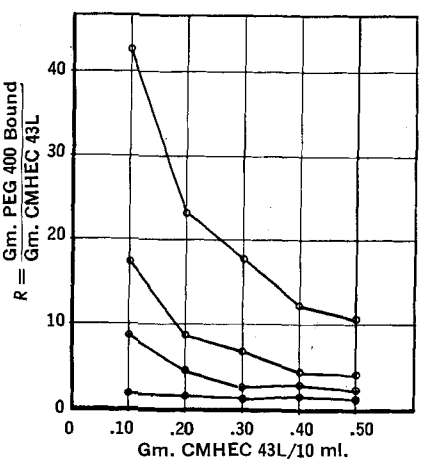

Fig. 2.—The ratio, *R*, of grams of PEG 400 bound per gram of CMHEC 43L as a function of CMHEC 43L concentration at 25°C. Key: ●, 1% PEG 400; ◒, 3% PEG 400; ◐, 5% PEG 400; ○, 10% PEG 400.

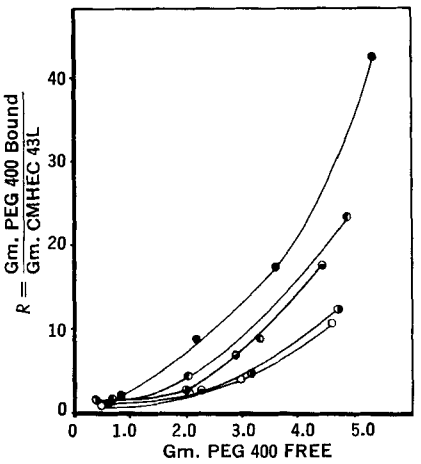

Fig. 3.—The ratio, R, of grams of PEG 400 bound per gram of CMHEC 43L as a function of the grams of free PEG 400 at 25°C. Key: ●, 1% CMHEC 43L; ◐, 2% CMHEC 43L; ◒, 3% CMHEC 43L; ◑, 4% CMHEC 43L; ○, 5% CMHEC 43L.

TABLE I.—TOTAL SOLUBILITIES OF THE PARABEN ESTERS IN MOLES/LITER × 10^{-2} WITH CMHEC 43L AFTER 48 HOURS AT 25°C.

Paraben Ester	Cellulose Polymer and Concn. 1%	2%	3%
CMHEC 43L			
Methylparaben	1.63	1.70	1.67
Propylparaben	0.19	0.24	0.23
Controls in Distilled Water			
Methylparaben	1.55	...	...
Propylparaben	0.19	...	...

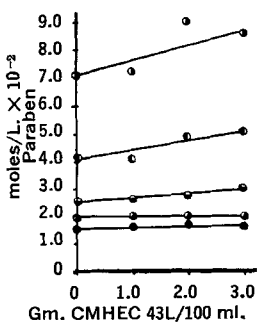

Fig. 4.—Total solubility of methylparaben in the presence of CMHEC 43L–PEG 200 systems in distilled water at 25° C. Key: ●, control (distilled water); ◕, 5% PEG 200; ◒, 10% PEG 200; ◐, 20% PEG 200; ◑, 30% PEG 200.

was contained in a 20-ml. test tube. After cooling, the tubes were inoculated with a 2-weeks growth of the respective organism. The tubes were stored at a constant temperature of 25° and observed during a 14-day period.

RESULTS AND DISCUSSION

Dialysis Study.—The dialysis of CMHEC 43L and polyethylene glycol 200 or 400 was investigated to determine the degree of interaction between the CMHEC–polyethylene glycol by determining the dialyzed free polyethylene glycol. It was assumed that it would be possible to establish a trend relating the increased η' (an indicator of viscosity from the power law equation) values to the increased binding of the polyethylene glycol to the CMHEC 43L polymer using the low molecular weight polyethylene glycols.

The assay procedure used for the determination of the polyethylene glycols was readily reproducible. The data are expressed in Fig. 1 for the PEG 200 systems and in Fig. 2 for the PEG 400 systems. R is the grams of PEG bound (not found in the assay) per gram of CMHEC 43L placed in the system originally. The general trends indicated that as the concentration of the cellulose polymer is increased at a particular concentration of polyethylene glycol, the total amount of polyethylene glycol binding to the polymer increases, but the relative amount of binding as given by the R value decreases. The amount of binding increases with increasing concen-

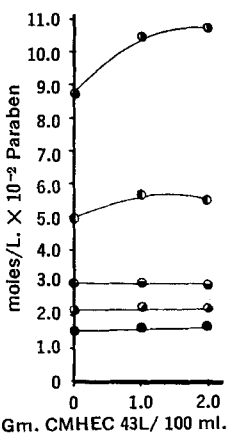

Fig. 5.—The total solubility of methylparaben in the presence of CMHEC 43L–PEG 400 systems in distilled water at 25° C. Key: ●, control (distilled water); ◕, 5% PEG 400; ◒, 10% PEG 400; ◐, 20% PEG 400; ◑, 30% PEG 400.

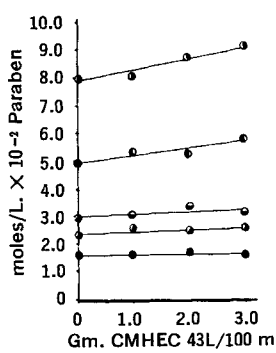

Fig. 6.—The total solubility of methylparaben in the presence of CMHEC 43L–PEG 4000 systems in distilled water at 25° C. Key: ●, control (distilled water); ◕, 5% PEG 4000; ◒, 10% PEG 4000; ◐, 20% PEG 4000; ◑, 30% PEG 4000.

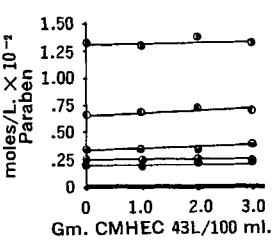

Fig. 7.—Total solubility of propylparaben in the presence of CMHEC 43L–PEG 200 systems in distilled water at 25°C. Key: ●, control (distilled water); ◕, 5% PEG 200; ◒, 10% PEG 200; ◐, 20% PEG 200; ◑, 30% PEG 200.

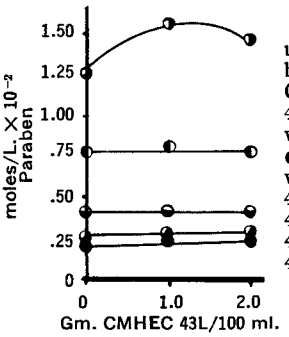

Fig. 8.—Total solubility of propylparaben in the presence of CMHEC 43L–PEG 400 systems in distilled water at 25°C. Key: ●, control (distilled water); ◕, 5% PEG 400; ◒, 10% PEG 400; ◐, 20% PEG 400; ◑, 30% PEG 400.

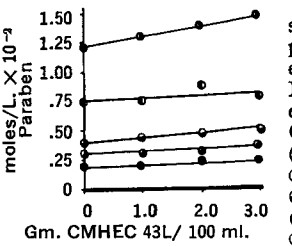

Fig. 9.—The total solubility of propylparaben in the presence of CMHEC 43L–PEG 4000 systems in distilled water at 25° C. Key: ●, control (distilled water); ◕, 5% PEG 4000; ◒, 10% PEG 4000; ◐, 20% PEG 4000; ◑, 30% PEG 4000.

trations of the polyethylene glycol at a particular concentration of CMHEC 43L.

Figure 3 was plotted in an attempt to determine if there is any change in the mechanism of polyethylene glycol binding at higher CMHEC concentrations. This figure also shows that as the concentration of CMHEC is increased, the relative amount of polyethylene glycol bound decreases. More significantly, however, Fig. 3 suggests that the mechanism of polyethylene glycol binding varies with the concentration of CMHEC, since the various lines of Fig. 3 are quite divergent.

Solubility Method.—In the present study the extent of paraben preservative binding was determined in the presence of two component systems (CMHEC 43L and polyethylene glycol). The control samples consisting of only polymer and the respective total paraben ester solubilities are given in Table I.

Figures 4 to 9 show the total solubilities of the respective paraben esters in the presence of the CMHEC 43L–polyethylene glycol systems. Figures 4 to 6 show the total solubility plots for the methylparaben ester and the respective CMHEC 43L–polyethylene glycol systems. Figures 7 to 9 show the total solubility plots for the propylparaben ester and the respective CMHEC 43L–polyethylene glycol systems. Large increases in the paraben ester solubility were noted, particularly with the PEG

TABLE II.—TOTAL SOLUBILITIES OF THE PARABENS (MOLES/LITER AT 25°C.) IN THE PEG 200 CONTROL SYSTEMS, CMHEC 43L–PEG 200 SYSTEMS, AND THE CONCENTRATIONS USED IN THE BACTERIOLOGICAL STUDY

Compn.	5% PEG	10% PEG	20% PEG	30% PEG
		Methylparaben Series		
PEG control[a]	0.0191	0.0253	0.0417	0.0716
PEG–CMHEC 43L[b]	0.0200–0.0208	0.0259–0.0309	0.0410–0.0509	0.0728–0.0907
Bact.–PEG concn.[c]	4.0%	8.0%	16.0%	24.0%
Bact.–paraben concn.[d]	0.0155	0.0253	0.0417	0.0716
		Propylparaben Series		
PEG control[a]	0.0025	0.0034	0.0066	0.0133
PEG–CMHEC 43L[b]	0.00248–0.00275	0.00346–0.00400	0.00688–0.00721	0.0129–0.0132
Bact.–PEG concn.[c]	4.0%	8.0%	16.0%	24.0%
Bact.–paraben concn.[d]	0.0019	0.0027	0.0052	0.0104

[a] Polyethylene glycol 200 aqueous solutions. [b] CMHEC 43L (1, 2, 3%) and polyethylene glycol 200 systems. [c] Concentration of polyethylene glycol 200 used in the bacteriological study with 1, 2, and 3% CMHEC 43L. [d] Concentration of the respective paraben ester used in the bacteriological study.

TABLE III.—TOTAL SOLUBILITIES OF THE PARABENS (MOLES/LITER AT 25°C.) IN THE PEG 400 CONTROL SYSTEMS, CMHEC 43L–PEG 400 SYSTEMS AND THE CONCENTRATIONS USED IN THE BACTERIOLOGICAL STUDY

Compn.	5% PEG	10% PEG	20% PEG	30% PEG
		Methylparaben Series		
PEG control[a]	0.0213	0.0293	0.0493	0.0877
PEG–CMHEC 43L[b]	0.0222–0.0227	0.0296–0.0300	0.0546–0.0569	0.1045–0.1075
Bact.–PEG concn.[c]	4.0%	8.0%	16.0%	24.0%
Bact.–paraben concn.[d]	0.0190	0.0187	0.0379	0.0778
		Propylparaben Series		
PEG control[a]	0.00264	0.00396	0.00773	0.01249
PEG–CMHEC 43L[b]	0.00278–0.00283	0.00429–0.00426	0.0078–0.00822	0.0146–0.0158
Bact.–PEG concn.[c]	4.0%	8.0%	16.0%	24.0%
Bact.–paraben concn.[d]	0.0018	0.0031	0.0065	0.0130

[a] Polyethylene glycol 400 aqueous solutions. [b] CMHEC 43L (1, 2, 3%) and polyethylene glycol 400 systems. [c] Concentrations of polyethylene glycol 400 used in the bacteriological study with 1, 2, and 3% CMHEC 43L. [d] Concentration of the respective paraben ester used in the bacteriological study.

400 series and methylparaben, which is less pronounced with PEG 200 and PEG 4000, an indication that greater solubilization takes place when PEG 400 is present in the system. The same pattern was noted with propylparaben.

The general trend noted with increasing polymer and polyethylene glycol concentration was that solubilization of the paraben esters increased. This is somewhat analogous to the increases in η' (indicator of viscosity) where the increases in η' were greatest with the low molecular weight glycols present in the systems. η' also increases with increasing polymer and polyethylene glycol concentration (25).

However, with the total solubility data shown in Figs. 4 to 9 of these select systems, the investigation of whether these systems can be preserved with the usual concentrations of the methyl- and propylparaben must be determined.

Bacteriological Study.—Tables II and III show the respective concentrations of the paraben esters used in this study and the total solubilities of the paraben esters in the CMHEC 43L–polyethylene glycol systems for comparison purposes. In the polyethylene glycol 200 series, growth was absent in all instances; however, in the polyethylene glycol 400 series, growth was evident at the 2 and 3% CMHEC 43L concentrations and the lower two concentrations of propylparaben used.

The growth indicated inactivation of the preservative by the system. The growth of the organism used was noted readily by the black mycelial growth shown, and microscopic examination was unnecessary.

The limited bacteriological study of the systems shows that there is some bacteriostatic inactivation of the respective methyl and propyl parabens in the presence of CMHEC 43L–polyethylene glycol systems, despite large increases in the solubilization of the paraben esters studied. The effective concentration of the paraben esters used to inhibit growth in these systems must be in excess of the normal preservative concentrations; those concentrations needed for the adequate preservation may be determined from a solubility and bacteriological study.

CONCLUSIONS

The dialysis study showed an increase in the total binding of polyethylene glycol to CMHEC 43L as a function of cellulose polymer concentration and increasing polyethylene glycol concentration. This finding tends to add substance to the fact that large η' increases were obtained in the previous study (25), with the lower molecular weight polyethylene glycols and CMHEC 43L. The increases were a function of the interaction between these materials, increasing in general with concentration increases of both the polyethylene glycol and CMHEC 43L.

Relatively large increases in the solubility of methyl and propyl paraben are shown by CMHEC 43L–polyethylene glycol 200, 400, and 4000 systems. The increased solubility relative to the total solubility in distilled water at 25° is greatest with CMHEC 43L–polyethylene glycol 400 systems.

The bacteriological study of CMHEC 43L and polyethylene glycol 200 and 400 with *A. niger* and *R. nigricans* showed that inactivation of the respective paraben esters is indicated by large increases in paraben solubility in the polymeric systems. The systems may, however, be preserved adequately (according to the limited bacteriological study) by using concentrations of the parabens considerably excess of normal paraben concentrations usually used for preservation. The findings of this aspect of the study somewhat contrast the findings of the early investigators that the polyethylene glycols alone (6–8) do not inactivate markedly the preservative activity of the paraben esters.

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