Dilute Solution Properties of Hydroxyethyl Starch

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Synopsis

The dilute solution properties of hydroxyethyl starch (HES) were examined by using the techniques of osmometry, light scattering, and viscometry. The molecular weight range was approximately 2×10^6–0.06×10^6. Since HES is a branched molecule, its properties were compared with those of two linear counterparts, ethyl hydroxyethyl cellulose and hydroxyethyl cellulose. The branching index g was estimated to be about 0.3 when calculated from the intrinsic viscosity, radii of gyration, and second virial coefficients.

INTRODUCTION

About four years ago, Walton and Thompson reported the use of solutions of hydroxyethyl starch (HES) as a possible artificial colloidal plasma volume expander.[1] HES is a derivative of amylopectin, a "waxy" starch, containing only branched chains of glycoside molecules, devoid of a linear component. This polymer is prepared by subjecting the starch to acid hydrolysis and then reacting it with ethylene oxide in an alkaline solution until the degree of substitution is about 85%. In a 6% solution, the HES has approximately the same viscosity as the standard clinical dextran and is quite resistant to the action of amylase in the blood. After a great number of studies with laboratory animals, Walton and Thompson concluded that the HES used compared favorably with dextran. Since it has been shown recently that dextran may not have the required stability or shelf-life that is needed for a plasma expander,[2] the need for more work on HES appears advisable. To date, one clinical study with HES has been reported.[3]

With the current interest in HES, the present study was undertaken to examine some of the physicochemical properties of HES that may be of value to other investigators.

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EXPERIMENTAL

Fractionation

A crude sample of HES was fractionally precipitated from an aqueous solution by using both acetone and isopropanol. The polymer was generously provided by the Don Baxter Labs., Inc., of Glendale, California. This sample was designated as No. 4248 of the master batch of 4091. The analyses were provided by Dr. T. J. Schoch of Corn Products Company of Argo, Illinois.[4] It was shown to have a moisture content of 7.2% and residual NaCl content of 2.5%.

The precipitation was performed in a 25°C. constant temperature bath by dropwise addition, with constant stirring, of one of the precipitating agents to an aqueous solution that was initially 2.5% HES. After the addition of the precipitant, the cloudy mixture was stirred for at least 1 hr., and then the fraction was allowed to settle. The lower layer was not removed until it became clear. This usually took a period of 24 hr. The top solution was then decanted. The remaining precipitate was redissolved in water and added dropwise, with constant stirring, to a large excess of the precipitant, filtered, and dried under vacuum for 8 hr. The same procedure was repeated until all of the HES appeared to be removed.

The acetone and isopropanol series are designated by the letters A and I, respectively. The results of the two fractionations are given in Table I. The nature of the material lost was checked by calculating $\sum_i [\eta]_i w_i$ where $[\eta]_i$ is the intrinsic viscosity and w_i is weight fraction of the ith fraction. It appears that the isopropanol fractionation is more efficient. However,

TABLE I
Fractionation of Hydroxyethyl Starch[a]

	Precipitant: acetone				Precipitant: isopropanol		
Fraction	Per cent of total	k_H	$[\eta]$	Fraction	Per cent of total	k_H	$[\eta]$
1-A	0.94	—	—	1-I	7.87	—	0.440
2-A	9.53	0.76	0.420	2-I	14.53	0.69	0.415
3-A	13.06	1.39	0.345	3-I	12.66	0.76	0.362
4-A	12.53	0.87	0.311	4-I	10.26	0.57	0.324
5-A	7.60	1.03	0.267	5-I	7.33	0.52	0.298
6-A	6.00	0.94	0.250	6-I	8.27	0.36	0.274
7-A	9.07	0.85	0.222	7-I	7.47	0.60	0.236
8-A	7.00	0.62	0.205	8-I	7.67	0.50	0.210
9-A	5.60	0.63	0.178	9-I	4.40	0.55	0.171
10-A	5.13	—	0.155	10-I	2.80	0.56	0.163
11-A	2.67	—	0.139	11-I	1.73	—	0.152

$$\sum_i w_i [\eta]_i = 0.215 \qquad\qquad \sum_i w_i [\eta]_i = 0.267$$

[a] Initial concentration, 75 g./3000 ml. H_2O; $\bar{M}_n = 7.1 \times 10^4$; $[\eta] = 0.262$; $k_H = 0.474$; $\bar{M}_w = 4.35 \times 10^5$.

since this was the second attempt, the experimental technique was probably better. If one allows for the moisture content and residual NaCl, the total recovery is quite good.

It is believed that the large change in $\bar{M}_w/\bar{M}_n$ from the initial crude material to the ratios found after fractionation is due to the extreme difficulty in cleaning up these solutions for light scattering rather than an indication that the fractionation separated degrees of branching and molecular weights.

Intrinsic Viscosities in 0.9% NaCl Solution

The intrinsic viscosities of the polymer solutions were measured in a Cannon-Ubbelohde calibrated viscometer. The flow time for a 0.9% NaCl aqueous solution was 128 sec. at 25°C. The intrinsic viscosity was determined by an extrapolation of the best straight lines through the points given by a plot of η_{sp}/c and ln η_r/c versus c. In all cases, the solutions were sufficiently dilute that no curvature appeared in these graphs. The Huggins constants k_H are also given in Table I.

Osmotic Pressure

The number-average molecular weights $\bar{M}_n$ of the HES fractions were measured by osmometry. These determinations employed a Mechrolab Model 501 high-speed membrane osmometer. The membranes were supplied by Schleicher and Schuell Co. of Keene, New Hampshire. In this study, a B-20 cellulose acetate membrane with an average pore size of $0.005\,\mu$ was used. The membranes were kept moist at all times.

The osmotic pressure data were fitted to the equation[5]

$$\pi/c = (\pi/c)_0 \left[1 + \Gamma_2 c + (\Gamma_2 c)^2/4\right] \tag{1}$$

or

$$(\pi/c)^{1/2} = (\pi/c)_0^{1/2} \left[1 + (\Gamma_2 c/2)\right] \tag{2}$$

i.e., $(\pi/c)^{1/2}$ was plotted against c.

Light Scattering

The light-scattering measurements were made using a Brice-Phoenix Model 2000 universal light-scattering photometer which was modified for temperature control of the scattering solutions. The photometer was calibrated by using the Cornell standard polystyrene in toluene. All measurements were made at 4358 A. in 0.9% NaCl solutions at 25°C.

The solutions were clarified by centrifuging at 25,000 rpm for 90 min. in a Spinco Model E centrifuge. The samples were removed from the tubes by use of a pipet with a curved tip. These samples were then placed in a Pyrex ultrafine fritted filter and filtered under a pure, dry nitrogen pressure directly into the light-scattering cell. All dilutions were clarified in the same manner.

The refractive index increments of HES in 0.9% NaCl solution were determined by the use of a Brice-Phoenix differential refractometer thermostated at 25°C. The calibration was made by use of sodium chloride solutions.[6] The refractive index increment for HES in 0.9% NaCl was found to be 0.136 cm.^{3}/g. at 4358 A.

The Rayleigh ratio R_θ was calculated from the observed scattering ratios G_θ/G_0 by using the equation given by Tomimatsu and Palmer.[7] This equation corrects for back reflections.

RESULTS AND DISCUSSION

Viscosity

The intrinsic viscosities of the two fractionations are given in Table I. Shear corrections were found to be unimportant for these HES fractions.

Osmotic Pressure

The osmotic pressure data are plotted as $(\pi/c)^{1/2}$ versus c according to eq. (2). In order to obtain the best fit through all of the points, the method of least squares was employed. The $\bar{M}_n$, Γ_2 and $\Gamma_2/[\eta]$ values are given in Table II.

TABLE II
Summary of Osmotic-Pressure and Light-Scattering Data

| Fraction	$M_n \times 10^{-6}$	$M_w \times 10^{-6}$	Γ_2, cc./g. Osmometric	Γ_2, cc./g. Light scattering	$\Gamma_2/[\eta]$ (Osmometric)	$\langle \bar{S}^2 \rangle_z^{1/2}$, A.
2-I	0.752	1.92	69.7	191	168	445
3-I	0.665	1.11	67.0	95	185	324
4-I	0.418	0.855	49.8	59.1	154	282
5-I	0.362	0.552	51.1	63.7	171	249
6-I	0.279	0.469	47.2	30.4	172	259
7-I	0.165	—	30.8	—	131	—
8-I	0.121	0.191	30.1	79.4	143	191
9-I	0.0805	0.163	24.2	44.8	142	—
10-I	0.0537	—	15.5	—	95	—
2-A	0.891	—	81.8	—	195	—
3-A	0.644	—	66.1	—	192	—
4-A	0.435	—	50.0	—	161	—
5-A	0.384	—	58.4	—	216	—
6-A	0.317	0.518	58.0	112	232	207
7-A	0.218	0.319	47.7	61.8	215	142
8-A	0.145	0.212	36.9	35.0	180	236
9-A	0.0884	—	27.2	—	153	—
10-A	—	0.0646	—	6.6	—	169
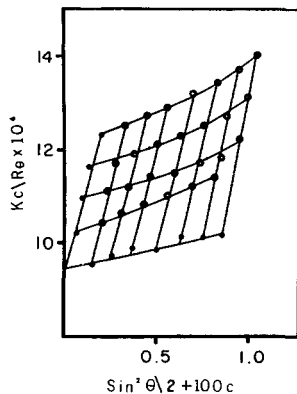
Fig. 1. Zimm plot for fraction HES-31.

Light Scattering

The weight-average molecular weight $\bar{M}_w$, the Z-average root-mean-square radii of gyration, $\langle S^2 \rangle_z^{1/2}$, and the second virial coefficients Γ_2 from light scattering are given in Table II for some of the fractions. Because of the heterogeneity that was shown to exist in dextran, a polymer that is very similar to HES, the calculation of $\bar{M}_w$, $\langle S^2 \rangle_z^{1/2}$ and Γ_2 is not altogether straightforward. Here we chose the technique used by Hunt[8] for cellulose trinitrate fractions. The details of the extrapolation methods, both to zero angle as well as to infinite dilution, are given in considerable detail in this paper.[8] This approach to the evaluation of the desired parameters proved to be very satisfactory. For compact illustrative purposes, the data for HES-3I are shown in Figure 1 by a Zimm plot.

The molecular weight dependence of the intrinsic viscosity was determined for both the weight-average and number-average molecular weights as:

$$[\eta] = 5.29 \times 10^{-3} \, \bar{M}_w^{0.300} \tag{11}$$

and

$$= 3.27 \times 10^{-3} \, \bar{M}_n^{0.351} \tag{12}$$

The size of the exponent of the molecular weight is typical for branched molecules.[10-12]

The degree of branching g was evaluated by comparing the mean square radii, the intrinsic viscosities, and second virial coefficients of HES with those of a linear counterpart. In this study, hydroxyethyl cellulose (HEC)[13] and ethyl hydroxyethyl cellulose (EHEC)[14] were used. The schematic diagrams of the repeating units are shown in Figure 2. In Table III the results of the branching parameters are given. These values were calculated by using

TABLE III
Branching Parameters

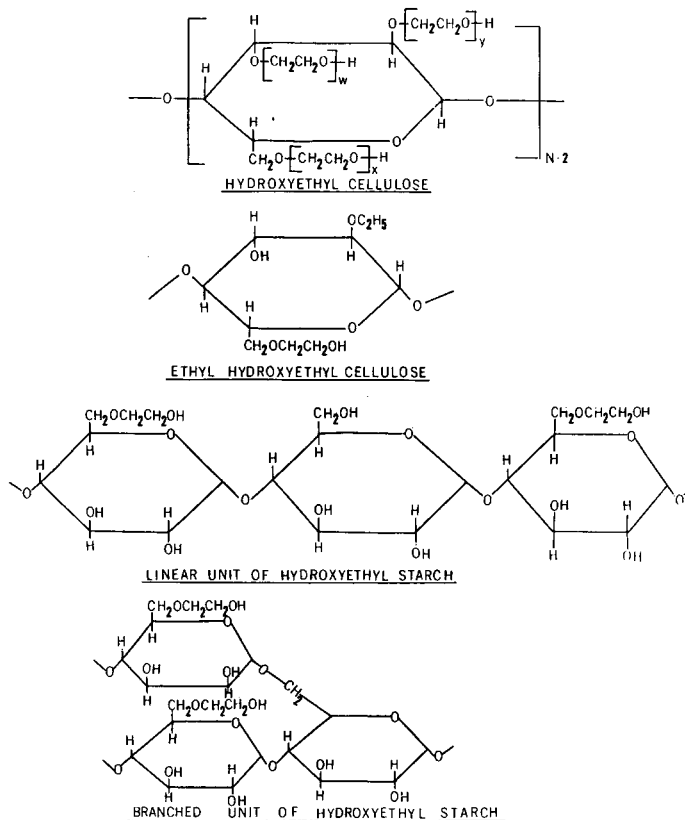| Fraction	$g^{1/2}$ [eq. (13)]		h [eq. (14)]		$g^{1/2}$ [eq. (15)]	
	EHEC	HEC	EHEC	HEC	EHEC	HEC
2-I	0.266	0.293	0.310	0.308	0.49	0.089
3-I	0.295	0.306	0.297	0.296	0.47	0.094
4-I	0.312	0.314	0.292	0.297	0.42	0.115
5-I	0.339	0.326	0.316	0.318	0.41	0.123
6-I	0.343	0.325	0.318	0.326	0.38	0.137
8-I	0.419	0.356	0.342	0.375	0.31	0.197
9-I	0.433	0.362	0.346	0.383	0.28	0.235
6-A	0.343	0.327	0.316	0.321	—	—
7-A	0.379	0.324	0.328	0.346	—	—
8-A	0.309	0.266	0.341	0.369	—	—
7-I	—	—	—	—	0.34	0.172
10-I	—	—	—	—	0.26	0.279

Fig. 2. Schematic structural diagrams of hydroxyethyl cellulose (HEC), ethyl hydroxy-ethyl cellulose (EHEC), and hydroxyethyl starch (HES).
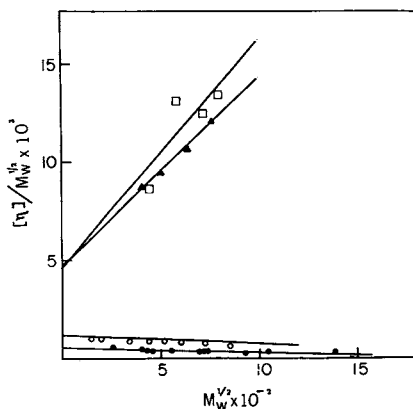
Fig. 3. Test of the equation of Stockmayer and Fixman, $[\eta]/M^{1/2} = K + 0.51\Phi M^{1/2}$: (□) HEC; (▲) EHEC; (○) dextran; (●) HES.

$$g = \bar{S}_{br}^2/\bar{S}_l^2 \tag{13}$$

$$h^3 = g^{3/2} = [\eta]_{br}/[\eta]_l \tag{14}$$

and

$$g^{1/2} = A_{2br}/A_{2l} \tag{15}$$

It is believed that the uncertainty in the values of g from eq. (15) is quite large due to the errors involved in A_2 for all components.

The values of the expansion factor were calculated from the expression given by Orofino and Flory[15]

$$A_2\bar{M}_n/[\eta] = (2^{5/2} \pi N/3^3\Phi) \ln [1 + (\pi^{1/2}/2) (\alpha^2 - 1)] \tag{16}$$

the value of 2.63×10^{21} for Φ being used instead of 2.20×10^{21} as suggested by Senti and co-workers.[16]   The technique of Fixman and Stockmayer[17]
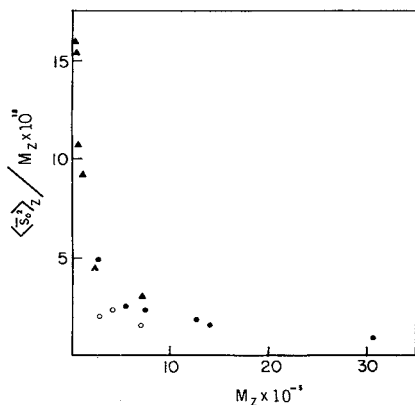

Fig. 4. Dependence of $<\bar{S}_0^2>_z/\bar{M}_z$ on $\bar{M}_z$: (▲) dextran; (○) HES-A; (●) HES-1.

was tried but yielded a negative slope, making the evaluation of α meaningless. This is shown in Figure 3. The values of the expansion factor α are listed in Table IV.

The Z-average molecular weight was estimated by using the ratios $\bar{M}_w/\bar{M}_n$ and a Schulz type distribution,[18] $\bar{M}_z:\bar{M}_w:\bar{M}_n = (y + 2):(y + 1):y$. These values are combined with the expansion factors and the radii of gyration to calculate the parameter $\langle S_0^2 \rangle_z / \bar{M}_z$, (Table IV). Some values for dextrans fractions[19] are also presented. These data are shown graphically in Figure 4.

Linear polymer chains exhibit the familiar relation $[\eta]_l = KM^a$. Zimm and Kilb[20] propose that for branched molecules $[\eta]_{br} = K' M^{a-1/4}$. This is shown in Figure 5. The solid line adjusts for the exponent while the dotted line also includes using Φ_{br} instead of Φ in K.

It is gratifying to note the comparison of the branching parameters obtained both from radii of gyration and the intrinsic viscosities. It is not surprising to find that the same parameter as measured from the second virial coefficients is not in complete accord. First of all, the manner in which the second virial coefficient is related to the branching and molecular weight distributions is much more complex than it is for the intrinsic

TABLE IV
Configuration Parameters

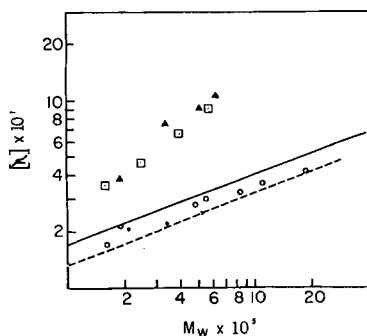| Fraction	α [eq. (16)]	y	$\bar{M}_z \times 10^{-6}$	$(\langle S_0^2 \rangle_z / M_z)$ $\times 10^{18}$
2-I	1.82	0.645	3.09	1.05
3-I	1.93	1.78	1.41	1.57
4-I	1.73	0.952	1.29	1.88
5-I	1.84	1.89	0.745	2.33
6-I	1.84	1.47	0.657	2.45
7-I	1.60	—	—	—
8-I	1.67	1.72	0.262	4.88
9-I	1.66	—	—	—
10-I	1.41	—	—	—
2-A	1.99	—	—	—
3-A	1.98	—	—	—
4-A	1.78	—	—	—
5-A	2.14	—	—	—
6-A	2.27	1.59	0.718	1.53
7-A	2.14	2.17	0.419	2.36
8-A	1.90	2.17	0.279	2.07
9-A	1.73	—	—	—
Dextran				
C-26	—	—	0.011	16
U-3	—	—	0.025	15.4
C-26	—	—	0.0524	10.7
C18A10	—	—	0.106	9.2
NRRL-3136-54-1	—	—	0.230	4.4
NRRL-3136-70-1	—	—	0.700	3.0

Fig. 5. Log–log plot of intrinsic viscosity $[\eta]$ vs. weight-average molecular weight M_w: (▲) HEC; (□) EHEC, $[\eta] = 2.40 \times 10^{-3} \bar{M}_w^{0.619}$; (●) HES-A; (○) HES-I; (———) $[\eta] = 2.40 \times 10^{-3} \bar{M}_w^{0.319}$; (— — — —) $[\eta] = 2.00 \times 10^{-3} \bar{M}_w^{0.319}$.

viscosity for example. Also, the accuracy of the A_2 values is much less than that of the intrinsic viscosities. It should also be pointed out that for both the HEC and the EHEC the molecular weight ranges were very limited (HEC, $\bar{M}_n = 380\text{--}125 \times 10^3$; $\bar{M}_w = 625\text{--}190 \times 10^3$; EHEC, $\bar{M}_n = 176\text{--}77.6 \times 10^3$; $\bar{M}_w = 570\text{--}160 \times 10^3$). However, it is believed that within the limits set by the linear counterparts the measure of branching and the description of HES is satisfactory.

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Résumé

Les propriétés de solutions diluées d'amidon hydroxyéthylé (HES) ont été examinées en utilisant les techniques de pression osmotique, de diffusion lumineuse et de viscosimétrie. Le domaine de poids moléculaire était approximativement de 2 à 0.06 × 10⁶. Puisque HES est une molécule ramifiée ses propriétés sont en rapport avec celles de composés linéaires la cellulose éthyle hydroxyéthylée et la cellulose hydroxyéthylée. L'indice de ramification g a été estimé être environ égal à 0.3 lorsqu'on le calcule au départ de la viscosité intrinsèque, des rayons de gyration et des coefficients de second viriel.

Zusammenfassung

Die Eigenschaften von Hydroxyäthylstärke (HES) in verdünnter Lösung wurden durch Messung des osmotischen Druckes, der Lichtstreuung und Viskosität untersucht. Der Molekulargewichtsbereich betrug etwa 2 bis 0,06 × 10⁶. Da HES ein verzweigtes Molekül ist, wurden seine Eigenschaften mit denjenigen zweier linearer Gegenstücke, Äthylhydroxyäthylcellulose und Hydroxyäthylcellulose verglichen. Der Verzweigungsindex, g, wurde aus Viskositätszahl, Gyrationsradius und zweitem Virialkoeffizienten zu etwa 0,3 berechnet.