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Determination of the molecular weight distribution of hydroxyethylcellulose by gel filtration chromatography

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Due to the importance of the knowledge of the molecular weight distribution of hydroxyethylcellulose, (HEC), in predicting its performance, a study on the use of gel filtration chromatography for the determination of this information has been carried out. Results presented demonstrate that it is possible to use gel filtration columns with 100% aqueous eluents for the size separation and gel filtration analysis of a representative selection of water-soluble HEC samples. Data obtained correlate with the viscosity grading supplied with the samples by the manufacturer.

INTRODUCTION

Hydroxyethylcellulose (HEC) is a non-ionic water-soluble polymer derived from cellulose (Kennedy *et al.*, 1984). It is used for a range of applications in the paint, building and textile industries as a stabilizing, thickening and emulsifying agent and more recently it has been incorporated with drilling muds for the recovery of petroleum (Beckers, 1987). Aqueous solutions of HEC exhibit pseudoplastic behaviour, i.e., the solution viscosity will vary depending upon the amount of stress applied. The extent of this pseudoplastic behaviour of hydroxyethylcellulose solutions is, however, very dependent upon the molecular weight distribution of the sample. A fast reproducible and reliable method for the determination of the molecular weight distributions is, therefore, essential for production quality control and end-use performance prediction.

This paper describes the results of the study performed on the suitability of a gel filtration (GFC) column system to determine the molecular weight distributions of a representative selection of hydroxyethylcellulose samples. Two aqueous solvent systems have been evaluated.

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EXPERIMENTAL

Samples

The six hydroxyethylcellulose samples were commercial grades identified as HEC 1–6. Samples HEC 1, 2, 3 and 4 were samples quoted as being of low viscosity and HEC 5 and 6 were high viscosity grades.

Eluents

With the exception of sodium azide which was of laboratory grade, all salts used for eluent preparation were of analytical grade quality. Water used for eluent preparation was purified using a reverse osmosis system.

Gel filtration chromatography

Calibrants: Well characterized HEC reference materials are not commercially available for the calibration of the gel filtration column system. Therefore, pullulan polysaccharides with a narrow molecular weight distribution which were previously characterized for molecular weight averages were used (Polymer Laboratories Ltd, Church Stretton, UK). The quoted molecular weights

for these reference materials were: 853,000, 380,000, 186,000, 100,000, 48,000, 12,200 and 5800. Glucose with a molecular weight of 180 was used as a marker for total permeation. No pullulan polysaccharide calibrant was available of higher molecular weight to determine the column exclusion limit. However, from information received from the manufacturer it is predicted that these columns will have approximately 48% pore volume.

Columns

Three PL-GFC gel filtration columns (300 × 7·5 mm I.D.) were operated in series to optimize the resolution achieved for these HEC samples, the pore sizes of the individual columns being 4000 Å, 4000 Å and 300 Å. They were coupled in order of decreasing pore size (Polymer Laboratories Ltd, Church Stretton, UK).

HPLC system

An HPLC system consisting of a ConstaMetric 111 pump and refractive index detector (LDC/Milton Roy Ltd, Stone, UK) and Rheodyne 7125 injection value (Rheodyne Inc., Cotati, USA) was used. The detector output was monitored using a twin pen strip chart recorder (Knauer GmbH, Berlin, Germany).

Sample and calibrant preparation

The calibrants were prepared in the eluents to be used at a concentration of 1 mg/ml. After shaking to disperse the standards were left a minimum of 2 h to ensure complete dissolution. The HEC samples were also prepared at a concentration of 1 mg/ml. However, the eluent was added in four equal aliquots and a vortex mixer used to aid dissolution. The sample solutions were left a minimum of 15 h before analysis. All reference standard and sample solutions were filtered through a 0·45 μm membrane filter (Millipore Ltd, Watford, UK) prior to analysis.

Calibration and molecular weight calculation

The column set was calibrated using the pullulan polysaccharide standards prepared in the eluent used for the analysis. The flow rate in all cases was 0·5 ml/min. Calibration curves for each eluent were constructed as the log of the pullulan polysaccharide molecular weight vs the elution volume. Pullulan polysaccharide molecular weight averages for the HEC samples were calculated based on the concentration of material present within equidistant segments of the chromatogram (which represents the molecular size distribution) and the pullulan polysaccharide molecular weight represented by each segment as determined by the calibration curve.

RESULTS AND DISCUSSION

Hydroxyethylcellulose is normally classified as a water-soluble derivative of cellulose; however, it is also soluble in some aggressive organic solvents. Previously molecular weight information has been obtained for this type of polymer by using organic phase GPC, poly(styrene/divinylbenzene) packing materials and dimethyl formamide/dimethyl sulphoxide eluent systems (Kennedy *et al.*, 1990). However, these eluents are viscous and harmful and a salt, lithium bromide is needed not only to aid dissolution but also to reduce sample/sample and sample/packing material interactions. Elevated temperatures may also be required to achieve high performance separations. The use of gel filtration chromatography would be preferred for the molecular weight determination of this sample type. The samples evaluated in this study were representative of those commercially available and in all cases sample preparation was straightforward. No aggressive solvents or heat were necessary to achieve dissolution and, therefore, it is anticipated that sample degradation during solution preparation and analysis when 100% aqueous buffers of neutral pH were used, would be kept to a minimum.

Two aqueous eluents were evaluated in this study: (a) 0·05 M sodium dihydrogen orthophosphate, 0·25 M sodium chloride, pH 7·0; and (b) 0·1 M sodium nitrate, 0·02% sodium azide, pH 7·0. The pullulan polysaccharide calibration curves for both eluent systems are shown in Fig. 1. As would be expected the calibration curves are relatively similar but slightly off-set. This is due to the difference in the hydrodynamic volume of the calibrants in the two eluent systems, the gel filtration separation mechanism being based purely on the relative size of the molecules in solution. The set of six HEC

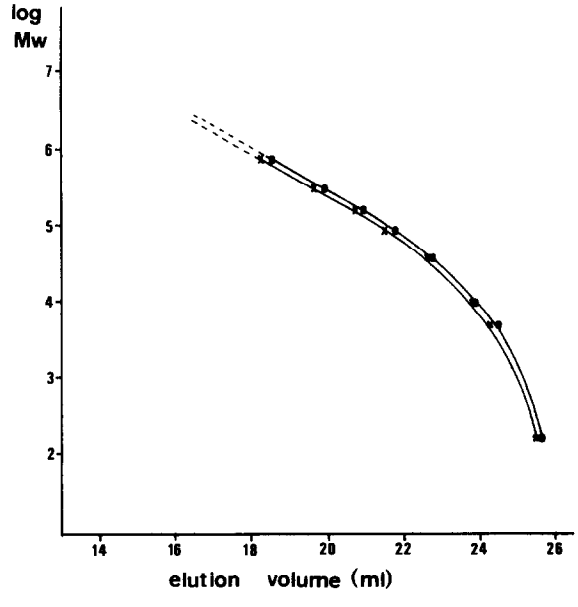

Fig. 1. Pullulan polysaccharide calibration curves. Columns: 2 × PL-GFC 4000 Å + 1 × PL-GFC 300 Å 8 μm 300 × 7·5 mm I.D.; detector, refractive index; flow rate, 0·5 ml/min; temperature, ambient; eluent (a) 0·05 M sodium dihydrogen orthophosphate, 0·25 M sodium chloride, pH 7·0 (x); and (b) 0·1 M sodium nitrate, 0·02% sodium azide, pH 7·0 (•).

samples were also analysed using both solvent systems. Figures 2 and 3 show the profiles obtained for the HEC samples using eluent a, the 0·5 M sodium dihydrogen orthophosphate, 0·25 M sodium chloride, pH 7·0 and eluent b, 0·1 M sodium nitrate, 0·02% sodium azide, pH 7·0, respectively. By comparing the elution volumes of the HEC samples in both eluent systems with the elution volumes of the reference standards used to produce the calibration curve it can be seen that in both cases part of the samples elute outside the upper limit of the calibrated fractionation range of the columns. In order to calculate the pullulan polysaccharide equivalent molecular weight averages for the HEC samples it was, therefore, necessary to extrapolate the calibration curve as no higher molecular weight calibrants were available.

The calculated molecular weight averages for these samples in both eluent systems based on the respective calibration curves shown in Fig. 1, are given in Table 1. Although the actual pullulan polysaccharide equivalent

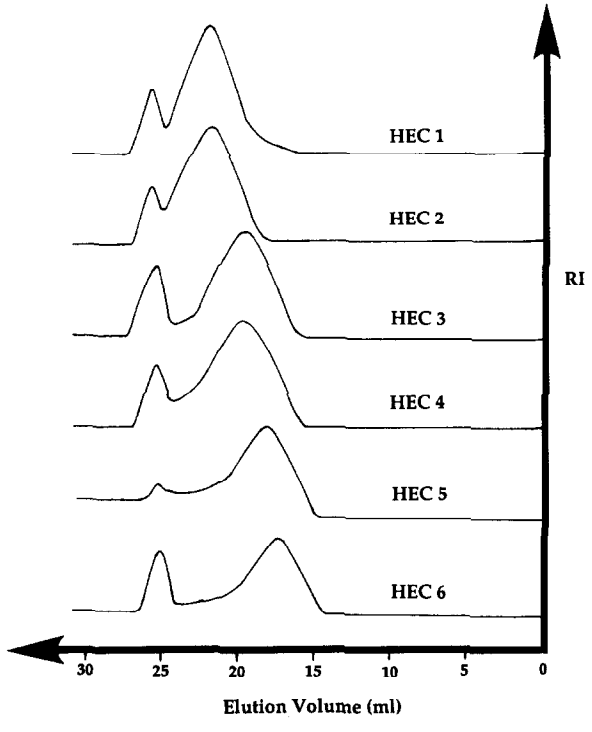

Fig. 2. Elution profiles obtained for the six HEC samples using eluent a. Chromatographic conditions as in Fig. 1.

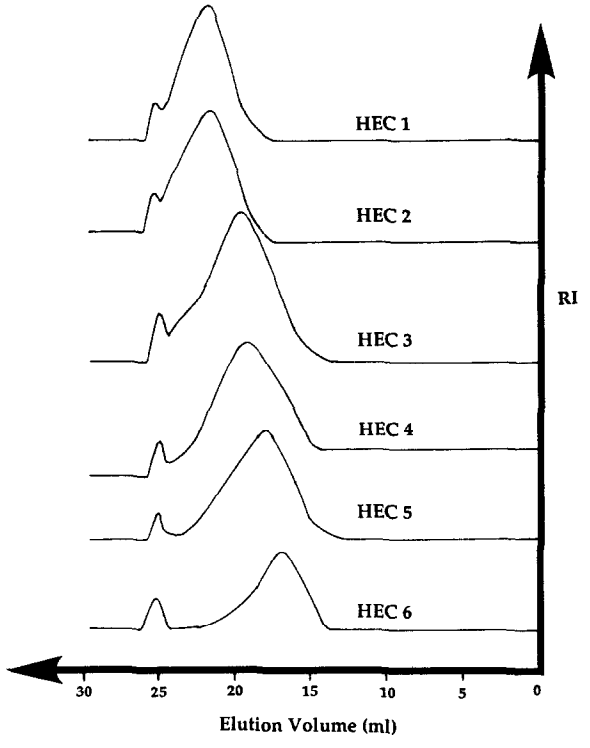

Fig. 3. Elution profiles obtained for the six HEC samples using eluent b. Chromatographic conditions as in Fig. 1.

Table 1. Pullulan polysaccharide molecular weight averages obtained for the six commercial samples of HEC

Sample	Eluent[a]	Mn[b]	Mw[c]	Mp[d]	D[e]	Area
HEC 1	a	23,000	134,000	96,000	5·8	4·6
	b	22,000	140,000	95,000	6·4	4·8
HEC 2	a	11,400	150,000	95,000	10·1	4·6
	b	19,000	175,000	120,000	9·2	5·0
HEC 3	a	250,000	701,000	484,000	2·8	4·2
	b	160,000	864,000	484,000	5·4	6·4
HEC 4	a	250,000	770,000	484,000	3·1	4·3
	b	230,000	690,000	420,000	3·0	3·8
HEC 5	a	660,000	1,645,000	1,120,000	2·5	3·1
	b	444,000	2,530,000	1,620,000	5·7	5·4
HEC 6	a	840,000	1,900,000	1,300,000	2·2	2·5
	b	460,000	2,300,000	1,900,000	5·0	3·1

[a]Eluent a: 0·05 M sodium dihydrogen orthophosphate, 0·25 M sodium chloride, pH 7·0. Eluent b: 0·1 M sodium nitrate, 0·02% sodium azide, 7·0.
[b]Mn is the number average molecular weight.
[c]Mn is the weight average molecular weight.
[d]Mp is the peak molecular weight.
[e]D is the polydispersity Mw/Mn.

molecular weight averages calculated for the HEC samples differ for the two eluent systems the trend within each set is similar — the differences in the calculated averages for the two eluent systems being due to the difference in the hydrodynamic volume of the pullulan polysaccharides and the HEC molecules in the different ionic strength aqueous buffer.

Both sets of data correlate with the viscosity grading given by the manufacturer (HEC samples 5 and 6 being of high viscosity and HEC samples 1, 2, 3 and 4 being of low viscosity). However, by using gel filtration chromatography it is possible to establish differences in the molecular size profiles within the two grades of samples in addition to the differences between grades.

Effect of sample concentration

To study the effect of sample concentration on the molecular size profiles and polysaccharide equivalent molecular weights three concentrations of HEC sample 3 were prepared (0·45, 0·97 and 2·04 mg/ml) in eluent 1. Data in Table 2 show that at concentrations of 0·45 and 0·97 mg/ml the values obtained for the molecular weight averages and polydispersity are within the experimental error of the method of calculation but at the higher concentration of 2·04 mg/ml there is a significant difference in the values obtained. The sample profiles (Fig. 4) obtained at the three concentrations clearly show the difference in the peak elution volume. Increasing the concentration increases the peak elution volume and hence reduces the calculated molecular weight averages. These results show that at a concentration of approximately 1 mg/ml there are no significant sample viscosity effects but at higher concentrations a distortion in the distribution and hence calculated averages occurs. The 1 mg/ml concentration would, therefore, appear to be optimum to obtain concentration-independent data whilst providing maximum detector response and hence sensitivity.

CONCLUSION

Results presented here demonstrate that an aqueous gel filtration system can be used for the determination of molecular weight averages and distributions for a representative set of water-soluble HEC samples. The data obtained using the two eluent systems correlate well with the viscosity grading provided by the manufacturer. However, in addition to establishing differences between the grades it is also possible using the gel filtration chromatography system to determine significant variations within the grades. Although there are no characterized HEC samples which could be used to calibrate the gel filtration system by using the pullulan polysaccharide standards it is possible to obtain comparative information for these samples. Owing to the differences in the molecular hydrodynamic volumes in the two eluent systems evaluated it is necessary to calibrate the system using the same eluent as for the analysis of the HEC samples and to quote the eluent composition and calibrants used. It would appear from this work that it is possible to use both aqueous eluent systems to generate molecular weight information for the HEC samples.

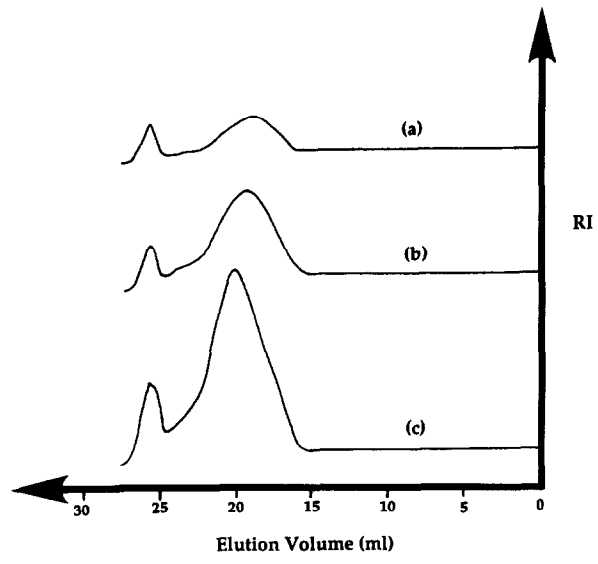

Fig. 4. Elution profiles obtained for HEC sample 3 using eluent a. Chromatographic conditions as in Fig. 1. Concentration of sample (a) 0·45 mg/ml; (b) 1·0 mg/ml; (c) 2·04 mg/ml.

Table 2. Effect of sample concentration on the molecular weight averages calculated for HEC 3 using eluent 1

Sample concentration (mg/ml)	Mn	Mw	Mp	D[a]
0·45	180,000	470,000	501,000	2·6
1·07	202,000	440,000	501,000	2·2
2·04	89,000	570,000	422,000	6·4

[a]D is the polydispersity Mw/Mn.

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