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On the characterization principles of some technically important water soluble non-ionic cellulose derivatives

Stefan Nilsson[a] *, **Lars-Olof Sundelöf**[a] & **Bedrich Porsch**[b]

[a]*Physical Pharmaceutical Chemistry, Uppsala University, Uppsala Biomedical Center, P.O. Box 574, S-751 23 Uppsala, Sweden*
[b]*Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, 162 06 Prague, Czech Republic*

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Principles are developed and discussed for a detailed characterization of water soluble polymers (cellulose ethers) as to hydrodynamic and thermodynamic behaviour, molecular size in solution in solvents of varying hydrophilicity and aggregation. In particular, the application of dynamic light scattering (DLS) and size exclusion chromatography is discussed and compared with static light scattering and viscometry. Results are given for eight non-ionic cellulose ether samples concerning phase separation properties, intrinsic viscosity, Huggins' constant, weight and number average molecular weight, second virial coefficient, radius of gyration, molecular weight distribution, and hydrodynamic particle size parameters from DLS. The results are also discussed in terms of chemical properties such as degree and type of substitution and cloud point. Values of refractive index increments have been measured for all samples in the two solvents used.

INTRODUCTION

Water soluble polysaccharides, both uncharged and charged, play an important role in many technical applications as well as in medicine and pharmacy (Florence & Attwood, 1988). Cellulose derivatives have for a long time been used in the pharmaceutical and food industry, owing to their ability to serve as emulsifiers, colloidal stabilizers and flow controllers but also due to their non-toxic nature (Greminger & Krumel, 1980). Dextran has similar applications but is also directly used in medicine, for instance as a plasma substitute (Grönwall & Ingelman, 1945). Some charged polysaccharides like the carrageenans have found applications similar to uncharged cellulose derivatives (Guiseley *et al.*, 1980). Hyaluronan has a well documented biological importance and a wide medical application (Laurent & Fraser, 1992). Even in the solid or semi-solid form, cellulose and cellulose derivatives have found wide use as adsorbents, components in release regulating films (Greminger & Krumel, 1980; Daul & Mitchell, 1971) etc.

For optimal use of such polymers, properties such as chain length (molecular weight), molecular size in relation to chain length, type of conformation, size and conformation as a function of solvent and temperature as well as their tendency to form aggregates or otherwise interact on the molecular level, constitute essential elements in their characterization as required in the applications. Although much work has been done on the improvement of principles of characterization, there is a lack of knowledge regarding satisfactorily precise and dependable procedures for such an analysis. Furthermore, the solution state of many of the substitution products of cellulose have been found to require a sophisticated use of several independent characterization methods on the same sample. The question of aggregation, for instance, requires considerable attention if the results are not to be misleading.

The present paper is intended firstly to serve as a guideline for future work on precise characterization of water soluble polymers; and secondly to provide characterization data for, and a comparative discussion of a set of non-ionic cellulose ethers also used in other studies (Holmberg *et al.*, 1992; Nilsson *et al.*, 1994, 1995; Hammarström & Sundelöf, 1993). A wide selection of methods have been applied including viscometry, static light scattering (LS), dynamic light scattering (DLS), size exclusion chromatography (SEC) with both refractive index (RI) and low angle laser light scattering (LALLS) detection. It has also been found

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*Author to whom correspondence should be addressed.

MATERIALS AND METHODS

Materials

Data on the non-ionic cellulose derivatives used in this study are presented in Table 1. All of the ethyl(hydroxyethyl)cellulose (EHEC) fractions were kindly supplied by Berol Kemi, Stenungsund, Sweden. Hydroxyethylcellulose (HEC) and methylcellulose (MC) were obtained from Aldrich-Chemie, Steinham, Germany. Hydroxypropylcellulose (HPC) was supplied by Hercules Inc., Wilmington, USA and hydroxypropyl(methyl)cellulose (HPMC) was obtained from Colorcon Ltd, West Point, UK. Two typical cellulose ether structures are shown in Fig. 1a and b.

The cloud point (CP) of aqueous cellulose ether solutions was determined by visual observation in glass tubes and taken as the temperature when the last visible sign of clouding in the solution disappeared upon cooling (Nilsson *et al.*, 1994).

The standardized procedure adopted to make an aqueous stock solution of a cellulose ether is described elsewhere (Holmberg *et al.*, 1992). The stock solutions were dialysed in tube membranes (cut-off 12-14000, Spectra/Por®, Spectrum Medical Ind., LA, USA) against Milli Q water (Millipore) for 1 week to remove salts and low molecular weight material. Ultimately, the stock solutions were filtered through 0.8 μm filters (Millex-AA, Millipore SA, Molsheim, France) to remove undissolved substances, microgels and dust particles before the concentrations were determined by drying the samples to constant weight at 105°C.

All solutions of the cellulose ethers were prepared by weight at least 24 h before an experiment was performed, in order to reach equilibrium and to allow any time-dependent effect to settle (Nilsson *et al.*, 1994). Concentrations of the cellulose ether samples are expressed in per cent by weight %(w/w). Either water or a methanol/water mixture (50% methanol/50% 10 mM NaCl in water) served as solvent for the cellulose samples. Analytical grade sodium chloride and methanol were obtained from Merck, Darmstadt, Germany and used without further purification. Milli Q water was used in the preparation of all solutions and the temperature of measurements was 20°C throughout this study.

Viscosity measurements

The viscometric measurements were performed in ordinary Ostwald capillary viscometers (solvent-water; flowtime of approx. 100 s) which were immersed in a water thermostat. Viscometric data were calculated as reduced specific viscosity (η_{sp}/c) and plotted against the polymer concentration using the expression

$$\eta_{sp}/c = [\eta] + k_H[\eta]^2 c + \ldots$$

where higher terms have been omitted. k_H is the Huggins' constant and c is the polymer concentration. From such plots the intrinsic viscosities ([η]) of the different cellulose ethers were determined both in water and in a methanol–water mixture (50% methanol/50% 10 mM NaCl in water) by extrapolation to zero polymer concentration. Densities were determined in a digital densitometer DMA O2C from Anton Paar K.G., A-8054 Graz, Austria according to Kratky *et al.* (1973).

SEC/LALLS/RI measurements

Three stainless steel columns (250×8 mm I.D., supplied by Tessek Ltd, Prague, Czech Republic) connected in series, were packed with diol modified LiChrospher 300, 1000 and 4000 packings prepared according to a recently described procedure (Regnier & Noell, 1976; Porsch, 1993). Sample concentrations were 0.1–0.3% (w/w) depending on the level of response from the detectors and the mobile phase used was a methanol–water mixture (50% methanol/50% 10 mM NaCl in water). Apart from the columns, the SEC equipment consisted of a VCR 40 HPLC pump (CSAS Development Works, Prague, Czech Republic), an injection valve Rheodyne 7125 (Rheodyne Inc., Cotati, CA, USA) with a 200 ml loop, a Waters R 401

Table 1. Data for the non-ionic cellulose derivatives used in this study

Polymer	Trade name	CP[a] (°C)	DS_{alkyl}[b]	$MS_{alkyleneoxide}$[b]
EHEC	Bermocoll CST-103	28	1.5	0.7
EHEC	Bermocoll DVT-87014	28	1.4	0.9
EHEC	Bermocoll E230G	65	0.9	0.9
EHEC	Bermocoll E411G	58	1.2	1.7
HEC	—	> 100	—	~3
MC	—	65	~2	—
HPC	Klucel LF	42	—	~0.4
HPMC	Methocel E4 EP	55	~2	~0.4

[a] Refers to 0.5% (w/w) aqueous solution.

[b] Values for the EHEC fractions are determined by the producer and values for HEC, MC, HPC and HPMC are estimated.

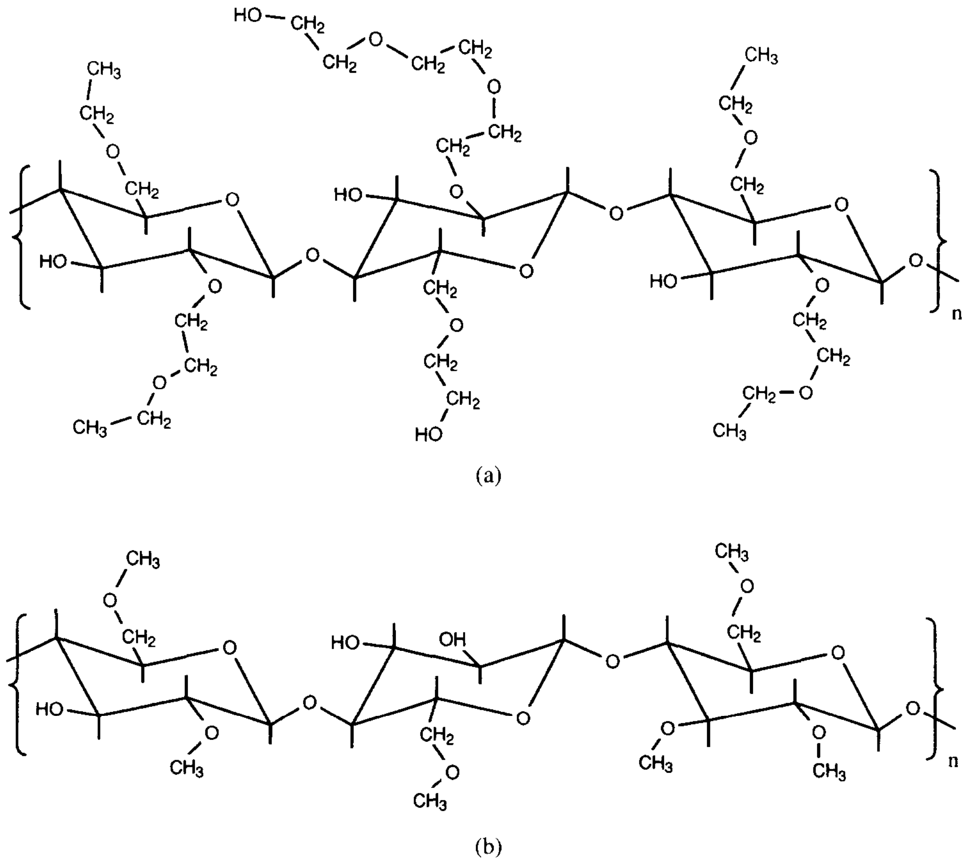

Fig. 1. (a) Possible structure segment in EHEC with $MS_{\text{alkylene oxide}} = 2$, $DS_{\text{alkyl}} = 1.3$ and DP (degree of polymerization) = $3n$. (b) Possible structure segment in MC with $DS_{\text{alkyl}} = 2$ and DP = $3n$.

differential refractometer (Waters Assoc., Milford, MA, USA) and a Chromatix KMX-6 LALLS detector (LDC/MiltonRoy, Sunnyvale, CA, USA) operated at 6° measuring angle. Both detectors were connected through a Black Star 2308 A/D converter (Black Star Ltd, Huntingdon, UK) to an IBM-compatible computer.

DLS measurements

Measurements of scattered light were made in high grade 10 x 10 mm cells (Hellma G.m.b.H., Mullheim Baden, Germany) at a fixed 90° angle with respect to the illuminating beam using as light source an argon-ion laser (Coherent Innova 70-2, Coherent Laser Division, Palo Alto, CA, USA) with a nominal power output of 2 W and tuned to 514.5 nm. The laser beam was spatially filtered before being focused into the solution. A set of field and aperture stops together with a lens defined the scattering characteristics into a photomultiplier unit (Brookhaven Instruments Corp., NY, USA) where the raw signal was amplified, discriminated and given proper characteristics for further handling in the electronics. These 'shaped' photon counting signals were then processed in a digital correlator (BI 8000 AT, Brookhaven Instr. Corp.). The Brookhaven particle size distribution software package contains five of the most common distribution analytical procedures. Brookhaven CONTIN (version 3.0) was mainly used here to process the time correlation functions measured. The details of DLS can be found elsewhere (Chu, 1974; Berne & Pecora, 1976). All optical components were mounted on a high quality optical table (Newport Research Corp., CA, USA). All measurements were performed using 64 channels with linear mode, the sampling time 5 or 10 μs and the duration of an experiment approximately 15 min.

For the majority of DLS measurements the solutions were filtered through 0.2 μm filters (Millex-FG, SLFG 025 BS, Millipore, Molsheim, France); 0.5 μm filters (Millex-SR, SLSR 025 NS) were used for the sample E411G, into the carefully cleaned sample cells. The solvent consisted of either water or a methanol–water mixture (50% methanol/50% 10 mM NaCl in water).

LS measurements

Static light scattering measurements at 20°C (room temperature) were made with a modified Sofica 42000 instrument at 11 fixed angles (30–150°) and at a wavelength of 633 nm (helium/neon laser light source). Five polymer solutions with concentrations in

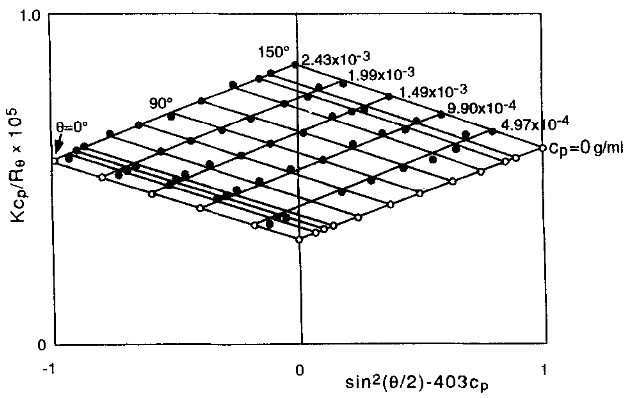

Fig. 2. Zimm plot for MC in '50% methanol/50% 10 mM NaCl in water' at 20°C. Scattering angles and polymer concentrations measured are shown. The molecular weight of this sample is 2.98×10^5.

the approximate range 0.05–0.3%(w/w) were prepared in the solvent 50% methanol/50% 10 mM NaCl in water. Prior to LS measurements, each solution was clarified and made dust-free by filtration through a 0.22 μm filter (Millex-GS, SLGS 025 BS, Millipore) directly into the carefully cleaned cylindrical cell. The Zimm plot method (Zimm, 1948) was used to determine the weight average molecular weight, M_w, the radius of gyration, R_g, and the second virial coefficient, A_2. In constructing a Zimm plot experimentally determined values of Kc_p/R_θ are plotted vs $\sin(\theta/2)+kc_p$. An example is shown in Fig. 2, where R_θ is the reduced intensity of scattered light (excess Rayleigh factor) at an angle q, k is an arbitrary scaling constant (−403 in Fig. 2), c_p is the polymer concentration and K is the optical constant. The resulting gridlike plot allows extrapolation to both $c_p=0$ and $\theta=0°$ and from such a plot, values of the molecular weight, M_w, the radius of gyration, R_g, and the second virial coefficient, A_2, are determined. Further details of the LS technique can be found elsewhere (Huglin, 1972; Kratochvil, 1987).

Refractive index increment measurements

Refractive index increments, dn/dc, of the cellulose derivatives in the two solvents were determined at 20°C using an Optilab differential refractometer (Optilab Multiref. 902B, Sweden) which was connected to a thermostated water bath. The instrument was calibrated with aqueous KCl and sucrose solutions of known refractive index (Huglin, 1972). The refractometer had a very high sensitivity, hence the concentrations of the cellulose derivatives had to be rather low and were kept in the range 0.003–0.015%(w/w). Refractive index increments were determined both in water and in the methanol–water mixture (50% methanol/50% 10 mM NaCl in water) and they were used in the LS and SEC/LALLS/RI experiments.

RESULTS AND DISCUSSION

In substitution derivatives, such as the ones investigated here, the overall properties, so important in applications, depend intimately on the detailed chemical characteristics of the substituents, the degree of substitution, the degree of polymerization and the distribution of substituents along the backbone chain. A detailed chemical analysis is an enormous task and can only be performed in certain model cases. On the other hand, the properties of practical importance can, with good confidence, be extracted from studies in solution of molecular size, interpolymer interaction (both thermodynamic and hydrodynamic), tendency to phase separate, mobility, size distribution, tendency to aggregate, filtrability, etc. Comparison of size parameters derived from different techniques can, due to basic differences in their response to molecular characteristics, reveal important aspects of the polymer behaviour. The present study attempts a comparative approach on a broad base.

From Table 1 it is obvious that the chemical properties of the derivatives investigated are fairly different and hence the physical appearance could be expected to show considerable differences. The substitution of the cellulose ethers is characterized by the numbers DS_{alkyl} (degree of substitution) and $MS_{\mathrm{alkylene\ oxide}}$ (molar substitution). DS_{alkyl} is the average number of hydroxyl groups on the anhydroglucose unit which has been substituted by alkyl groups ($0< DS_{\mathrm{alkyl}} <3$). $MS_{\mathrm{alkylene\ oxide}}$ is the average of the total number of alkylene oxide groups (hydroxyethyl or hydroxypropyl) per anhydroglucose unit. Due to formation of oligo(alkylene oxide) chains, the quantity $MS_{\mathrm{alkylene\ oxide}}$ could in principle be almost any positive number. In practice, the highest value encountered is only a few units. The numbers DS and MS are only averages and do not reveal anything about the distribution of substituents along the chain. This distribution is certainly quite decisive for the real properties. In this respect, the value of CP is somewhat complementary to DS and MS (Table 1). CP depends on a number of factors such as the type of substituents and degree of substitution (Sarkar, 1979; Dönges, 1990) (which determine the hydrophilic/hydrophobic balance of the cellulose derivative), molecular weight (Sarkar, 1979) and concentration (Jullander, 1957; Sarkar, 1979; Dönges, 1990; Nilsson *et al.*, 1994) of the cellulose ether in solution. A rough estimation of the heterogeneity in the distribution of the substituents on a cellulose ether can be performed using enzymatic cleavage of the chain in combination with viscosity measurements (Gelman, 1985). High resolution NMR spectroscopy (Parfondry & Perlin, 1977; Zadorecki *et al.*, 1987) and chromatographic analysis in combination with mass spectroscopy (Lindberg *et al.*, 1988) can also be utilized to pinpoint more precisely the detailed chemical structure. The substances used here are of technical origin, however, and are, therefore, less amenable to analysis by these more elaborate techniques.

Because of the large variation in the 'ratio' between hydrophobic and hydrophilic groups, one would expect a great sensitivity to solvation, for instance. That this is the case is clearly revealed by the results described here. For the SEC experiments it was found that a mobile phase consisting of a 1:1 mixture of methanol and 10 mM NaCl in water provided a suitable hydrophobic/hydrophilic balance in solubility and prevented hydrophobic interaction of solutes with column packing. In this way, a true SEC performance with an excellent resolution was obtained. Such a mixture proved to be of value even with the other methods. A comparison between size data and interaction parameters obtained in the two solvents is of great help in the detailed analysis of polymer properties. As for the hydrodynamic properties, one should observe that some of the derivatives investigated have a conformational structure that is quite expanded leading to at least partial free draining of the coil. This leads to difficulties in assigning precise geometrical dimensions from hydrodynamic measurements for comparison with dimensions obtained from scattering techniques.

In fact, the only common feature of the substances listed in Table 1 is the cellulose backbone. The EHEC derivatives used carry, in the structural sense, two short but different substituents and the number of these substituents as well as their ratio varies. The rest of the samples contain one or two different substituents of a chemical structure different from EHEC. This variability of substitution should certainly result in differences in the intra- and intermolecular interactions that determine the hydrodynamics of different samples in solutions. Hence, simple scalings such as molecular weight to $[\eta]$ etc. can hardly be expected to be valid for the set of 'different polymers' in Table 1.

Obviously only a combination of several methods could be trusted to give a characterization that is dependable. In the course of this work, much was learned about how to interpret data on size and mass distributions. Initially, for instance, the DLS technique seemed to indicate a mixture of fast and slow modes at all concentrations, an indication of aggregate formation. However, detailed studies combining the DLS and the SEC techniques using filters with different hydrophobicity and stepwise filtration finally revealed that in sufficiently low concentrations these 'slow modes' could be removed and that no new ones developed.

Table 2 presents the intrinsic viscosities, $[\eta]$, in water and in the methanol–water mixture (50% methanol/50% 10 mM NaCl in water) of the set of cellulose ethers. Two typical examples (HPMC and MC in water) of intrinsic viscosity determinations are shown in Fig. 3. Obviously the curvature of the data for HPMC indicates a solute–solute interaction stronger than in the MC case, i.e. higher order terms of concentration dependence must be taken into account in the expression for η_{sp}/c even at fairly moderate polymer concentrations. Such aspects of system properties at higher concentrations are, however, not a topic of this paper but it can be noted that, in the case mentioned, the effect derives mainly from the fact that the HPMC/water system is close to a concentration where the coils begin to overlap, defined as $c^* = 1/[\eta]$ (Weisberg *et al.*, 1951). These values calculated in Table 2 for both solvents seem rather low for all polymers investigated. For comparison, in the case of dextran (M = 676,000) in water, poly(ethylene oxide) (M = 594,000) in water, sodium hyaluronate (M = 560,000) in 0.2 M NaCl and polystyrene (M = 411,000) in toluene, the values of c^* are 1.4, 0.25, 0.12 and 0.76%, respectively. HPMC and E411 samples from Table 2 fall into the molecular weight range of these examples (see below) and their c^*

Table 2. Intrinsic viscosity ($[\eta]$), viscosity equivalent sphere diameter (d_η), critical overlap concentration (c^*), and Huggins' constant (k_H) at 20°C in water and in '50% methanol/50% 10 mM NaCl in water' (mp^*) for the set of non-ionic cellulose ethers investigated

Substance	Water				mp^*				r_η
	$[\eta]$ (ml/g)	c^* (%)	k_H	d_η (nm)	$[\eta]$ (ml/g)	c^* (%)	k_H	d_η (nm)	
(A) EHEC									
CST-103	455	0.22	0.68	47.6	320	0.31	1.31	42.3	1.12
DVT-87014	290	0.34	1.03	36.5	244	0.41	1.25	34.5	1.06
E230G	410	0.24	0.48	65.1	270	0.37	1.19	56.7	1.15
E411	1000	0.10	0.62	99.6	980	0.10	0.44	98.9	1.01
(B) Others									
HPMC	740	0.13	0.54	65.4	620	0.16	0.80	61.7	1.06
HPC	134	0.74	0.83	26.1	110	0.91	1.80	24.5	1.07
MC	400	0.25	1.20	43.3	395	0.25	0.36	43.2	1.00
HEC	273	0.37	1.22	40.2	285	0.35	0.88	40.8	0.98

$r_\eta = d_\eta$ (water)/d_η(mp^*).

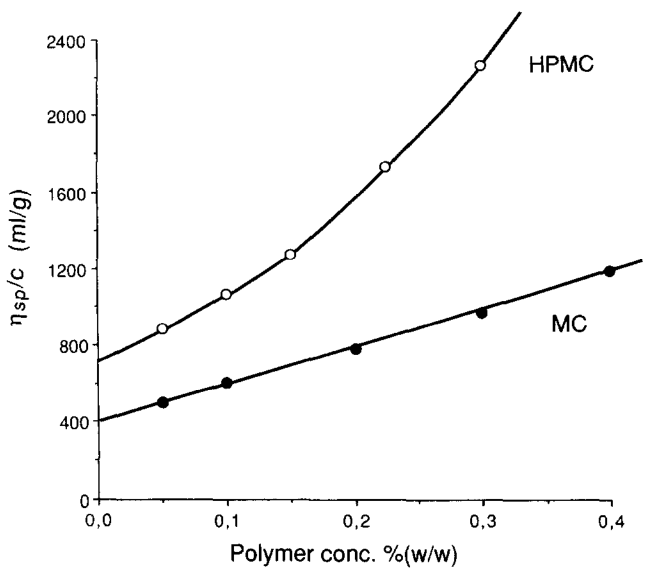

Fig. 3. Typical plot of η_{sp}/c as a function of c. The intrinsic viscosities ($[\eta]$) are 740 and 400 ml/g for HPMC and MC, respectively. The data are valid in H_2O and at 20°C.

compares with sodium hyaluronate, known to form greatly expanded and partially draining coils in solution (Fouissac *et al.*, 1992). This should be borne in mind when the viscosity diameters, d_η, in Table 2, calculated as $d_\eta = 1.08\ (M[\eta])^{1/3}$ (Vollmert, 1962), are interpreted and compared. The equivalent sphere diameter (d_η) relates to the hard sphere that would exhibit the same $[\eta]$ as the dissolved polymer coil, hence, it assumes the applicability of the non-draining behaviour of the polymer investigated (Flory, 1953). The ratio d_η(water)/$d_\eta(mp^*)$ (r_η in Table 2) illustrates the different behaviour of the samples when dissolved in water and in the methanol–water mixture (mp^* = 50% methanol/50% 10 mM NaCl in water). An almost unchanged equivalent sphere diameter is seen in three cases, three samples moderately decrease their size and in two cases (CST-103 and E230G), the decrease is pronounced. This clearly indicates the close relationship between the structural differences of the samples and interactions that determine coil size and shape.

Table 3 reports results from static light scattering (LS) in terms of weight average molecular weight (M_w), radius of gyration (R_g) and second virial coefficient (A_2) in the 50% methanol/50% 10 mM NaCl in water mixture at 20°C for the set of cellulose ethers investigated. In Fig. 2, a typical Zimm plot is shown for MC in the 50% methanol/50% 10 mM NaCl in water mixture.

A structural parameter, $r = R_g/R_H$, where R_H is the hydrodynamic radius obtained from a DLS experiment, was shown to be related to coil density, i.e., to reflect branching (Burchard, 1983). Unfortunately, the effect of sample polydispersity on r values in the case of broad distributions overwhelms the structural effect; the r values may then easily increase up to ~2, especially for asymmetrical distributions (Burchard, 1983; Patton *et al.*, 1989). From this point of view, the r values in Table 3 scattered around the value of two are quite reasonable when the polydispersity indices, M_w/M_n, of the samples studied are taken into account. The origins of a lower or a higher value of r are unclear; it might be related to differences in hydrodynamic behaviour and/or coil conformation.

Table 3. Static light scattering results at 20°C in '50% methanol/50% 10 mM NaCl in water' for the set of non-ionic cellulose ethers investigated

Substance	$M_w \times 10^{-5}$	R_g (nm)	$A_2 \times 10^4$ (ml·mol/g^2)	r
(A) EHEC				
CST-103	5.11	66	-0.04	2.06
DVT-87014	2.35	47	2.0	1.92
E230G	~50	—	-0.3	—
E411	11.0	78	5.1	2.16
(B) Others				
HPMC	3.85	64	3.3	2.17
HPC	2.62	33	9.8	2.64
MC	2.98	57	4.4	2.07
HEC	2.28	47	3.5	1.71

$r = R_g/R_H$.
R_H is the hydrodynamic radius ($d^1/2$ in '50% methanol/50% 10 mM NaCl in water' as determined from the DLS experiment (Table 6).

The E230G sample does not allow a proper evaluation of the LS experiments and the data reported should be taken with reservation. However, the small negative value of A_2 probably gives an indication of the thermodynamic 'situation' in the solution. The high molecular weight and the low value of A_2 indicate an 'incipient phase separation condition' for the E230G sample in the 50% methanol/50% 10 mM NaCl in water mixture.

Figure 4 further highlights the aggregation discussion of E230G in the methanol–water mixture. In these experiments, the DLS equipment was used to obtain static light scattering intensities in water and in 50% methanol/50% 10 mM NaCl as a function of E230G concentration in solution. It is clear from Fig. 4 that there is a dramatic difference in scattering intensity from the polymer in the two solvents. The magnitude of this difference is concentration-dependent and increases with polymer concentration. It is also worth noting that the system behaves in a 'normal' way only at concentrations equal to or less than 0.025%(w/w) in both solvents. Dynamic light scattering experiments were then performed on the solutions presented in Fig. 4 and the CONTIN analysis gave a well developed bimodal distribution, indicating aggregation in the system at concentrations higher than 0.025% of E230G, the extent of aggregation being much higher in the methanol/water system. Similar topics are under further investigation for another system, (cf. Porsch & Sundelöf, 1995).

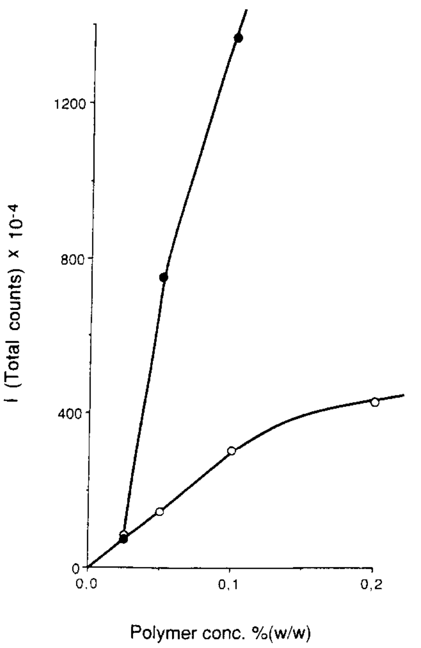

Fig. 4. Scattered intensity of light (I) expressed as the total number of counts during a fixed duration (60 s) of an experiment as a function of polymer concentration for EHEC/E230G in water (●), and in '50% methanol/50% 10 mM NaCl in water' (○).

Table 4. Refractive index increments, d*n*/d*c*, at 20°C in water and in '50% methanol/50% 10 mM NaCl in water' (*mp) for the set of non-ionic cellulose ethers investigated**

Substance	d*n*/d*c* (ml/g)	
	Water	*mp**
(A) EHEC		
CST-103	0.145	0.123
DVT-87014	0.143	0.125
E230G	0.141	0.124
E411	0.141	0.123
(B) Others		
HPMC	0.135	0.119
HPC	0.143	0.124
MC	0.135	0.120
HEC	0.141	0.121

Refractive index increments, d*n*/d*c*, for the different cellulose ethers were determined both in water and in the 50% methanol/50% 10 mM NaCl in water mixture at 20°C and are presented in Table 4. As could be expected, the d*n*/d*c* values are 15–20% lower in the methanol–water mixture than in water and they are in fair accordance with literature data (Brandrup & Immergut, 1975; Neely, 1963; Lewis & Robinson, 1970; Manley, 1956). The d*n*/d*c* data were used to convert the direct signals to actual molecular weights in the LS and SEC/LALLS/RI experiments.

In Table 5 the SEC/LALLS/RI results are presented. A typical example of a SEC/LALLS/RI chromatogram for CST-103 is shown in Fig. 5, the molecular weight calibration curve in Fig. 6 and the corresponding differential molecular weight distribution (MWD) curve in Fig. 7. The great advantage of a SEC/LALLS/RI experiment, once the conditions for its performance have been properly adjusted, is that it gives a fairly dependable molecular weight distribution curve. From this the weight average molecular weight, M_w, and the number average molecular weight, M_n, can be obtained and hence also the polydispersity index, M_w/M_n. Invariably the SEC data give values of M_w lower than those from static light scattering (Tables 3 and 5), on average by a factor of two, but for the E230G sample by a factor of nine. The difference between SEC and static LS results probably has its origin in the different concentration intervals over which the two methods operate. From the DLS measurements, discussed below, and from the discussion of the results in Fig. 4, it appears, namely, as if an aggregation takes place that increases with increasing concentration. In the SEC experiments, the injected sample is soon diluted substantially below 0.05% due to both polydispersity and eddy diffusion and the main part of the analytical

Table 5. SEC/LALLS/RI results at 20°C for the set of non-ionic cellulose ethers

Substance	$M_w \times 10^{-5}$	$M_n \times 10^{-5}$	M_w/M_n	$\log([\eta]M)$	$M_w^* \times 10^{-5}$	V_e^{**}(ml)
(A) EHEC						
CST-103	1.89	0.90	2.1	7.782	6.54	19.3
DVT-87014	1.33	0.74	1.8	7.512	3.54	19.8
E230G	5.35	2.97	1.8	8.160	3.69	20.2
E411	7.85	4.79	1.6	8.886	12.48	17.7
(B) Others						
HPMC	3.01	1.37	2.2	8.271	14.57	17.9
HPC	1.06	0.53	2.0	7.068	1.67	21.8
MC	1.62	0.88	1.8	7.806	5.97	19.1
HEC	1.89	0.39	4.8	7.732	5.17	20.7

The mobile phase used in the experiments was '50% methanol/50% 10 mM NaCl in water' (*mp**).
$\log([\eta]M)$, values of $[\eta]$ for the different cellulose derivatives in *mp** are found in Table 2.
M_w*, SEC/RI, poly(ethylene)oxide calibration curve used.
V_e**, the retention volume (V_e) at the maximum of the RI vs V_e curve.

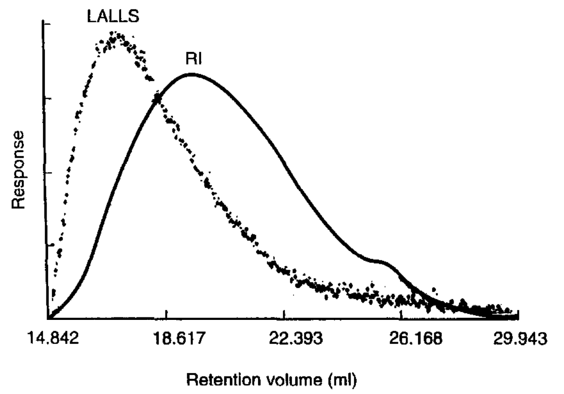

Fig. 5. Typical SEC/LALLS/RI chromatogram for EHEC/CST-103 in '50% methanol/50% 10 mM NaCl in water'.

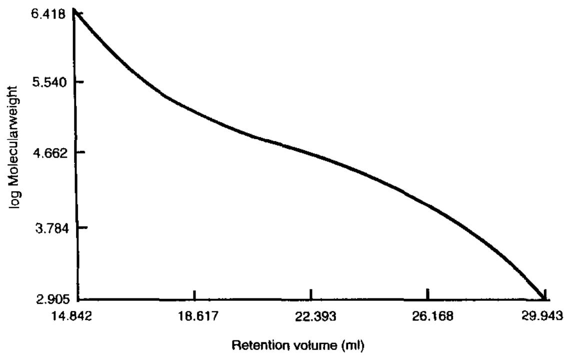

Fig. 6. Molecular weight calibration curve for EHEC/CST-103 (in Fig. 5).

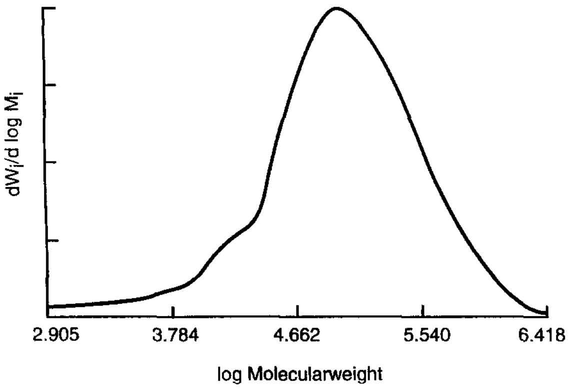

Fig. 7. The differential molecular weight distribution curve calculated from data of Figs 5 and 6 (EHEC/CST-103). $M_w = 1.89\times10^5$, $M_n = 0.90\times10^5$ and $M_w/M_n = 2.1$.

separation in the column, followed by the detection, takes place at concentrations below the onset of measurable aggregation (see Fig. 4 and the discussion above). Alternatively, the column set used has been shown to adsorb hydrophobic impurities (microdroplets) that may be contained in water soluble polymers (Porsch & Sundelöf, 1995). Some indication of the presence of such an impurity in the samples investigated was observed during the DLS experiments (see below). As static LS solutions were not filtered by hydrophobic filters to remove this impurity, the presence of such microdroplets in the sample might also bias static LS molecular weight values.

The HEC sample (Table 5) is found to give a much broader molecular weight distribution and hence a much higher polydispersity index ($M_w/M_n = 4.8$) than the other samples. This will also influence results from other measurements. The rest of the samples have polydispersity indices in the range 1.6–2.2 and distributions of a similar shape. Even if this indicates molecular weight distributions that are certainly not narrow, the overall similarity in polydispersity makes comparison between different techniques more dependable. To illustrate the real meaning of the sometimes practised determination of molecular weight relative to a different polymer in a calibration standard set (with RI detection only), one column in Table 5 contains M_w values calculated using a broad standard calibration procedure with a poly(ethylene oxide) standard set (Porsch & Sundelöf, 1994). The values indicate the level of failure of this approach.

The universal calibration plot in terms of log([η]M) vs elution volume (V_e) falling on a single line has been shown to apply for a variety of linear and branched polymers and copolymers (Grubisic *et al.*, 1967) as well as for some flexible water soluble polymers and proteins (Dubin & Principi, 1989). The basic assumption of its applicability is the validity of a non-draining behaviour of dissolved macromolecules. It is well known (Flory, 1953) that coil draining decreases when molecular weight increases and the non-draining approximation begins to apply at rather low molecular weights (approx. $M_w > 50{,}000$) at least in the case of synthetic polymers in organic solvents that are highly flexible. Contrary to that, as the major part of the samples in Table 5 have rather low molecular weights (with the exception for E230G and E411 where $M_w > 500{,}000$) and their viscosity and DLS behaviour indicate large and highly expanded coils with high internal stiffness (see above), it is reasonable to expect partially draining behaviour of the samples studied. The universal calibration plot is constructed in Fig. 8 from the data in Tables 2 and 5. It can be seen that three points deviate from the common straight line. The HEC sample greatly differs from the rest of the samples, having an entirely different shape and width of distribution of molecular weights (Table 5). In this case, the peak value of V_e does not correspond to the same moment of the distribution as the V_e values corresponding to the rest of the samples. These less broad samples have similar distribution shapes and similar M_w/M_n (Table 5); hence, the average peak value of V_e may be considered to be related to the same (although unknown) moment of the distribution and the resulting universal plot should not be too biased. The last two deviating samples (E411, E230G)

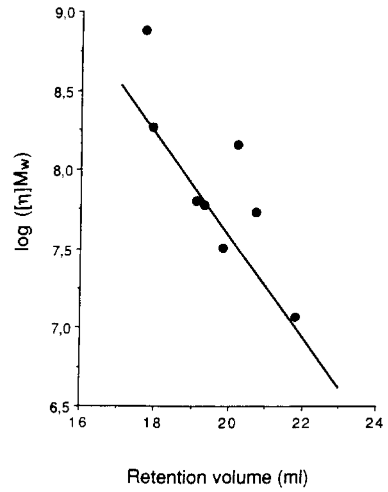

Fig. 8. Universal calibration plot for the cellulose ether set investigated.

have a substantially higher M_w than the rest of the samples, where with the exception of HPMC, the M_w values are quite similar (Table 5). The coil draining should certainly decrease when the molecular weight increases, and this may conveniently explain the failure of the plot in Fig. 8, in the case of these samples. Although this result should be taken as a preliminary one, similarity to the data of Dubin & Principi (1989) obtained for DNA and the polysaccharide schizophyllan supports the above explanation, either in terms of draining or rod-like shape of molecules with lower M_w. Our data thus indicate another example of failure of the universal calibration in the case of macromolecules characterized by high stiffness of the backbone.

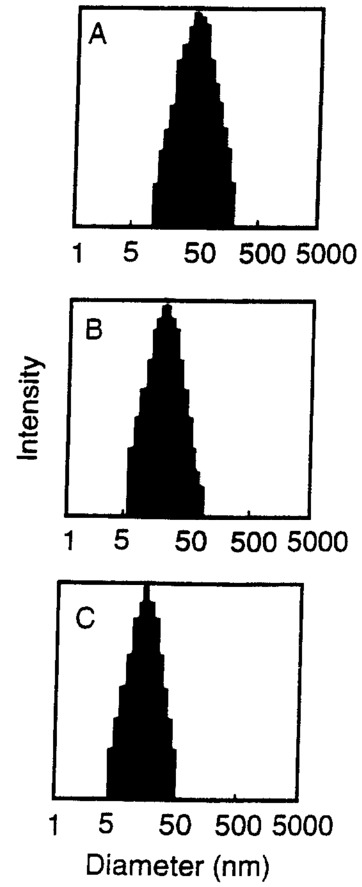

Fig. 9. Typical size distributions of the non-ionic cellulose ethers from DLS calculated with CONTIN. (A) EHEC/E411G in water ($d^1 = 66$ nm); (B) HPC in water ($d^1 = 34$ nm); and (C) HPC in '50% methanol/50% 10 mM NaCl in water' ($d^1 = 25$ nm).

Table 6 contains the dynamic light scattering (DLS) results for the set of cellulose ethers investigated both for water and for the 50% methanol/50% 10 mM NaCl in water mixture at 20°C. Figure 9 gives three typical examples of size distributions obtained in the DLS experiments by the CONTIN algorithm. In general, the data have been analysed by the CONTIN procedure

Table 6. DLS results at 20°C in water and in '50% methanol/50% 10 mM NaCl in water' (*mp) for the set of non-ionic cellulose ethers investigated**

Substance	Water		*mp**		r_{DLS}
	d^1 (nm)	d^2 (nm)	d^1 (nm)	d^2 (nm)	
(A) EHEC					
CST-103	63	51	64	52	0.98
DVT-87014	55	44	49	43	1.12
E230G	56	44	42	34	1.33
E411	66	58	72	58	0.92
(B) Others					
HPMC	64	53	59	49	1.08
HPC	34	28	25	22	1.36
MC	51	42	55	42	0.93
HEC	55	42	55	42	1.00

d^1, the average particle (molecule) diameter as calculated from CONTIN.
d^2, the particle diameter corresponding to the maximum of the distribution frequency curve as obtained from CONTIN.
$r_{DLS} = d^1(\text{water})/d^1(mp*)$.

from which average size parameters can be calculated: d^1, the average particle (molecule) diameter (defined as the $1/z$ average of the equivalent sphere diameter (Pusey, 1973)), and d^2, the particle diameter corresponding to the maximum of the distribution frequency curve, as obtained from CONTIN. The agreement between the values of the diameters (d^1,d^2) is good and generally $d^1 > d^2$ as it should be for these broad samples. Large equivalent sphere diameters are obtained from the DLS experiments for relatively low values of molecular weights similarly to the viscosity radius (Table 2). The different behaviour of samples after change of solvent is seen here as well. A comparison of $d_\eta(\text{water})/d_\eta(mp^*)$ (r_η in Table 2) and of $d^1(\text{water})\ /d^1(mp^*)$ (r_{DLS} in Table 6) shows the same viscosity and friction behaviour only in two cases (HPMC and HEC). In two other cases (MC and E411), where almost no change in viscosity diameter in both solvents is seen, an increase of DLS size is observed. The CST-103 sample exhibits a decrease of the viscosity size and almost no change of the DLS size. The rest of the samples are characterized by a decrease of both d^1 and d^2 when going from water to the methanol/water mixture. These differences in viscosity and diffusion (DLS) experiments, as expressed in coil size changes, again confirm the complex hydrodynamic behaviour of the samples studied. Nevertheless, the reasonable values of the r parameter (Table 3) indicate good consistency of static LS and DLS data. The advantage of a DLS experiment is that aggregation phenomena can be studied if the aggregates have a size sufficiently different from the non-aggregated molecules and that distributions of both the molecularly dispersed and the aggregated material can be obtained in one experiment. It must be mentioned, however, that in order to obtain dependable data by the DLS technique, extensive measurements must be performed where channel distribution, interval of channels used in the calculation, total interval in correlation time, sampling time, etc. are gradually changed and the results compared, for instance, between filtered and non-filtered solutions. There was also an indication of the presence of a similar impurity like that observed recently in poly(ethylene oxide) (Porsch & Sundelöf, 1995) resulting also in differences of DLS behaviour when hydrophilic and hydrophobic filters were used. In this way, one may successively approach a state of understanding through which the characteristics of the sample can be laid down. It was thus observed that all samples in the cellulose ether set gave bimodal distributions at elevated concentrations, both in water and in the mixture of 50% methanol/50% 10 mM NaCl in water. This effect was concentration-dependent and for the majority of samples, single mode distributions were observed at concentrations equal to or below 0.05%(w/w). For the E230G sample, this concentration limit was as low as 0.025%(w/w).

CONCLUSIONS

From the results presented and the accompanying discussions, the following conclusions can be drawn.

When all data are compared one must consider the differences in molecular weight, and in molecular weight distribution. The EHEC samples E411 and E230G have considerably higher molecular weights than the rest of the samples investigated. There is also some small variation in the polydispersity index, M_w/M_n, except that the HEC sample is considerably broader than the rest. It is only through the proper use of SEC that good polydispersity data can be obtained which helps considerably also as a general background for the judgement of data.

From Table 1, it is clear that values of DS and MS alone do not give a definite answer to the solution behaviour which results from a comparison of the four EHEC samples investigated. The importance of the substituent distribution was mentioned above. It seems, though, as if the DS values are more important for the solubility properties than the MS values. As long as on average, approximately two hydroxyl groups remain (i.e. DS~1) the cloud point (CP) stays fairly high for the EHEC samples. However, if we compare the CST-103 and the DVT-87014 samples, having an equal but low CP value, we know from preliminary results in our laboratory, that they differ in macroscopic behaviour when it comes to the interaction with other components like amphiphiles. This could be related to the difference in the value of the second virial coefficient, A_2, which indicates an almost non-existent excluded volume effect for the CST sample as compared to the DVT sample.

In the EHEC sample group one also notices that the sample with the highest CP, E230G, also having low values of DS and MS, has a small and possibly negative second virial coefficient indicating an attractive rather than a repulsive polymer–polymer interaction. This could perhaps be traced to a high probability of intra- and intermolecular hydrogen bond formation. This would fit with the observation that, in spite of a much higher molecular weight, the DLS size is smaller than that of the CST sample. Furthermore, the size decreases going from water to the more hydrophobic surroundings in the methanol/water mixture (50% methanol/50% 10 mM NaCl in water) and used as mobile phase (mp^*) in the SEC measurements. However, there is a difference, since for the CST sample it is only the intrinsic viscosity size that decreases, going from water to the methanol/water mixture. The DLS data indicate no observable change for CST but a considerable change for E230G, in the latter case almost in relation to the decrease in $[\eta]$. These observations probably indicate a difference in the polymer–solvent interaction for the two samples and hydrodynamic observations on the effect of polymer–amphiphile interactions have already shown such differences (Holmberg, 1995).

Noting that the MC sample with $M_w = 162{,}000$ has almost the same $[\eta]$ in water as E230G with $M_w = 535{,}000$, and in the methanol/water mixture the value of $[\eta]$ for MC is substantially higher than that of E230G, it is clear that the determination of $[\eta]$ itself is insufficient for a characterization and comparison of the samples. The same is true of the CST-103 and HEC samples which both have the same molecular weight. Unfortunately, even the universal calibration is questioned here which is evident from Fig. 8. This means that even the two-detector SEC system with RI and viscosity detection may be erroneous when used here for determination of molecular weights. It is believed that only SEC with a combined LS and RI detection, having the intrinsic advantage to remove some sample impurities, provides reliable molecular weights of these macromolecules. Static LS may also give a reliable molecular weight if all the impurities that might bias the output are properly removed.

The general discussion indicates that only a combination of chemical information (DS, MS, type of substituents), thermodynamic properties (CP, A_2), hydrodynamic parameters ($[\eta]$, k_H), geometric size parameters from scattering experiments (R_g, d), and molecular mass and size distributions will make it possible to provide sufficiently detailed information on the overall properties of complex polymers such as the substitution derivatives of cellulose, as required for many applications.

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REFERENCES

Berne, B.J. & Pecora, B. (1976). *Dynamic Light Scattering with Applications to Chemistry, Biology and Physics*. John Wiley and Sons, New York.
Brandrup, J. & Immergut, E.H. (1975). *Polymer Handbook*, 2nd edition. John Wiley and Sons, New York.
Burchard, W. (1983). *Adv. Polym. Sci.*, **48,** 1.
Chu, B. (1974). *Laser Light Scattering*. Academic Press, New York.
Daul, G.C. & Mitchell, R.L. (1971). In *Cellulose and Cellulose Derivatives*, Part V, eds N.M. Bikales & L. Segal. Wiley Interscience, New York, Chapter 19.
Dönges, R. (1990). *Br. Polym. J.*, **23,** 315.
Dubin, P.L. & Principi, J.M. (1989). *Macromolecules*, **22,** 1891.
Florence, A.T. & Attwood, D. (1988). *Physicochemical Principles of Pharmacy*, 2nd edition. Macmillan, London.
Flory, P.J. (1953). *Principles of Polymer Chemistry*. Cornell University Press, Ithaca, New York.
Fouissac, E., Milas, M., Rinaudo, M. & Borsali, R. (1992). *Macromolecules*, **25,** 5613.
Gelman, R.A. (1985). In *Cellulose and its Derivatives: Chemistry, Biochemistry and Applications*, eds J.F. Kennedy, G.O. Phillips, D.J. Wedlock & P.A. Williams. Ellis Horwood, Chichester, Chapter 25.
Greminger, G.K., Jr. & Krumel, K.L. (1980). In *Handbook of Water-Soluble Gums and Resins*, ed. R.L. Davidson. McGraw-Hill, New York, Chapter 3.
Grönwall, A. & Ingelman, B. (1945). *Acta Physiol. Scand.*, **9,** 1.
Grubisic, Z., Rempp, P. & Benoit, H. (1967). *J. Polym. Sci. B*, **5**, 753.
Guiseley, K.B., Stanley, N.F. & Whitehouse, P.A. (1980). In *Handbook of Water-soluble Gums and Resins*, ed. R.L. Davidson. McGraw-Hill, New York, Chapter 5.
Hammarström, A. & Sundelöf, L.-O. (1993). *Colloid Polym. Sci.*, **271,** 1129.
Holmberg, C. (1995). *Colloid Polym. Sci.*, submitted.
Holmberg, C., Nilsson, S., Singh, S.K. & Sundelöf, L.-O. (1992). *J. Phys. Chem.*, **96,** 871.
Huglin, M.B. (1972). *Light Scattering from Polymer Solutions*. Academic Press, London.
Jullander, I. (1957). *Ind. Eng. Chem.*, **49,** 364.
Kratky, O., Leopold, H. & Stabinger, H. (1973). In *Methods in Enzymology*, Vol. 27, eds C.H.W. Hirs & S.N. Timasheff. Academic Press, New York.
Kratochvil, P. (1987). Classical light scattering from polymer solutions. In *Polymer Science Library 5*, ed. A.D. Jenkins. Elsevier, Amsterdam.
Laurent, T.C. & Fraser, J.R.E. (1992). *FASEB J.*, **6,** 2397.
Lewis, K.E. & Robinson, C.P. (1970). *J. Colloid Interface Sci.*, **32,** 539.
Lindberg, B., Lindquist, U. & Stenberg, O. (1987). *Carbohydr. Res.*, **170,** 207; *Carbohydr. Res.*, **176,** 137.
Manley, R.St.J. (1956). *Arkiv Kemi*, **9**, 519.
Neely, W.B. (1963). *J. Polym. Sci. A*, **1**, 311.
Nilsson, S., Holmberg, C. & Sundelöf, L.-O. (1994). *Colloid Polym. Sci.*, **272,** 338.
Nilsson, S., Holmberg, C. & Sundelöf, L.-O. (1995). *Colloid Polym. Sci.*, **273,** 83.
Parfondry, A. & Perlin, A.S. (1977). *Carbohydr. Res.*, **57,** 39.
Patton, E.V., Wesson, J.A., Rubinstein, M., Wilson, J.C. & Oppenheimer, L.E. (1989). *Macromolecules*, **22,** 1946.
Porsch, B.J. (1993). *J. Chromatogr.*, **653,** 1.
Porsch, B.J. & Sundelöf, L.-O. (1994). *J. Chrom. A*, **669,** 21.
Porsch, B.J. & Sundelöf, L.-O. (1995). *Macromolecules*, submitted.
Pusey, P.N. (1973). In *Industrial Polymers: Characterization by Molecular Weight*, eds J.H.S. Green & R. Dietz. Transcripta Books, London, p. 26.
Regnier, F.E. & Noell, R. (1976). *J. Chromatogr. Sci.*, **14,** 316.
Sarkar, N. (1979). *J. Appl. Polym. Sci.*, **24,** 1073.
Vollmert, B. (1962). *Grundriss der Makromolekularen Chemie*. Springer, Berlin.
Weisberg, S.G., Simha, R. & Rothman, S. (1951). *J. Res. Natn. Bur. Stand.*, **47,** 298.
Zadorecki, P., Hjertberg, T. & Arwidson, M. (1987). *Makromol. Chem.*, **188,** 513.
Zimm, B.H. (1948). *J. Phys. Chem.*, **16,** 1099.