A new approach to characterising carboxymethylcelluloses by size exclusion chromatography

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Sodium carboxymethylcelluloses are analysed by size exclusion chromatography using three detectors online. First, the molecular weight distribution was established using either direct molecular weight determination or the universal calibration procedure. The agreement between the two series of data is good and seems to indicate that the K and a parameters of the Mark–Houwink relationship are not strongly affected by the degree of substitution (DS) at least in the range tested. In addition, it was demonstrated that dn/dc is independent of DS. The amount of salt rejected depends on the quantity of polymer injected and on its charge densities. A quantitative analysis of salt rejection was performed for three samples. The charge distribution was superimposed with the molecular weight distribution. The conductimetric detector was shown to be valuable for describing charge distributions only for low charge parameter ($\lambda < 1$). For the three samples tested we showed that the charge density does not change with molecular weight as reflected by the constancy of the ratio between the conductimetric and refractometric signals.

INTRODUCTION

Carboxymethylcelluloses, like many other polysaccharide derivatives, are very difficult to characterise due to their heterogeneity not only in molecular weight (M_w) but also in chemical composition. Size exclusion chromatography (SEC) allows the determination of the molecular weight distribution when a light scattering detector is used for direct molecular weight determination. The use of the universal calibration may be questionable when the hydrodynamic volume or the parameters K and a in the Mark–Houwink relationship depend on the degree of substitution (DS). Until now, the distribution of the carboxymethyl substituents on —OH—2, 3 or 6 positions has been obtained by NMR measurements (Zeller *et al.*, 1991) without relation to the molecular weight distribution.

The aim of this paper is to discuss the SEC experimental conditions to get information on the charge density of the different molecular weight molecules; for this purpose a conductimetric and a concentration detector were used.

EXPERIMENTAL

Commercial sodium carboxymethylcellulose (NaCMC) samples of various average MW ($\overline{M_w}$) and DS were purified by precipitation (Rinaudo & Milas, 1978).

Two different SEC separation systems were used.

(A) a simple SEC instrument composed of the pump, injector, differential refractometer (Waters, Milford, MA, USA), conductimetric detector (Wayne Kerr B642, Bognor Regis, UK) and two microcolumns Separon HEMA mono C60 G65 (Tessek, Prague, Czechoslovakia).

(B) a liquid chromatograph Waters 150 C with three detectors: differential refractometer (inbuilt), online viscometer (stainless-steel capillary equipped with two pressure detectors) and a low-angle light-scattering detector (LALLS, Chromatix CMX 100) equipped with two Shodex OH Pak B 804 and B 805 columns (Tinland *et al.*, 1988).

The eluent in both instruments was 0·1 M NH_4NO_3 for the determination of the molecular weight distribution

(MWD) and 10^{-4} M NH_4NO_3 for measurement of the charge distribution using system A.

The conductimetric and refractometric signals have been calibrated. It was found that the unit used in standard conditions adopted in this work for the conductimetric signal area of the salt, $\Delta\chi_s$, corresponds to 3·78 × 10^{-7} equivalent of NH_4NO_3 and the unit of the refractometric signal area for the polymer, Δn_p, corresponds to 0·4 mg of CMC injected.

MWD

The samples of NaCMC were dissolved in water and filtered through a 0·2 μm filter before injection on the SEC equipment. First, it was demonstrated that the value of dn/dc is independent of the $\overline{DS}$ in the range investigated; for that purpose different quantities of materials were injected and the areas of the refractometric peaks Δn_p were determined (Fig. 1). A unique straight line was obtained in 10^{-4} M NH_4NO_3. It was assumed that this property is preserved in 0·1 M NH_4NO_3 and $dn/dc = 0·145$ was adopted for all samples (Brown et al., 1964).

M_w and MWD values were calculated using the universal calibration curve made with commercial fractions of poly(maltotriose) (PMT) (Polymer Laboratories, Church Stretton, Shropshire, UK). The Mark-Houwink constants for PMT of $K = 0·0262$ ml/g and $a = 0·654$ used for the construction of the universal calibration curve were determined for the same eluent. The Mark-Houwink constants for NaCMC with $\overline{DS} = 1·37$, were $K = 0·01384$ ml/g and $a = 0·84$, determined using system B. The plot of log $[\eta]$ versus log M_w is shown in Fig. 2.

The $(\overline{M_w})_A$, $(\overline{M_w})_B$ and $[\eta]_B$ values were calculated from the data obtained from both systems (Table 1). Table is is completed using values of $[\eta]_F$ determined separately with an Ubbelohde viscometer coupled to a Fica Viscomat automatic counter. The relatively good
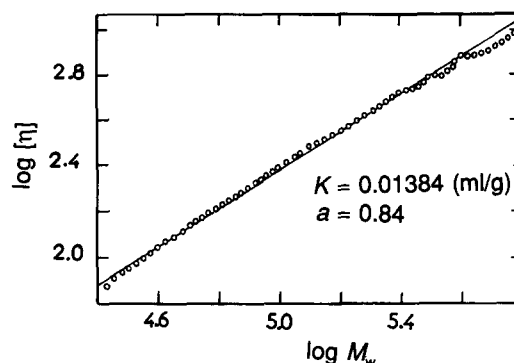

Fig. 1. Refractometric signal for the polymer (Δn_p) as a function of weight of material injected (w expressed in mg).

Fig. 2. Logarithm of intrinsic viscosity as a function of logarithm of M_w of NaCMC with $\overline{DS} = 1·37$ (experimental conditions B).

Table 1. Comparison of SEC and viscometric data[a]

Sample	$\overline{DS}$	$(\overline{M_w})_A$	$(\overline{M_w})_B$	$[\eta]_B$ (ml/g)	$[\eta]_F$ (ml/g)
1	1·00	55 930	55 830	118	—
2	1·01	233 440	226 050	559	—
3	1·08	247 510	289 210	427	414
4	1·37	115 510	101 680	177	162
5	2·00	550 830	578 580	790	—
6	2·35	56 840	57 240	63	65
7	2·90	41 310	—	—	43

[a] A, B — assignment of SEC system; F — viscometer Fica.

agreement found between $(\overline{M_w})_A$ and $(\overline{M_w})_B$ indicates that there is little influence, if any, of the DS on the K and a parameters of the Mark-Houwink relationship in the range of DS tested.

Salt rejection

The chromatogram has a characteristic form when a polyelectrolyte is eluted in a low salt content eluent (Fig. 3). The conductimetric signal consists of two parts: one corresponding to the polymer elution and the other to excluded salt elution for higher elution volume. This excluded salt was interpreted as coming from a Donnan equilibrium established in the pores of the column filling (Lindström et al., 1977; Domard et al., 1979; Rinaudo & Desbrières, 1980; Rochas et al., 1980). The amount of salt can be calculated using

$$Q_s = Q_p \frac{\phi}{4} \tag{1}$$

where Q_s is the number of salt equivalents excluded when Q_p (the number of carboxylic groups of polymer) is eluted; ϕ is the osmotic coefficient of the counterions.

Figure 4 displays the area of the salt peak obtained by the conductivity detector ($\Delta\chi_s$) as a function of the
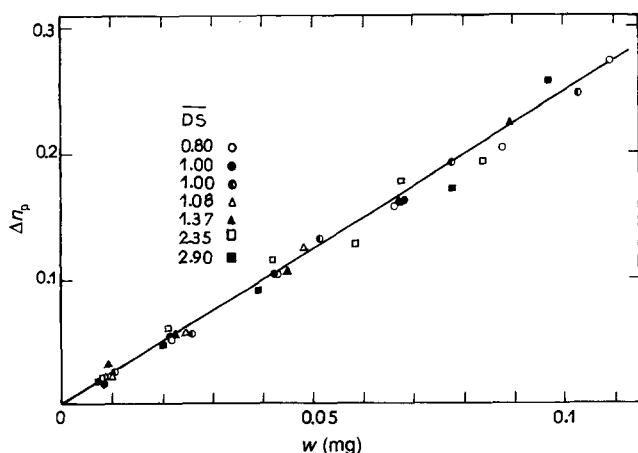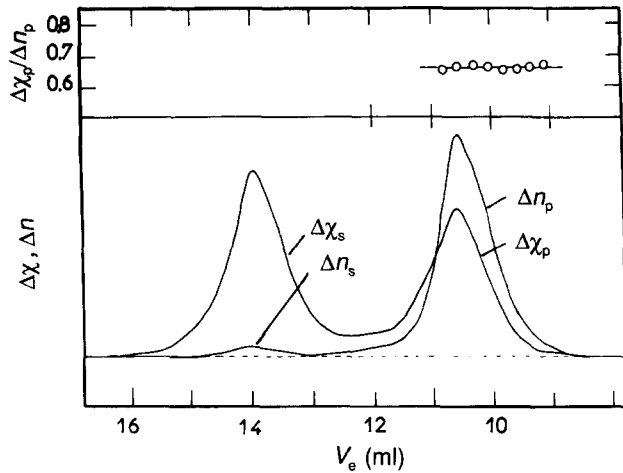
Fig. 3. Chromatogram obtained with NaCMC with $\overline{DS} = 1$ using conductimetric and refractometric detectors (eluent: 10^{-4} M NH_4NO_3, volume injected: $10\,\mu l$ of 5·155 g/litre).
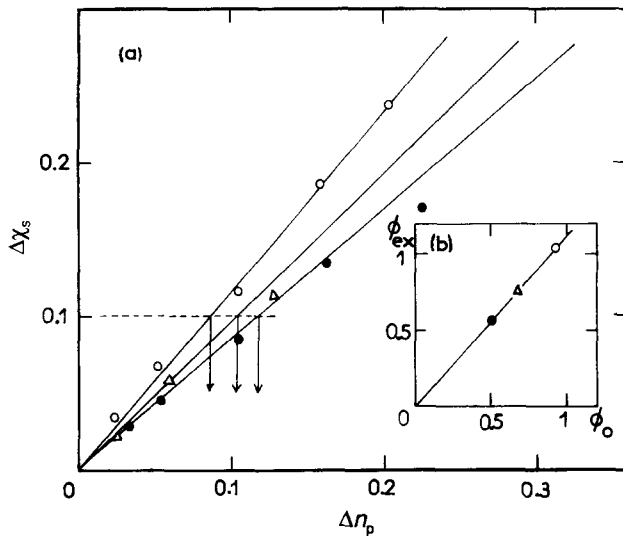

Fig. 4. (a) Conductimetric signal area ($\Delta\chi_s$) of excluded salt peak as a function of the refractometric signal area of the polymer (Δn_p) for different $\overline{DS}$: (O) $\overline{DS} = 0\cdot80$; ($\triangle$) $\overline{DS} = 1\cdot08$; ($\bullet$) $\overline{DS} = 1\cdot37$. Arrows indicate Δn_p needed for the determination of Q_p. (b) Comparison of experimental and theoretical values of the osmotic coefficient.

area of the refractometric signal for the polymer (Δn_p) for different quantities of polymer injected and different $\overline{DS}$. The conductimetric signal was calibrated by elution of well-defined salt samples and a value of Q_s could be thus determined from the $\Delta\chi_s$ area. From the characteristic curve relating Δn_p to the weight, w, of material injected (independent of $\overline{DS}$) (Fig. 1), the value of Q_p is deduced for each Δn_p. Q_p must be expressed as a number of carboxylic groups injected according to the following relationship:

$$Q_p = \frac{w\,\overline{DS}}{162 + 80\,\overline{DS}} \qquad (2)$$

Table 2. Relationship between salt rejection and polymer injected

Sample	$\overline{DS}$	$Q_s \times 10^8$ (equiv.)	$Q_p \times 10^7$ (equiv.)	ϕ_{ex}	$\phi_0{}^a$	ϕ_M
1	0·80	3·78	1·30	1·15	0·93	0·54
2	1·08	3·78	1·97	0·76	0·68	0·41
3	1·37	3·78	2·72	0·56	0·51	0·32

^{a}Experimental activity coefficients of Na^+ were found to be in good agreement with the values predicted by the Oosawa theory (Oosawa, 1971); the values of ϕ_0 given in this table are estimated for infinite dilution.

The values of Q_p obtained for the same Q_s excluded ($\Delta\chi_s = 0\cdot1$) are given in Table 2 for three NaCMC samples using Figs 1 and 4 and eqn (2) to express successively Δn_p, w and finally Q_p.

It is necessary to point out that the refractometric signal for the salt is much lower than for the polymer and thus the Δn_p signal of the polymer is assumed not to be perturbed by the salt effect.

From these data the direct relationship between the salt rejection and the nature and content of the polyelectrolyte injected is confirmed. Following eqn (1) $4Q_s/Q_p$ is estimated which must be equal to ϕ, the osmotic coefficient; these ratios are compared with the experimental data obtained previously (Rinaudo, 1974) using the Oosawa (1971) theory (ϕ_0) (Fig. 4(b)) and the Manning theory (ϕ_M) (Manning, 1974) (Table 2). The experimental data are in relatively good agreement with theoretical predictions (ϕ_0).

These results prove again that the salt exclusion is directly related to the quantity of polymeric charges injected.

Charge distribution in the polyelectrolyte

Following previous work (Rinaudo & Desbrières, 1980), a conductimetric detector was introduced in addition to the refractometric detector to determine the charge distribution and to superpose this with the MWD. Considering the Donnan exclusion when the polymer is eluted, the conductivity of the polymer peak to a first approximation is the sum of two contributions:

- a positive contribution of the polyelectrolyte corresponding to the content of polymeric charges; and
- a negative contribution due to the exclusion of salt (the corresponding area of this conductimetric contribution is equal to that of the salt rejected).

$$\Delta\chi_p = k\left(fQ_p\Lambda_p - \frac{\phi Q_p}{4}\Lambda_{el}\right) \qquad (3)$$

in which $\Delta\chi_p$ is the area of conductimetric signal produced by Q_p equivalents of polymer injected; f is the transport coefficient, ϕ the osmotic coefficient, Λ_p and

Λ_{el} are the equivalent conductivities of the polyelectrolyte and eluent, respectively. Assuming in the first approximation that the Λ values are equal then $\Delta\chi_p$ becomes directly proportional to $f - (\phi/4)$. As $f > \phi$, $\Delta\chi_p$ is always positive but its dependency on DS is based on the dependency of $(f - \phi/4)$ on DS.

Equation (3) may be rearranged to be expressed as a function of weight of the material injected (w). In addition, the area of the refractometric signal Δn_p is proportional to this quantity, i.e.

$$\Delta n_p = k' w \tag{4}$$

then, using eqn (2) and still assuming $\Lambda_p = \Lambda_{el}$, the following is found:

$$\frac{\Delta\chi_p}{\Delta n_p} = k'' \left(\frac{DS}{162 + 80\,DS}\right)\left(f - \frac{\phi}{4}\right) = k''Z \tag{5}$$

where $k'' = \Lambda_p(k/k')$ and Z is introduced for convenience later in this paper. This treatment is proposed for each fraction of material with a determined DS; the same relationship can also be adopted for each elution volume i, $\Delta\chi_{pi}/\Delta n_{pi}$, to analyse the chromatograms (Fig. 3) assuming that each fraction corresponds to a given DS.

From the Manning theory (Manning, 1974), the following expressions are obtained for f and ϕ:

$$\text{for}\,\lambda < 1: \quad f = 1 - \frac{0.55\lambda^2}{\lambda + 3.14}; \quad \phi = 1 - \frac{\lambda}{2}$$

$$\text{for}\,\lambda > 1: \quad f = \frac{0.87}{\lambda}; \qquad \phi = \frac{1}{2\lambda}$$

where λ is the charge parameter with

$$\lambda = \frac{ve^2}{hDkT}$$

proportional to the charge density v/h (where v is the number of charge on a chain with contour length h, e is the electronic charge, D is the dielectric constant and kT the Boltzmann term).

As the charge parameter λ of the CMC is directly related to DS by the relationship $\lambda = 1.38\,DS$ at $25°C$, f, ϕ and the function Z (Rinaudo, 1974)

$$Z = \frac{DS}{162 + 80\,DS}\left(f - \frac{\phi}{4}\right) \tag{6}$$

depend directly on DS and consequently, from eqn (5) the ratio $\Delta\chi_p/\Delta n_p$ becomes a function of DS (Fig. 5). Therefore, a change in the ratio $\Delta\chi_p/\Delta n_p$ corresponds to a change in DS.

From the variation of Z with DS shown in Fig. 5, it can be concluded that the conductivity detection must be very useful when $\lambda < 1$, since the ratio $\Delta\chi_{pi}/\Delta n_{pi}$ increases when DS increases (up to DS = 0.72); over $\lambda = 1$, since both $f \sim \lambda^{-1}$ and $\phi \sim \lambda^{-1}$, $\Delta\chi_{pi}/\Delta n_{pi}$
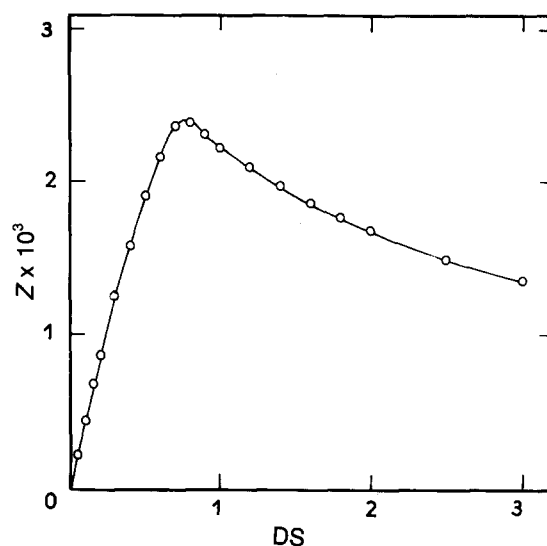

Fig. 5. Representation of calculated Z values proportional to the ratio $\Delta\chi_{pi}/\Delta n_{pi}$ as a function of DS.

decreases as $(162 + 80\,DS)^{-1}$. This is a serious limitation of this technique.

From Fig. 3, it is demonstrated that the ratio $\Delta\chi_{pi}/\Delta n_{pi}$ for each point is nearly constant which indicates a regularly charged polymer.

It has previously been shown that even where the eluent was 10^{-4} M the elution volume has the usual molecular weight dependency (Rinaudo & Desbrières, 1980). If it is assumed that the electrostatic elution is screened to a large extent, then it can be said that the chromatogram is related to the MWD even if not absolute analysis may be performed. Then, SEC using these two detectors allows the description of the charge density of the chains superposed with the MWD (at least for DS < 0.72).

CONCLUSION

The use of size exclusion chromatography can be very useful in characterising CMC. This paper shows that the molecular weight distribution obtained using the universal calibration procedure is in good agreement with a direct MWD. The DS of the CMC does not greatly affect these determinations. With a low concentration of salt in the eluent, it is shown that a conductimetric detector can be valuable in describing the charge distribution only for low substitution degree (DS < 0.7) of the CMC, which corresponds to the usual range for commercial samples.

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