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SEC of mono-carboxymethyl cellulose (CMC) in a wide range of pH; Mark–Houwink constants

T.E. Eremeeva*, T.O. Bykova

Institute of Wood Chemistry, 27 Dzerbenes str., LV-1006, Riga, Latvia

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Abstract

A method for determination of carboxymethyl cellulose (CMC) molecular weight (M_W) and chemical heterogeneity (degree of oxidation (DO)) using a bi-detector HPSEC (UV-detector online with refractometer) has been developed. It has been found that the use of 0.5 N NaOH or 0.4 M acetate buffer as the eluent ensures CMC separation according to M_W. It has been revealed that the universal calibration for the polyelectrolyte CMC and the neutral polymer dextran is valid under the conditions applied. The Mark–Houwink equations for CMC in 0.5 N NaOH and 0.4 M acetate buffer have been estimated to be $[\eta] = 5.37\text{x}10^{-4} M_W^{0.73}$ and $[\eta] = 2.24\text{x}10^{-4} M_W^{0.83}$ dl g^{-1}, respectively. The equation $\log K = 1.64 - 4.00\alpha$ ml g^{-1} for CMC has been estimated. An approach for determining DO from adsorption at 290 or 313 nm has been developed.

Keywords: Carboxymethyl cellulose; SEC; Polyelectrolyte effects; Mark–Houwink constants; Universal calibration; Degree of oxidation

1. Introduction

Carboxymethyl cellulose (CMC) has a wide range of applications, therefore determination of it's molecular weight distribution (MWD) is important. The SEC method continues to be particularly useful for MWD analysis of water-soluble polymers, including CMC. Substantial numbers of papers have been published, on aqueous SEC of CMC (Eriksson et al., 1968; Barth and Regnier, 1980, 1993).

Some difficulties were faced in the MWD analysis of CMC by SEC because of the polyelectrolyte properties of this polymer. Much effort has been made to suppress the polyelectrolyte expansion in solution and the secondary polyelectrolyte effects such as ion exclusion by varying the ionic strength and/or pH of the mobile phase and using different types of columns. Buytenhuys and Van der Maeden (1978) found that 0.5 M Na acetate buffer was required to eliminate these effects on high-resolution LiChrosphere columns. Barth and Regnier (1980) evaluated 1.0 M phosphate (pH 6–7) as well as 0.7 M acetate buffer (pH 3.7) for Synchropak and glucerylpropylsilyl derivatized LiChrosphere columns. The SEC analyses of Na CMC using 0.5–0.7 M Na acetate buffer as the eluent on TSK HW 55 and TSK SW was performed by Hamacher and Sahm (1985) and Barth and Regnier (1993). The use of an agarose gel and Sephadex with a strong alkaline eluent cadoxen (pK of ca. 13) has also been reported (Eriksson et al., 1968).

It is important to realise that SEC is a relative technique that requires column calibration in order to determine the MWD of polymers. This is generally achieved by a so-called universal calibration procedure which uses Mark–Houwink coefficients (K and α) derived from both the polymers under investigation and calibration. This procedure was applied by Rinaudo et al. (1993) for CMC samples characterisation by M_W using 0.1 M NH_4NO_3 as the eluent and two different SEC column systems: set of Separon HEMA mono C60 + G65 microcolumns and set of Shodex OH Pak B 804 + 805 columns. However, the elimination of polyelectrolyte effects using eluent of low ionic strength was not experimentally confirmed.

It is well known that the accuracy of MWD data obtained from such a calibration method can be quite variable. It is ultimately dependent on the correctness of the Mark–Houwink constants K and α for the polymer/solvent combination, especially in the case of polyelectrolytes whose hydrodynamic properties are strongly effected by the solvent ionic strength. The Mark–Houwink constants for CMC in cadoxen (Phillipp et al., 1987; Brown et al., 1963), NaCl solutions (Brown and Henley, 1964; Kurata

* Corresponding author

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et al., 1975), 1.5 N NaOH (Schurz et al., 1956) and in 0.1 M NH_4NO_3 (Rinaudo et al., 1993) were available from the literature. However, cadoxen and 1.5 N NaOH are extremely aggressive eluents (pK > 13) for chromatography equipment and, therefore, their application in the SEC practice is rather limited. The constants in salt solutions were estimated at a low ionic strength, while to suppress the polyelectrolyte expansion of CMC macromolecules the ionic strength of solution must be of ca. 0.2 M (Brown et al., 1964). Moreover, CMC formed molecular associates in salt solutions (Gruber, 1979). Unfortunately, Mark–Houwink constants in acetate or phosphate buffer suitable as eluents for SEC of CMC were not reported.

Thus, notwithstanding the good results obtained with the suppression of polyelectrolyte effects during SEC of CMC, the validity of universal calibration and the Mark–Houwink constants for CMC in solvents suitable as eluents continues to be questionable. The present work deals with the development SEC analyses of MW and chemical heterogeneity of CMC. The universal calibration and the Mark–Houwink constants are the aim of this paper.

2. Experimental

2.1. Chemicals and materials

Reagent grade sodium hydroxide, acetic acid, sodium acetate and double distilled water were used. Dextran standards T10, T20, T40, T70, T170, T500 and T2000 were from Pharmacia (Fine Chemicals A B, Uppsala, Sweden). Industrial CMC samples with a degree of substitution of 0.7–0.8 and a DP_V of 700 and 450 were used. Samples of different M_W were obtained by degradation as described by Ozolinsh and Pernikis (1989). Carbonyl groups' contents were determined by iodometry (Kuznetsova, 1981).

2.2. SEC conditions

Chromatographic analyses were performed on a liquid chromatograph GPC (Laboratory Instruments, Prague, Czech Republic) with a refractometric detector in line with a UV-detector. Separon HEMA 1000 (10 μm) prepacked stainless-steel column (250x8 mm I.D.) (TESSEK, Czech Republic) was used. An aqueous 0.5 N NaOH as well as an acetate buffer (0.2 M sodium acetate/0.2 M acetic acid) were used as the solvent and the eluent. 25 μl of 0.2% CMC solution was injected. The analyses were carried out at ambient temperature with an eluent flow-rate of 0.5 ml min^{-1}. It should be noticed that CMC in NaOH solution was stable for at least 6 h. The chromatograms obtained immediately after a full dissolution time of ca. 10 min were identical to those obtained after storage for 6 h in basic media.

2.3. Viscometry

Intrinsic viscosity determination was carried out with an Ubbelohde viscometer at 25 ± 0.05°C in 0.5 N NaOH and in 0.4 M acetate buffer.

3. Results and discussion

3.1. Elution behaviour in water

The curves presented in Fig. 1 illustrate the elution behaviour of a CMC using water as the eluent. The chromatogram is dependent on the concentration. With decreasing injected concentration from 0.5% to 0.05%, the elution volumes V_e decrease. This effect is caused by the polyelectrolyte expansion of macromolecules in solution. Moreover, samples of different M_W eluted at the void volume V_o of the column as determined by dextran T2000. This phenomenon may be explained by ion exclusion, i.e. by electrostatic repulsion of a charged polyion from a similarly charged packing. The co-operative action of coil expansion and ion exclusion are responsible for earlier elution of CMC from the column and the interference of their separation by M_W. This elution behaviour of CMC in water is typical for polyelectrolytes in polar solvents of a low ionic strength. These polyelectrolyte effects for water-soluble polymers during SEC were described by Barth (1980).

3.2. SEC in alkaline conditions

Bao et al. (1980) shown that 0.5 N NaOH was very useful

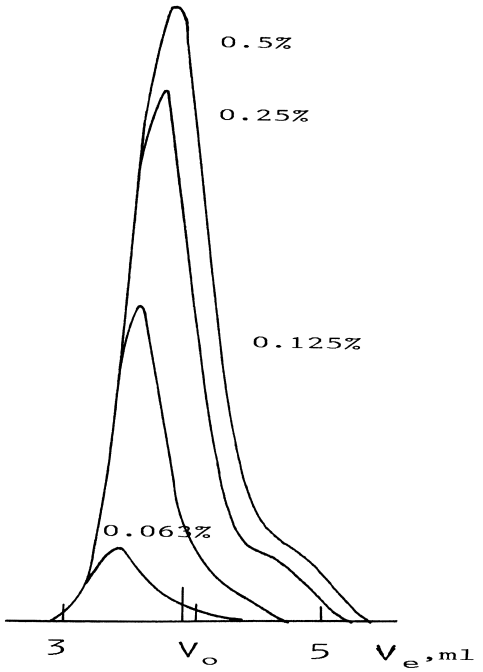

Fig. 1. Effect of injected CMC (M_V = 8000) concentration on elution volume using water as eluent.

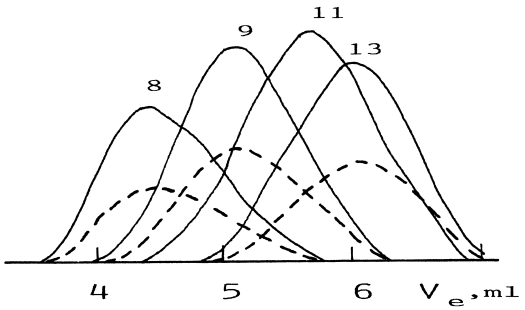

Fig. 2. Experimental chromatograms of CMC samples of M_V: 8-98 000; 9-34 000; 11-17 000; 13-8000 (numbers as in Table 1) using 0.5 N NaOH as eluent. ——, 0.2%; – – –, 0.1% solution.

as the mobile phase for alkali-soluble polysaccharides analysis. In our previous work (Eremeeva and Bykova, 1993), it was established that the separation of charged and neutral hemicelluloses on Separon HEMA 1000 using 0.5 N NaOH as eluent followed their M_W without any non-exclusion effects. Hence, to eliminate the polyelectrolyte effects observed with CMC on this packing, 0.5 N NaOH was used as the eluent. Applying the same SEC conditions for CMC we pursued the additional objective of unifying SEC conditions for different cellulosics and related polysaccharides.

Fig. 2 shows that neither concentration effects nor aggregate formation were found with CMC under these conditions. All CMC samples have monomodal and nearly symmetrical distribution curves. The samples elute according to their M_W determined by viscometry. The relationship between V_e and M_W was established using CMC fractions. The M_W and elution characteristics of calibration standards and CMC fractions are listed in Table 1. The calibration curve for CMC presented in Fig. 3 is linear over the range from 5 kDa to 100 kDa.

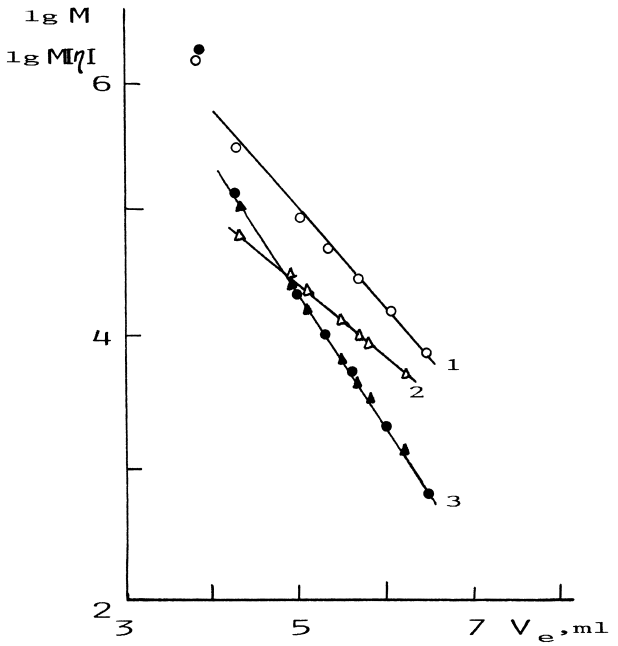

Fig. 3. Calibration graphs log M vs. V_e of Separon HEMA 1000 column with dextran (1), CMC (2), and universal calibration log $M[\eta]$ vs. V_e (3) for CMC (▲) and dextran (●) in 0.5 N NaOH.

Characterised fractions are not easily available, therefore we examined the applicability of the universal Benoit's calibration log $M[\eta]$ vs. V_e, using dextran standards as calibrants. The calibration curve obtained (Fig. 3) is linear from 8 kDa to 500 kDa. To construct the universal calibration curve the parameter log $M[\eta]$ for dextran were calculated using the Mark–Houwink constants $K = 1.32 \times 10^{-3}$ and $\alpha = 0.478$ given by Bose et al. (1982). The experimental intrinsic viscosity $[\eta]$ values for CMC in 0.5 N NaOH were

Table 1
Molecular mass and elution characteristics of dextrans and CMC samples

N	Sample	$M_V \times 10^3$	$M_p^a \times 10^3$	$[\eta]$	Elution volume V_e (ml)	
				(dl g^{-1})	0.5 N NaOH	0.4 M acetate buffer
1	DT 2000	2000	1414	1.40[b]	3.87	4.03
2	DT 500	506	310	0.72[b]	4.58	4.83
3	DT 170	168		…	…	5.33
4	DT 70	66.3	49.1	0.27[b]	5.33	5.80
5	DT 40	39.4	30.4	0.21[b]	5.67	6.20
6	DT 20	20.9	17.1	0.15[b]	6.15	…
7	DT 10	9.5	6.8	0.11[b]	6.37	7.10
8	CMC 450	98.0[c]	65.9	2.20[d]	4.33	4.80
9	CMC 250	48.2[c]	30.8	1.26[d]	4.95	5.38
10	CMC 170	34.0[c]	23.2	0.98[d]	5.08	5.63
11	CMC 100	20.0[c]	14.1	0.67[d]	5.50	…
12	CMC 85	17.0[c]	11.3	0.60[d]	5.71	6.20
13	CMC 70	14.0[c]	9.3	0.52[d]	5.83	…
14	CMC 40	8.0[c]	5.6	035d	6.25	6.67

[a] Peak M_W, calculated as $(M_W \times M_n)^{1/2}$.
[b] Solvent 0.4 M acetate buffer.
[c] Viscose-average M_W.
[d] Solvent 0.5 N NaOH.

used to determine the log $M[\eta]$ of this polymer. As can be seen from Fig. 3, the calibration by universal parameters for dextran and CMC is valid. In some papers (Bose et al., 1982; Fishman et al., 1984), it has been suggested that a correction coefficient to Benoit's calibration has to be introduced as for polyelectrolytes. Our results have shown that this is not required for CMC under alkaline conditions.

3.3. SEC in acetate buffer (pH 5)

An acetate buffer of high ionic strength is usually used as the eluent for the MW analysis of CMC. In the present work the applicability of this buffer with a Separon HEMA column has been investigated.

Fig. 4 shows the effects of the Na acetate buffer ionic strength on the CMC elution behaviour. The elution volume of samples increased until the ionic strength of the eluent reached 0.4 M. The chromatograms are characterised by a nearly symmetrical profile, using a 0.4 M mobile phase (except for the first high M_W sample partly excluded from the column). The samples eluted in accordance with their M_W. The concentration effects were absent under these conditions, as well. This indicates that the polyelectrolyte effects during SEC are eliminated, and the ionic strength of Na acetate buffer equal to 0.4 M is required to achieve the separation of CMC by M_W on Separon HEMA. The calibration curve for CMC presented in Fig. 5 is linear over the range from 100 kDa to 5 kDa.

However, it should be noted that CMC associates in buffer solution in contrast to the 0.5 N NaOH solution.

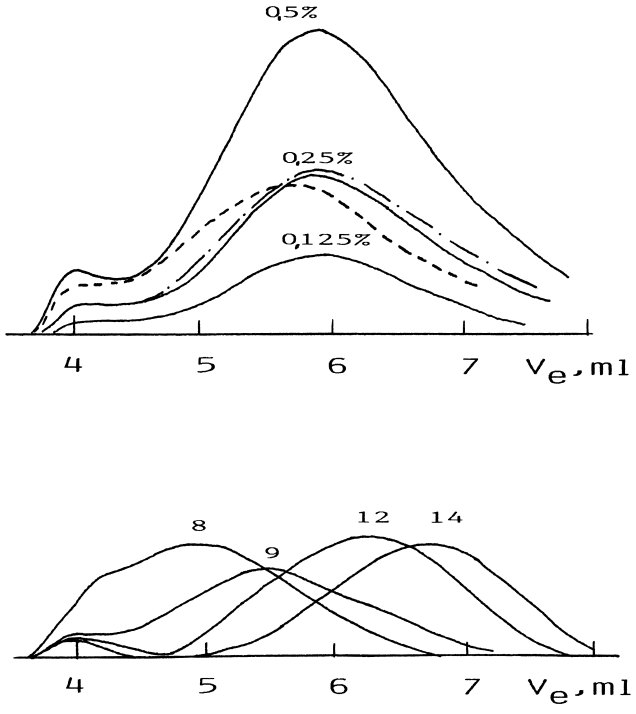

Fig. 4. Elution behaviour of CMC samples in acetate buffer. - - -, 0.3 M; ————————, 0.4 M; – · –, 0.45 M; numbers as in Table 1.

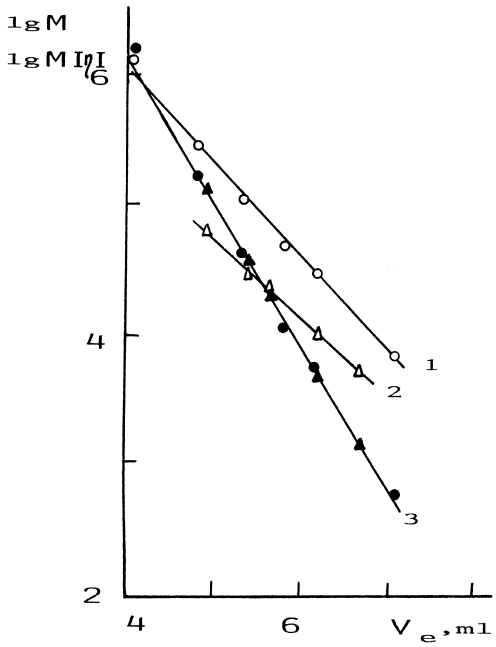

Fig. 5. Calibration of the Separon HEMA 1000 column in 0.4 M acetate buffer pH 5.0 (symbols as in Fig. 3).

The associates tend to elute in the void volume of the column and appear as a small prehump on the chromatograms (Fig. 4). The preparation of aggregate-free solutions of water-soluble polysaccharides is a generally known problem (Rinaudo, 1989). The SEC technique in contrast to other methods allows M_W parameters to be obtained omitting the prehump on the chromatogram. The data summarised in Table 2 illustrate that the M_W values obtained in the acetate buffer when this prehump is omitted show good agreement with that determined in alkali.

To evaluate the universal calibration for acetate buffer conditions, the Mark–Houwink constants for CMC, $K = 2.24 \times 10^{-4}$ dl g^{-1} and $\alpha = 0.83$, were calculated using the procedure described by Dobkowski (1984). The Mark–Houwink equation for dextran was obtained by viscometry:

$$[\eta] = 13.8 \times 10^{-4} M^{0.48} \text{ dl g}^{-1}$$

The plot of log $M[\eta]$ vs. V_e depicted in Fig. 5 shows that the universal calibration principle between CMC and dextran in acetate buffer is valid.

As regards the elution behaviour of dextrans, no complications were met. However, dextrans in the acetate buffer elute somewhat later than in NaOH (Table 1), whilst the Mark–Houwink equation evaluated in the acetate buffer is practically identical to that in NaOH, i.e. the dextran hydrodynamic volume is almost the same in both the solvents. The observed increase in V_e is obviously due to varying the void and total pore volumes of the column. Fig. 6 shows that the separation of CMC and dextran by distribution coefficients $k_{AV} = (V_e - V_o)/(V_t - V_o)$ in different solvents is in good agreement with each other. This curve additionally

Table 2
Comparison of the MM parameters of CMC samples determined in 0.5 N NaOH and 0.4 M acetate buffer

N	Carbonyl	0.5 N NaOH			0.4 M acetate buffer		
	content (%)	M_w	M_n	M_w/M_n	M_w	M_n	M_w/M_n
1	0.08	92 200	41 430	2.20	93 800	42 140	2.10
2	0.23	30 640	14 550	2.11	29 910	14 260	2.10
3	0.50	19 370	9810	1.97	19 890	9700	2.05
4	0.84	12 900	6820	1.89	13 460	7010	1.92
5	1.20	7630	4310	1.77	7400	4260	1.73

confirms that undesirable interactions between CMC and packing are eliminated in both the solvents.

3.4. Mark–Houwink constants

In order to apply the universal calibration approach to M_W analysis, the Mark–Houwink constants of the analysed polymer and the calibrant are necessary. Those for dextran in both the solvents under investigation and CMC in the acetate buffer were cited above. The equation obtained by the double logarithmic plot of $[\eta]$ in 0.5 N NaOH vs. M_W for CMC is as follows:

$$[\eta] = 5.37 \times 10^{-4} M_w^{0.73} \text{ dl g}^{-1}$$

It was assumed (Brown et al., 1964) that CMC macromolecules in aq. NaOH and cadoxen solutions would have the same hydrodynamic properties. Indeed, some insignificant deviations of K and α values are observed comparing the data of the present work with those obtained by Brown et al. (1963, 1964) (Table 3). However, it should be noted that there is a discrepancy between the constants for the same solvent cadoxen determined by different authors. Thus, Brown et al. (1963) established a value of 0.73 for the exponent, whereas Phillipp et al. (1987) found a higher α value of 1.0. To re-examine the reliability of Mark–Houwink constants, a relationship between K and α has been used. log K has been found by Aharoni (1977) to be directly proportional to α for the same polymer in different solvents over a wide range of temperatures.

Fig. 7 shows that the data of the K–α pairs given in the literature for CMC in different solvents fall on a straight line including the ones evaluated in the present work. The only exception is the data of Phillipp et al. (1987) for CMC in cadoxen. A linear regression analysis of the experimental data listed in Table 3 was carried out giving:

$$\log K = 1.64 - 4.00\alpha \text{ ml g}^{-1}$$

It should be noted that the log $K(\alpha)$ dependencies were evaluated by Aharoni mainly for neutral polymers and those for polyelectrolytes were not widely investigated. Although a number of α values for CMC were higher than 0.8 (Table 3) and were evaluated under conditions of a partially suppressed polyelectrolyte expansion of CMC

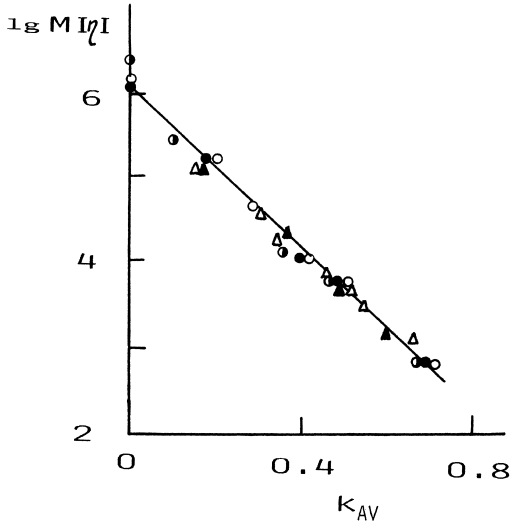

Fig. 6. Plot of log $M[\eta]$ vs. k_{AV} for Separon HEMA 1000 column. ●,DT/NaOH; ○, DT/acetate buffer; ◑, DT/H_2O; ▲, CMC/NaOH; △, CMC/acetate buffer.

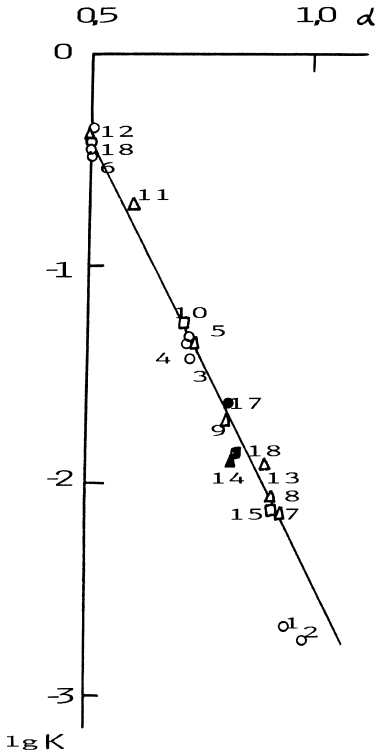

Fig. 7. Relationship between the Mark–Houwink constants log K vs. α for CMC (numbers as in Table 3).

Table 3
Mark–Houwink constants for CMC in different solvents

N	Solvent	$K \times 10^{-3}$ ($cm^3\ g^{-1}$)	α	DS	References
1	Cadoxen	2.14	0.96	0.9	Philipp et al., 1987
2	Cadoxen/water 1:1	0.193	1.00	0.9	Philipp et al., 1987
3	Cadoxen	3.68[a]	0.73	0.95	Brown et al., 1963
4	Cadoxen	4.22[a]	0.73	0.44	Brown et al., 1963
5	Cadoxen	4.55[a]	0.73	0.21	Brown et al., 1963
6	Cadoxen	35.0	0.50		Brown et al., 1963
7	NaCl 0.005 M	0.72	0.95	1.06	Brown and Henley, 1964
8	NaCl 0.01 M	0.81	0.92	1.06	Brown and Henley, 1964
9	NaCl 0.05 M	1.90	0.82	1.06	Brown and Henley, 1964
10	NaCl 0.2 M	4.30	0.74	1.06	Brown and Henley, 1964
11	NaCl infinite M	19.00	0.60	1.06	Brown and Henley, 1964
12	NaCl	42.00	0.50	1.06	Brown and Henley, 1964
13	NaCl 0.1 M	1.23	0.91	…	Kurata et al., 1975
14	HCl 4 M	1.19	0.83	…	Kurata et al., 1975
15	NaOH 1.5 M	0.73	0.93	…	Schurz et al, 1956
16	NaOH 0.5 M	5.37	0.73	0.76	Present work
17	Acetate buffer 0.4 M	2.24	0.83	0.76	Present work
18		43.6	0.50		Present work
19	NH_4NO_3 0.1 M	1.38	0.84	1.37	Rinaudo et al., 1993

[a] Recalculated from $[\eta] = K'DP^{\alpha}$ with taking into account the DS.

macromolecules in solution, the Aharoni equation, $\log K = A - B\alpha$, is valid for polyelectrolytes, as well. A value of 0.436 ml g^{-1} for K_θ, which is invariant for the given polymer, was obtained by the extrapolation of $\log K$ vs. α to $\alpha = 0.5$. This value is in good agreement with that determined by Brown et al., (1963, 1964) from hydrodynamic theory (Table 3). Thus, each of the K–α pairs that are on this straight line (of course, under the theory in force) may be considered as reliable and recommended for M_W calculation.

3.5. Degree of oxidation

SEC is a powerful technique for polymer investigation allowing a simultaneous estimation of M_W and chemical non-uniformity. Recently, the paper by Rinaudo et al. (1993) concerning the determination of charge distribution along the MWD applying the SEC method with a conductometric detector has been published. In our work, in addition to a refractive index detector (RI), a UV-detector at different wave lengths was applied to study the CMC chemical heterogeneity and, in particular, the degree of oxidation.

In Fig. 8, the experimental UV-chromatograms of CMC samples at wave lengths of 290 and 313 nm using an acetate buffer as the eluent are presented. The absorbance at 290 and 313 nm is usually attributed to the carbonyl groups. In the present work, the carbonyl groups were the result of the oxidation of CMC during destruction. A linear correlation between the UV-chromatogram areas and the carbonyl groups content determined chemically was found (Fig. 9(a)).

Conventional chemical methods give the total amounts of oxidized groups, while the UV-SEC shows the chromophores distribution over the MWD. As can be seen from Fig. 8, with decreasing M_W, the UV- absorption of the samples increases. The contribution of low M_W fractions ($M < 2000$) into total sample absorption is significant. This increases from 30% in the initial sample to 70% in the sample with the M_v 8000, whereas the role of the absorption of fractions with the M_W higher than 2000 decreases.

The mechanism of CMC oxidation during H_2O_2 destruction was not a special aim of this work. However, it is possible to demonstrate an approach for study of similar processes. The method is based on the ratio of the UV-chromatogram area to the RI one for each sample (marked R_{DO}). The UV/RI ratio is a relative characteristic of the

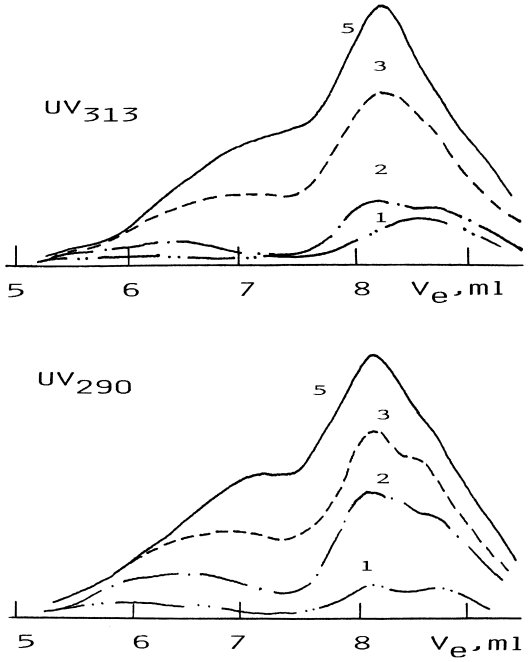

Fig. 8. Experimental UV-chromatograms of CMC samples in 0.4 M acetate buffer (numbers as in Table 2).

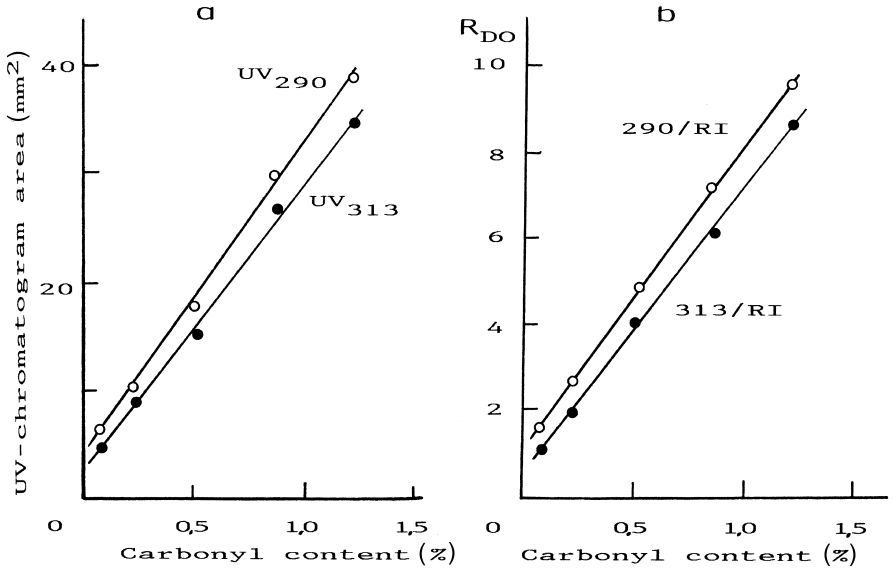

Fig. 9. Dependence of UV-chromatogram areas (a) and R_{DO} (b) on carbonyl group content in CMC samples.

amounts of the chromophores absorbing at a given wave length per mass unit. Fig. 9(b) shows a linear increase of R_{DO} values of both the 290/RI and 313/RI with an increase in the carbonyl group content during the destruction of CMC. Hence, the applied relative parameters R_{DO} reflect the degree of oxidation (DO) of the sample and may be used to study the dynamics of any oxidation process without carrying out a preliminary chemical analysis.

4. Conclusions

The studies on the CMC elution behaviour in a wide range of pH show that MMD analysis of this polymer under alkaline conditions is preferable. Firstly, no undesirable effects were observed. Secondly, the lower hydrodynamic volume of macromolecules in alkali than in acetic buffer enables the analysis of samples with a higher M_W. The Separon HEMA 1000 column is suitable for CMC analysis of M_W up to 200 kDa, i.e. with a DP of ca. 600.

Polyelectrolytes do not always obey the classical Benoit calibration. However, the present work shows that log $M[\eta]$ of the neutral polymer dextran and the polyelectrolyte CMC fall rather well on the same straight line. It is obvious that only a full elimination of polyelectrolyte interactions in the 'polymer–solvent–packing' system ensures the validity of the Benoit's calibration.

The Aharoni relationship $\log K = A - B\alpha$ evaluated for neutral polymers was also confirmed for CMC. This relationship can be used for polyelectrolytes to make a choice of reliable Mark–Houwink constants for MWD determination.

UV-SEC analysis enables the distribution of carbonyl groups over the MWD to be obtained.

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