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ISOTACHOPHORESIS OF SYNTHETIC ION-CONTAINING POLYMERS

CHARACTERIZATION OF THE CARBOXYMETHYL DISTRIBUTION IN CARBOXYMETHYLCELLULOSE

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SUMMARY

Isotachophoresis is used to evaluate the degree of substitution and the distribution of substitution of carboxymethyl groups in carboxymethylcellulose. Commercial samples ranged from very homogeneous to heterogeneous and included samples that appeared to be mixtures of carboxymethylcellulose polymers with different degrees of substitution.

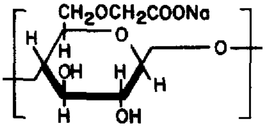
The measurements are made by separating the carboxymethylcellulose into zones inside a small capillary according to electrophoretic mobilities. The isotachophoretic separation mechanism is independent of polymer molecular weight. However, significant binding of counterions with the carboxyl groups is observed, which decreases the effective charge on fully neutralized carboxymethylcellulose. The extent of ion binding is proportionately higher for the polymer with higher degree of substitution, causing both shorter zone lengths and lower electrophoretic mobilities. This behavior conforms to generally accepted theoretical treatments for polyelectrolytes, resulting in isotachophoretic separations of carboxymethylcellulose that can be evaluated both quantitatively and qualitatively.

INTRODUCTION

Chemical heterogeneity in synthetic polymers offers a challenge to the analytical chemist to devise sensitive techniques for the characterization of these chemical distributions. Many synthetic copolymers consist of a collection of polymer chains that differ in their compositions. This distribution of repeat-unit composition from chain to chain can significantly influence the bulk chemical and physical properties of such polymers. A thorough characterization of a copolymer sample would include a description of the average composition and the composition distribution.

Carboxymethylcellulose (CMC) is prepared from a homogeneous natural polymer, cellulose. However, the derived polymer can be heterogeneous in substitution of carboxymethyl groups, because cellulose contains both amorphous and crystalline domains. Crystalline regions in the orginal cellulose sample might be less highly substituted, whereas the amorphous material is likely to be more rapidly swollen and receive a greater degree of substitution, leading to nonuniformity of carboxymethylation. A wide variety of processes are used to repare CMC; some are crude and give non-uniform substitution, whereas other processes give more uniform substitutions. A common synthesis for CMC utilizes alkaline chloroacetate:

$$\text{cellulose} + NaOH + ClCH_2COONa \rightarrow$$



$+ NaCl + H_2O$ (1)

DS = 1

The degree of substitution (DS) can vary between 0 and 3.0 (all three hydroxyl groups substituted). A value of 0.4 is needed to impart water solubility, and a value of 1.4 is the maximum that is readily available from commercial sources[1].

The application of isotachophoresis to evaluate chemical heterogeneity in synthetic ion-containing polymers has not been described in the literature. Isotachophoresis is, however, used to separate proteins and peptides[2,3], where the separations occur because of differences in the effective electrophoretic mobilities of the macromolecules. This technique offers a number of advantages over other techniques for examining heterogeneity in ion-containing polymers. The separation occurs without the aid of support medium or column packing. This eliminates the source of secondary polymer–substrate interactions that can complicate the interpretation of chromatographic separations. The ion-containing polymer does not have to be chemically altered or processed before the separation. The self-sharpening nature of the boundary between migrating zones ensures resolution of components that is not compromised by molecular diffusion effects. Each zone is easily detected, regardless of its composition, with a potential-gradient detector. The separated components can be quantitated by standard calibration techniques.

Isotachophoresis theory and practice are described in several review articles[2–6] and in a book by Everaerts[7]. Briefly, a solution of the polymer sample is introduced at the interface between a leading electrolyte of high effective mobility and a trailing electrolyte of low effective mobility. Sample ions of different electrophoretic mobilities separate into individual zones in direct relation to their effective mobilities and the zones migrate past a potential-gradient detector with a response that is proportional to the effective mobility and to the amount of ion present.

EXPERIMENTAL

Instrumentation

The separations were performed on a Shimadzu IP-2A isotachophoretic analyzer (Shimadzu Scientific Instruments, Columbia, MO, U.S.A.) equipped with a 60 mm O.D. × 1.0 mm I.D. PTFE first-stage capillary tube and a 100 mm O.D. × 0.5 mm I.D. fluorinated poly(ethylenepropylene) second-stage capillary tube. The compartment holding the capillaries and the potential-gradient detector cell was kept at 25°C by Peltier elements using a fluorinated-hydrocarbon cooling fluid. Zones were detected by sensing the potential gradient developed at the interface between migrating zones with two closely spaced platinum electrodes near the end of the capillary migration tube. The current-controlling anode and the cathode were operated at potentials of 2000–10 000 V from a high-voltage power supply maintaining a stabilized current at 125 or 150 μA. Operating parameters are given in the figures with each isotachopherogram.

Electrolytes

The leading electrolyte was either 0.01 *M* or 0.005 *M* chloride, buffered at pH 7.0–7.5 with imidazole (Kodak Laboratory Chemicals, Rochester, NY, U.S.A.), which had been recrystallized three times from ethanol. The 0.01 *M* chloride electrolyte was prepared by diluting 100 ml of 0.1 *N* hydrochloric acid and 5 g of Triton X-100 surfactant (Rohm and Haas, Philadelphia, PA, U.S.A.) to *ca.* 900 ml with distilled water, adding imidazole (*ca.* 0.4 g) until the solution pH increased to 7.5, and diluting to 1 l. The surfactant minimized electroosmotic flow.

The terminating electrolyte was 0.01 *M* *n*-hexanoic acid (Kodak) containing 0.5% Triton X-100 surfactant. The *n*-hexanoic acid was purified by fractional distillation. The Triton X-100 surfactant was purified by dialysis (1000 molecular weight cut-off cellulose membrane) to remove low-molecular-weight anionic impurities. The terminating electrolyte was prepared by dissolving 1.16 g of *n*-hexanoic acid and 5 g of Triton X-100 surfactant in distilled water and diluting to 1 l without pH adjustment.

Carboxymethylcellulose samples

Samples of CMC from Hercules (Wilmington, DE, U.S.A.) were dialyzed to

TABLE I

DESCRIPTION OF CMC MATERIALS

Designation	*Lot No.*	*Degree of substitution*		*MW*
		Nominal	*Analysis*	
Hercules (samples dialyzed)				
4M6SF	69626	0.4	0.44	
7M1	71346	0.7	0.79	
7H	46212	0.7	0.74	
7H3S	40121	0.7	0.74	
7M8SF	79038	0.7	0.67	
9M8	48686	0.9	0.66	
9M31	56387	0.9	0.72	
12M8	72563	1.2	1.20	
12M31	58330	1.2	1.15	
Polysciences (samples not dialyzed)				
6140	S137-2	0.7	0.60	80 000
6138	0112	0.7	0.70	250 000
6139	0113	0.7	0.72	700 000

remove low-molecular-weight salts. Three samples from Polysciences (Warrington, PA, U.S.A.) with weight-average molecular weights of 80 000, 250 000 and 700 000 daltons were used without dialysis. Table I summarizes the sample designations, lot numbers, nominal degree of substitution, and actual degree of substitution (determined by potentiometric titration).

Sample preparation

Samples were prepared for injection by dissolving 10 or 50 mg of CMC in 10 ml of distilled water. For high-viscosity CMC (MW 700 000) the lower concentrations were used, and injection volumes were increased proportionately. No significant advantage on the appearance of zone shapes was obtained when samples were dissolved in leading electrolyte as is often recommended[7].

Determination of average degree of substitution by potentiometric titration

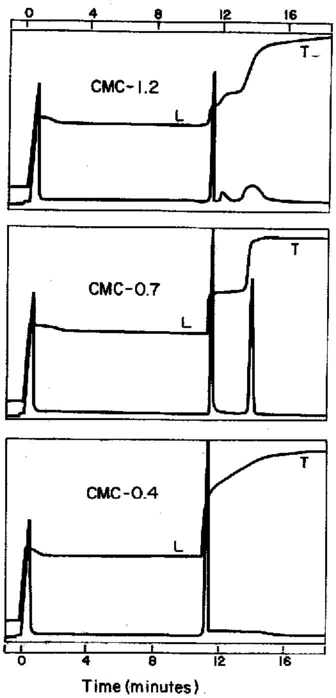
The CMC samples were analyzed for actual DS by potentiometric titration of the sodium carboxylate group. For the analysis, 10–20 mg of CMC were dissolved in 15 ml of distilled water and diluted to 50 ml with acetonitrile (ACS Reagent grade, Kodak). To ensure that all the carboxyl groups were neutralized, excess base (0.2 ml of 0.1 *M* sodium hydroxide) was added. The solution was titrated with 0.1 *M* perchloric acid on a Metrohm automatic potentiograph. The mequiv./g carboxylate was calculated from the difference between the first end-point (excess sodium hydroxide) and the second end-point (sodium carboxylate).

RESULTS AND DISCUSSION

CMC with various degrees of substitution

Isotachopherograms were obtained on CMC with DS of 0.4, 0.7, and 1.2. The effective mobility of these materials is governed by three factors: the actual number of carboxylic acid groups per polymer chain, the degree of acid-group ionization α, and the extent of counterion binding or charge condensation that occurs with most polyelectrolytes in water. This third factor, the extent of counterion binding for CMC, is discussed in detail in below, since it influences the interpretation of the isotachopherograms, and isotachophoresis provides a method for its measurement. The value of α is controlled by the pH of the leading electrolyte through which the polymer migrates. By potentiometric titration, the apparent pK_a values of CMC in water were 4.0, 4.3, and 4.6 for DS of 0.4, 0.7, and 1.2, respectively. At pH 7–7.5, ionization of these groups is essentially complete.

Isotachopherograms of the three polymers are shown in Fig. 1. The appearance of each isotachopherogram was diagnostic of both DS and the distribution of substitution. The 1.2-DS sample has the highest effective mobility, and in addition, two zones are evident, suggesting that this sample is a mixture of two materials with different DS. A second lot of 1.2-DS material did not show two zones (see Fig. 5). The 0.7-DS sample has a single, intermediate mobility with sharp zone boundaries, suggesting it is the most homogeneous of the three samples. The 0.4-DS sample has the lowest mobility and a much broader range of substitution, as evidenced by the gradually sloping potential-gradient detector trace towards lower-mobility polymer.



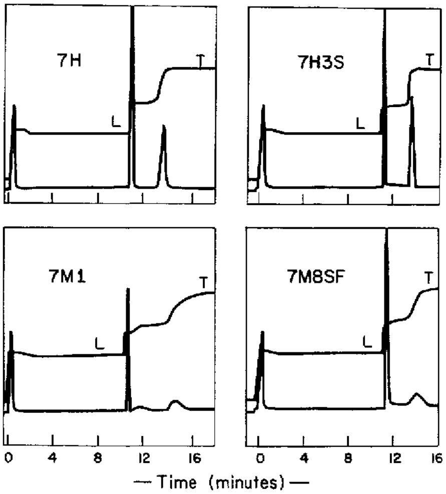


Fig. 1. Isotachopherograms of CMC with DS of 0.4, 0.7, and 1.2. Leading electrolyte (L): 0.005 *M* Cl^-, pH 7.0 imidazole buffer, 0.5% Triton X-100 surfactant. Terminal electrolyte (T): 0.01 *M* *n*-hexanoic acid, 0.5% Triton X-100 surfactant. Migration current, 150 μA; sample, 30 μl of 1000 ppm solutions in water; temperature, 25°C.

Fig. 2. Isotachopherograms of four grades of CMC with DS of 0.7. Conditions as in Fig. 1.

Comparison of grades of CMC

Four grades of Hercules Chemical CMC with nominal DS of 0.7 were examined for differences in distribution of substitution. The isotachopherograms are shown in Fig. 2. The detector traces suggest that differences in distribution exist among the grades although the major portion of each sample has the same mobility and, consequently, a similar DS. The observed differences can be summarized as follows:

Grade 7H3S is very homogeneous in distribution of carboxymethyl substitution. Grade 7H is nearly as homogeneous, with evidence in the PG detector trace of slightly higher-DS material but no lower-DS material. Grade 7M8SF is less homogeneous, with evidence of some lower-DS polymer seen as a broadened trailing zone and a broad derivative detector trace. Grade 7M1 is heterogeneous, and is a mixture of two CMCs with DS = *ca.* 0.7 and 0.9, as estimated from their relative electrophoretic mobilities.

Two grades of CMC (9M8 and 9M31) with reported DS of 0.9 were also examined. The isotachopherograms are nearly identical to that of the 7H3S polymer (DS = 0.74) (see Fig. 6, sample 9M31), suggesting no significant differences in substitution. This was confirmed by the potentiometric titration analyses for average

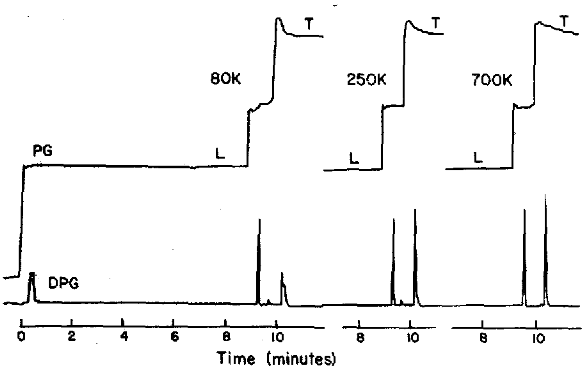


Fig. 3. Isotachopherograms of CMC of different molecular weights. Leading electrolyte (L): 0.01 *M* Cl^-, pH 7.3 imidazole buffer, 0.5% Triton X-100 surfactant. Migration current, 125 μA; temperature, 15°C; sample, 15 μl of 1000 ppm solutions in water. Other conditions as in Fig. 1.

DS, which were 0.7 ± 0.1 for the 9M8 and 9M31 materials, the same as predicted by the isotachopherograms.

Effect of CMC molecular weight on the isotachophoretic separation

The molecular-weight dependence on the isotachophoretic separation was evaluated for the three samples of CMC from Polysciences, with nominal molecular weights of 80 000, 250 000, and 700 000 daltons. By potentiometric titration, the DS values were 0.7 ± 0.1. The isotachopherograms are shown in Fig. 3. No significant difference in apparent mobilities was observed from this range of molecular weights. This behavior is consistent with reported observations of a lack of molecular-weight dependence on polyelectrolyte mobility determined by other electrophoretic techniques[8–10].

Quantitation of isotachopherograms

For quantitative evaluation of an isotachopherogram, zone lengths displayed

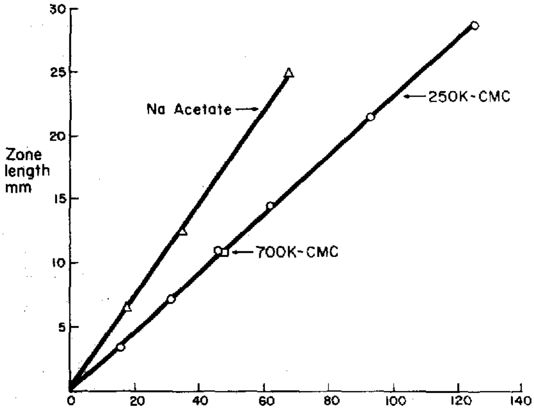


Fig. 4. Zone-length calibration of CMC and sodium acetate.

on the recorder tracing are measured. The zone lengths are best taken as the distance in millimeters between peak maxima of the differential potential-gradient detector signal. The relationship between amount of CMC (DS-0.7) injected and zone length was evaluated from a calibration curve based on injections of 5–40 μg of CMC with molecular weights of 250 000 and 700 000. The zone lengths are plotted *vs.* equivalents of carboxyl group (rather than sample weight) in Fig. 4, showing excellent linearity of the calibration curve for the polymer with no dependence on CMC molecular weight.

A similar plot for sodium acetate is also given in Fig. 4. The zone lengths for sodium acetate are substantially longer than those for identical equivalents of CMC carboxyl groups. Since both zones contain the same total number of carboxyl groups, the difference in zone lengths is attributed to a lower concentration of ionized carboxyl groups in the CMC sample. This behavior is caused by counterion binding of otherwise fully ionized carboxyl groups in polyelectrolytes. Because of ion binding, the actual charge on a polyion is less than the number of ionized groups present. This behavior is important in isotachophoresis measurements, since zone length is proportional to the number of ionized charged groups in the sample zone. Ion binding will influence the quantitative interpretation of zone lengths of polyelectrolytes such as CMC and therefore was examined further.

Effect of DS on CMC zone length and counterion binding

We evaluated the effect of DS on zone length by preparing solutions of sodium acetate and CMC with DS = 0.4, 0.7, and 1.2 at the same molar concentration of carboxyl group, based on the analytically determined mequiv./g of carboxyl group. In this way, the observed zone length should be equal for equal-volume injections. The isotachopherograms are shown in Fig. 5. The zone lengths are plotted *vs.* equivalents of carboxyl group in Fig. 6 for each DS and for sodium acetate. The plot shows a linearly decreasing zone length with increased DS or charge density on the cellulose polymer. With the higher-charge-density polymer, more of the carboxyl groups appear as if they are not ionized. We determined that this behavior was not caused by incomplete neutralization of the carboxyl groups because of low electrolyte

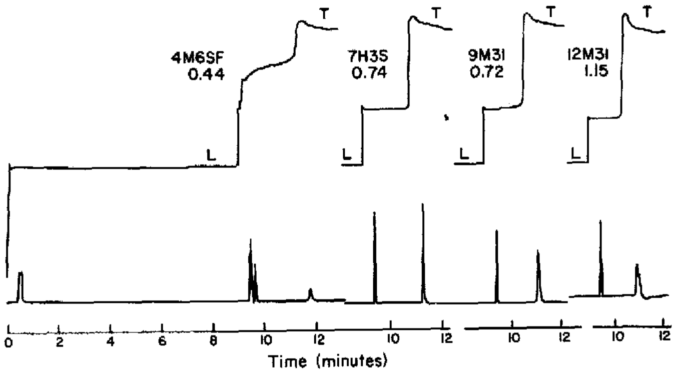


Fig. 5. Isotachopherograms of CMC with different DS for evaluation of zone-length dependence on DS. Leading electrolyte (L): pH 7.5 imidazole buffer, 0.5% Triton X-100 surfactant. Sample, 20 μl of CMC at 0.005 equiv./l carboxyl group. Other conditions as in Fig. 3.

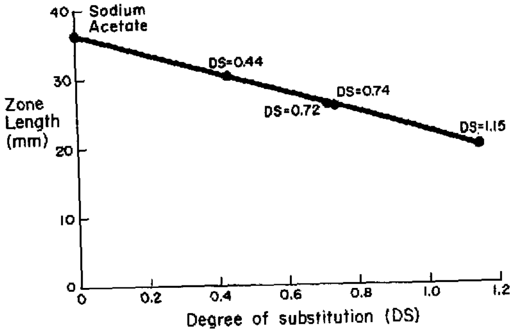


Fig. 6. CMC zone length *vs.* DS. (Each sample is 0.1 μequiv. of carboxyl group.)

pH by obtaining the isotachopherograms in a leading electrolyte buffered at pH 8.5 with tris(hydroxymethyl)aminomethane (Kodak). The relative zone lengths for acetate, methoxyacetate, and the CMC samples did not change, showing that no increased ionization of the carboxyl groups in CMC had occurred. The fraction of counterion bound to the polyelectrolyte was calculated from the ratio of zone lengths for CMC to acetate when equivalent carboxyl concentrations were injected.

The experimental sample zone concentrations were first adjusted to have an ion concentration equal to that of the leading ion, using the Kohlrousch regulating function[7], which forms the basis of isotachophoresis. The equation is

$$C_i = C_L \frac{m_i\,(m_L + m_c)}{m_L\,(m_i + m_c)} \tag{2}$$

where c_i is the sample zone concentration, C_L is the leading ion concentration, and m_i, m_L, and m_c are the effective mobilities of sample ion, leading ion, and counterion, respectively. For monovalent ions, this equation gives the concentration of ions in the sample zone resulting from the concentration discontinuity that exists between leading and sample ions as these ions migrate at constant velocity in a constant current. Both m_L and m_c have known values, $79 \cdot 10^{-5}$ and $29.5 \cdot 10^{-5}$ cm^2/V s at 25°C, respectively[11]. The value of m_i is readily determined from the step height dimensions in the isotachopherogram[11,12], using the equation

$$m_i = m_L(E_L/E_i) = m_L(h_L/h_i) \tag{3}$$

where E_L and E_i are the potential gradients and h_L and h_i are the step heights in millimeters of the leading and sample zones, respectively.

The fraction of counterions bound to CMC, φ, was calculated from the equation

$$\varphi = 1 - \frac{(\text{zone length}/\mu\text{equiv. CMC})C_{\text{cmc}}}{(\text{zone length}/\mu\text{equiv. acetate})C_{\text{acetate}}} \tag{4}$$

where C_{cmc} and C_{acetate} are mol/l calculated by using eqn. 2, and the zone lengths are

TABLE II

FRACTION OF COUNTERION BOUND TO CMC

Sample	*Fraction of counterion bound**	
	pH 7.5 (imidazole buffer)	*pH 8.5 (Tris buffer)*
Acetate, sodium salt	0	0
Methoxyacetate, sodium salt	0	0
CMC DS-0.4	0.22	–
CMC DS-0.7	0.27	0.28
CMC DS-1.2	0.43	0.44

* Calculated from the ratio of zone lengths of sample to sodium acetate for identical molar amounts of carboxyl group. Counterion is imidazole–H^+ or tris(hydroxymethyl)aminomethane–H^+.

the distances in millimeters between peak maxima of the differential detector signal trace divided by the μequiv. of carboxylic group injected. The results of these calculations are given in Table II.

We have observed this type of counterion behavior in isotachophoretic separations on other polyelectrolytes containing sulfonate and carboxylate groups. The counterion-binding behavior for these polymers wil be described in a separate publication.

This behavior of polyelectrolytes is consistent with the counterion-binding theory described by Manning[13–15] and others and confirmed experimentally for several types of polyelectrolytes by Okubo and Ise[16], Record *et al.*[17], and others, using electrophoretic light scattering, tracer-ion diffusion-coefficient measurements, and osmotic-pressure techniques. The high charge density on polyelectrolytes is "self-lowered" through counterion condensation by site-bound or territorially bound counterions that exist near the fixed charges on the polymer. These counterions move with the polyion in an electric field. The theory suggests that the extent of counterion binding is dependent on several parameters, the most important being the charge spacing along the polymer chain and the solvent dielectric constant.

Counterion binding has considerable influence on the interpretation of isotachopherograms of CMC. As has been demonstrated with CMC, the effects of counterion binding can be evaluated accurately by using well-characterized polymer samples.

CONCLUSION

Isotachophoresis is a sensitive and useful analytical technique for characterizing carboxymethyl substitution and the distribution of substitution in CMC. The distributions in several commercial samples ranged from very homogeneous (for the DS-0.7 material) to heterogeneous (for the DS-0.4 material). Two samples of CMC appeared to be mixtures of polymers with different degrees of substitution. Two other grades of CMC were labeled DS-0.9, yet were indistinguishable from material labeled DS-0.7, based on both isotachophoresis and potentiometric titration analysis.

The isotachophoretic separation mechanism is independent of polymer molecular weight. Significant binding of counterions with carboxyl groups was observed, which decreases the effective charge on CMC. The extent of counterion binding is proportionately greater for higher-DS polymer. This behavior causes shorter zone lengths for CMC with higher charge density. This behavior conforms to the generally accepted theoretical treatments for polyelectrolytes of Manning[13–15] and others[16,17].

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