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Problems in the size-exclusion chromatography of cellulose nitrates: non-exclusion effects and universal calibration

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ABSTRACT

It is shown that cellulose nitrates (CN) (degree of substitution from 2.1 to 2.97) in pure tetrahydrofuran (THF) during size-exclusion chromatography (SEC) on silanized silica gel exhibit polyelectrolytic properties and their behaviour is complicated by several non-exclusion effects. Substitution heterogeneity and ionogenic groups affect the properties of CN solutions and their SEC behaviour. Addition of 0.01 mol/l of acetic acid to the eluent suppresses the non-exclusion effects and leads to validity of a universal calibration between CN and polystyrene. The Mark–Houwink equation parameters for CN in THF–0.01 mol/l acetic acid were calculated to be $\alpha = 0.73$ and $K = 6.44 \cdot 10^{-4}$ dl/g.

INTRODUCTION

Cellulose nitrates (CN) are traditionally used to determine the molecular mass parameters (MMP) and molecular mass distribution (MMD) of cellulose. Although the CN have been the subject of extensive size-exclusion chromatographic (SEC) studies [1–11], a series of problems still remain. It is believed that the separation of CN during SEC occurs by a merely steric mechanism. If the SEC columns are applied with tetrahydrofuran (THF) as the mobile phase, the chromatogram obtained is considered to correspond to the MMD of CN. However, in practice the CN separation is accompanied by several effects.

One of them is the concentration effect, *i.e.*, a change in the retention volume (V_e) with varying polymer concentration. This effect has been observed in the SEC of CN by several workers, using Styragel as the packing material and THF as the eluent [4–7]. Marx-Figini and Soubelet [7] noted this effect for CN in THF with passivated silica gel (LiChrospher CH 8) as the stationary phase. For CN the concentration effect is considerable even with low injected concentrations.

Another effect is partial adsorption of CN on the packing, noted when Styragel, silica gel or porous glass was applied [4,8,9].

Finally, the problem of the validity of the universal calibration concept has not been solved. When the MMP of CN are determined using a universal calibration procedure the results are usually higher then those obtained by absolute methods

[2,5,6,8]. At the same time, the application of direct calibration with CN fractions provides satisfactory agreement of the MMP values [10,11].

In fact, the universal calibration between CN and other polymers under different experimental conditions is not valid [2,5,7–9]. It is not known whether the failure to obtain a universal calibration is caused by erroneous Mark–Houwink constants or by violation of CN steric separation mechanism. There are no convincing explanations of the concentration effects taking place on different packings. This paper is devoted to the consideration of these problems.

EXPERIMENTAL

Materials

Chemical-grade cotton cellulose was nitrated with the mixture described by Alexander and Mitchell [12] for 0.5, 1, 2 and 4 h at 0°C. Nitrogen contents were determined by the semimicro Kjeldahl technique. Samples with nitrogen contents of 11.7, 12.4, 13.6 and 13.9% were chosen for the investigation of the elution behaviour of CN.

In order to calibrate the chromatographic system for different series of CN fractions, three groups of fractions, with nitrogen contents of 13.3–13.9%, 12.4% and 11.5% in the molecular mass (M_w) range 30 000–300 000, were selected. CN fractions were obtained by the precipitation fractionation technique, using a conventional scheme with acetone–water [13].

Peroxide-free THF, freshly distilled over KOH, was used as solvent and eluent.

Intrinsic viscosity measurements

These were carried out with a Ubbelohde viscometer at 25 $\pm$ 0.05°C in THF and in THF–0.01 mol/l acetic acid.

Size-exclusion chromatography

SEC was carried out on a GPC chromatograph (Laboratory Instruments Works, Prague, Czechoslovakia) with a refractometric detector, equipped with a Rheodyne 7125 fixed-loop (20-μl) injector and a set of bimodal Zorbax PSM S 1000 + 60 columns (DuPont) packed with silanized silica gel. The analyses were performed at the room temperature. The analysis time was 20 min at a flow-rate of 0.5 ml/min.

Solutions (0.5–0.05%) of CN samples in THF and in THF–0.01 mol/l acetic acid and 0.05% solutions of CN fractions in THF–0.01 mol/l acetic acid were prepared and shaken overnight. The samples were filtered through a 0.5-μm Millipore filter prior to use.

Polystyrene standards (Waters Assoc., Milford, MA, U.S.A.) and CN fractions with different nitrogen contents characterized according to MMP by ultracentrifugation [14] were used for calibration of the chromatographic system.

RESULTS AND DISCUSSION

Elution behaviour

During SEC, an unchanged elution behaviour of the polymer when its concentration in the injected sample is varied is a chromatographic demonstration of

a pure steric separation mechanism. In this case, the chromatogram is adequate to the differential MMD of the polymer under investigation. An influence of the sample concentration on the elution volume indicates a violation of the steric separation mechanism by some non-exclusion effects.

The elution behaviour of CN with a degree of substitution (DS) of 2.05–2.95 (11.7–13.9% N, respectively) in pure THF depends on the CN concentration. Thus, the V_e of the samples decreases with decrease in the injected concentration from 0.5 to 0.05 g/dl (Fig. 1). The fronts of the chromatograms with different concentrations almost coincide, but their ends tend to show a decrease in V_e with decrease in concentration. Such behaviour of the polymer during SEC is typical of polyelectrolytes [15]. Obviously, CN in THF on silanized silica behaves as a polyelectrolyte.

The dependence of V_e on CN concentration is non-linear in the investigated DS range (Fig. 2). This indicates that the observed concentration effects have a complex nature and are caused by a sum of phenomena.

The samples elute before the column exclusion limit (V_0), determined by the polystyrene standard with $M_w = 2.7 \cdot 10^6$. Hence the main phenomenon controlling the separation of CN on silanized silica gel in THF is ion exclusion.

CN are never considered as polyelectrolytes. However, cellulose preparations usually contain some amounts of ionogenic carboxyl groups, because cellulose
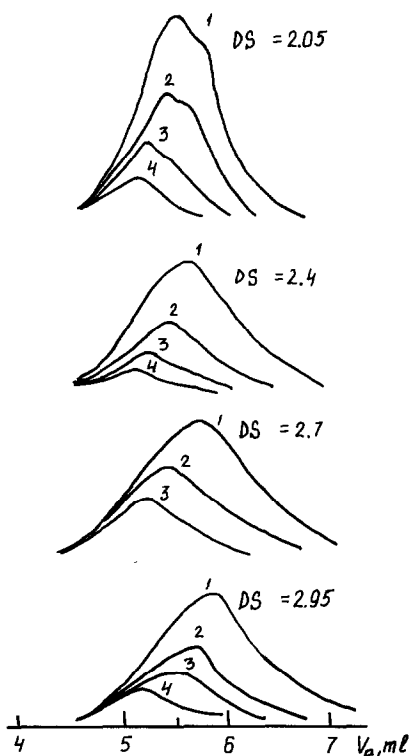

Fig. 1. Experimental chromatograms of CN with different DS in pure THF at injected concentrations of (1) 0.5, (2) 0.25, (3) 0.125 and (4) 0.05 g/dl.
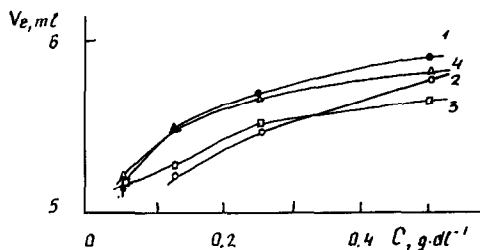
Fig. 2. Retention volume as a function of injected concentration of CN in THF.

undergoes oxidation during processing. Taking into account the fact that the carboxyl groups in THF dissociate [16] and the silica gel in neutral media has a negative charge (owing to dissociation of residual silanol groups), ion exclusion can take place. We assume that the concentration effects observed by other investigators during SEC of CN can be considered to be a consequence of the polyelectrolyte nature of CN solutions.

In this work, the viscometric properties of THF solutions of CN samples were also investigated. The dependence of specific viscosity on concentration, $\eta_{sp}/C = f(C)$, for CN in THF is non-linear (Fig. 3). There is a maximum for the highly substituted samples $(DS \geqslant 2.4)$ and η_{sp}/C tends to increase with decrease in the concentration of the sample with $DS = 2.05$.

A similar dependence for highly substituted CN was observed by Siochi and Ward [17], which they explained by the molecular associates present in the solution. Nevertheless, it is known [15] that this non-linear type of dependence is typical of polyelectrolyte solutions with insufficient solvent ionic strength. In our opinion, both the formation of associates and the presence of ionogenic groups in the CN macromolecules can be regarded as a reason for the observed effect.
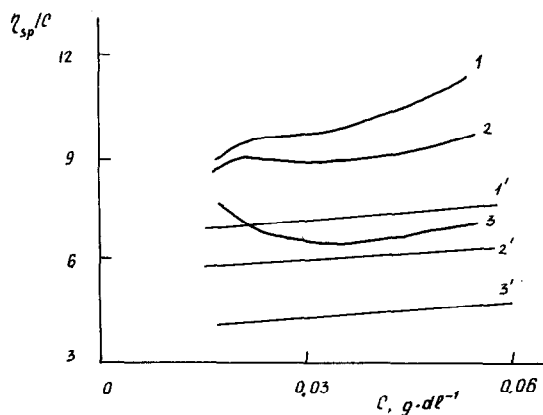

Fig. 3. Viscosity plots for CN with $DS = $ (1) 2.95, (2) 2.4 and (3) 2.05 in THF (1–3) and in THF–0.01 mol/l acetic acid (1'–3').

Effect of acetic acid

The presence of a charge in the polymer chain causes the coil to expand. A change in the ionic strength and/or pH of the solution results in suppression of the polyelectrolyte effects both in solution and during SEC [15].

In this work, the effect of adding acetic acid to the mobile phase on the elution behaviour of CN was studied.

The addition of acetic acid to the eluent resulted in an increase in the sample V_e irrespective of the *DS* of CN (Fig. 4). No shifts in V_e for the highly substituted CN sample were observed with addition of acetic acid at concentrations exceeding 0.005 mol/l, whereas for the samples with average and low DS 0.01 mol/l acetic acid was required. On adding 0.01 mol/l acetic acid to THF, no concentration dependence was observed.

The addition of acetic acid to CN solutions also influenced the viscosity. The dependence of η_{sp}/C on concentration became linear (Fig. 3), and the [η] values tended to decrease. The presence of acid in the solution suppresses the dissociation of ionogenic groups, leading to reduce macromolecule coil dimensions. The reduction of the effective hydrodynamic volume of CN macromolecules results in a corresponding shift in the chromatograms.

Universal calibration

The dependence of log $M[\eta]$ on V_e is nowadays accepted as a universal calibration. It is invariant for a given set of columns and eluent. If the universal calibration between different polymers is valid, it means that only steric exclusion of the polymers under investigation takes place.

In this work, the universal calibration procedure was carried out using CN fractions and polystyrene standards. It is well known that the properties of CN in solution depend on *DS*, and therefore the samples differed in nitrogen content (Table I). Experimental [η] values in THF–0.01 mol/l acetic acid were used to determine the CN (log M [η]).

Fig. 5 demonstrates that the elution behaviour of polystyrenes is not affected by the presence of acid in THF. This means that the hydrodynamic volume of
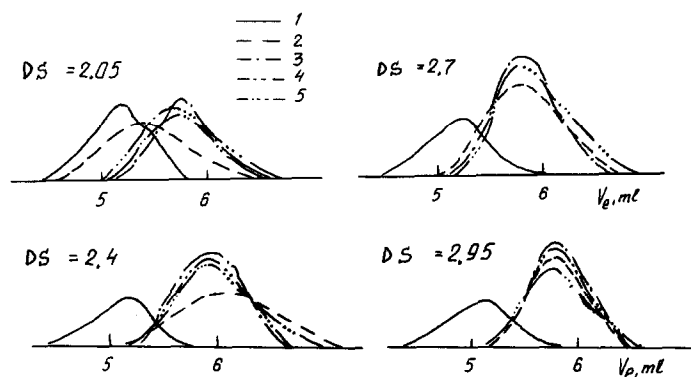

Fig. 4. Effect of acetic acid on CN elution behaviour. (1) Pure THF; (2–5) THF with acetic acid added: (2) 0.005; (3) 0.01; (4) 0.017; (5) 0.05 mol/l.

TABLE I

CHARACTERISTICS OF CN CALIBRATION SAMPLES

No.	N (%)	$M_w \cdot 10^{-3}$	M_w/M_n^c	$[\eta]$ (dl/g)	Log M $[\eta]^a$	V_e (ml)
1	14.0	508	1.35	10.27	6.65	5.55
2	13.6	250	1.40	5.94	6.10	6.04
3	13.9	86	1.30	2.62	5.30	6.50
4	13.3	87	1.40	2.65	5.29	6.50
5	13.3	30	1.24	1.23	4.52	7.00
6	13.9	25	1.25	1.00	4.35	7.07
7	12.4	282	1.65	—	6.06^b	6.02
8	12.4	192	1.25	—	5.87^b	6.14
9	12.4	172	1.25	—	5.78^b	6.19
10	12.4	110	1.25	—	5.45^b	6.40
11	12.4	68	1.25	—	5.08^b	6.62
12	11.3	316	1.48	7.02	6.26	5.93
13	11.6	187	1.45	5.00	5.89	6.10
14	10.9	147	1.40	3.80	5.67	6.27
15	11.6	77	1.60	2.40	5.16	6.60

^{a} Peak molecular mass, calculated as $1/2 \ln (M_w M_n)$.
^{b} Calculated by $[\eta] = 6.44 \cdot 10^{-4} M_{peak}^{0.73}$.
^{c} M_w = mass-average molecular mass. M_n = number-average molecular mass.

polystyrenes remains unchanged. Therefore, the log M $[\eta]$ values for polystyrenes were calculated using the Mark–Houwink constants $K = 13.4 \cdot 10^{-4}$ dl/g and $\alpha = 0.71$, which were established in pure THF [2]. The results obtained are presented in Table II.

As shown in Fig. 6 the universal calibration parameters of CN and polystyrene almost coincide if THF–0.01 mol/l acetic acid is used as the eluent. This means that the universal calibration concept between CN and polystyrene is valid when all non-exclusion effects are eliminated.

Although it was assumed that CN with different *DS* would have different calibrations in terms of log *M*, we found that the samples with differing nitrogen
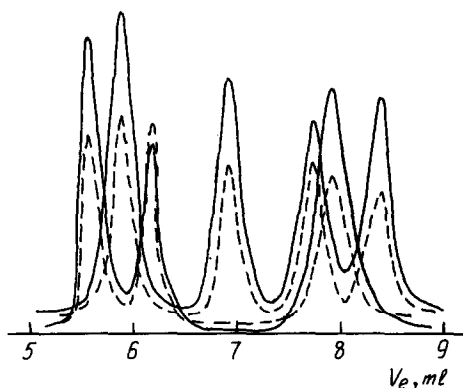

Fig. 5. Experimental chromatograms of polystyrene standards in THF (solid lines) and THF–0.01 mol/l acetic acid (dashed lines).

TABLE II

MMP AND CORRESPONDING RETENTION VOLUMES FOR POLYSTYRENE STANDARDS

M_w	Log M [η]	V_e (ml)
2 700 000	7.13	5.55
867 000	6.29	5.90
470 000	5.83	6.16
111 000	4.57	6.94
20 800	3.52	7.60
15 000	3.28	7.73
10 000	2.97	7.93
4000	2.20	8.40
2100	1.89	8.50

contents and equal molecular masses elute with the same V_e (Fig. 7). The variations in the hydrodynamic properties of this polymer depending on DS are connected with the different chain stiffnesses of high- and low-substituted CN [18]. At the same time, it is recognized that the chain stiffness is determined to a marked extent by the hydrogen bond system [19]. The observed levelling of CN in terms of DS during SEC in the presence of acid can be explained only if the influence of volume effects on the CN solution properties is taken into account. The added acid destroys the system of bonds formed in pure solvent and changes the nature of the interactions between incompletely substituted CN and THF.

If the size of the CN molecules decreases on adding acetic acid to THF, the Mark–Houwink constants for CN must change. The parameters K and α were
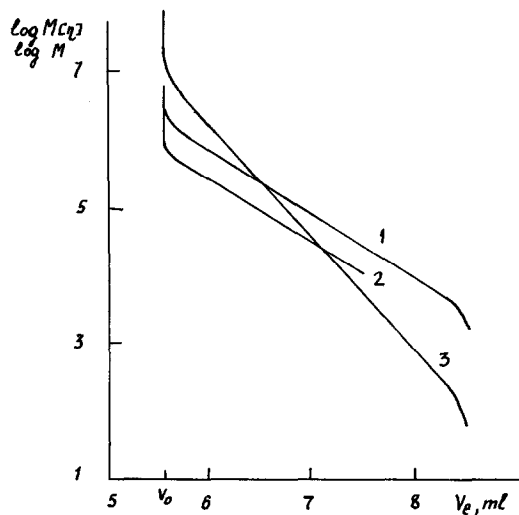

Fig. 6. Calibration graphs, log M = f(V_e), for (1) polystyrene and (2) CN, and (3) universal calibration in THF–0.01 mol/l acetic acid.
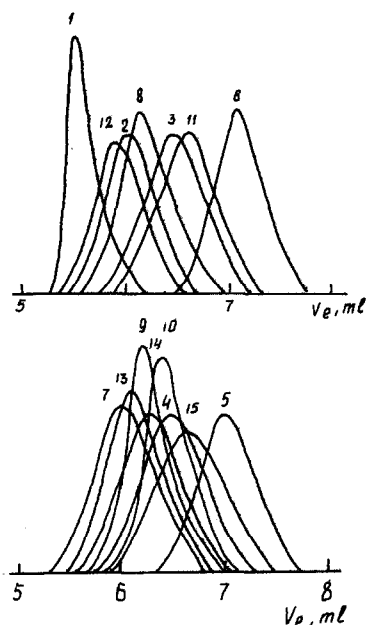
Fig. 7. Normalized chromatograms of CN used for calibration in THF–0.01 mol/l acetic acid. Numbers as in Table I.

established from the combined SEC and viscometric data according to the principle described by Dobbin et al. [20]. The Mark-Houwink equation is

$$[\eta] = 6.44 \cdot 10^{-4} \, M^{0.73} \; (\mathrm{dl/g})$$

The MMP of CN samples caculated using these constants and the universal calibration approach are in good agreement with the values determined by ultra-centrifugation and viscometry (Table III).

TABLE III

MOLECULAR MASS PARAMETERS OF CN SAMPLES OBTAINED BY SEDIMENTATION, VISCOMETRY AND SEC

Sedimentation[a]			Viscometry[b]: $M_v \cdot 10^{-3}$	SEC			
$M_w \cdot 10^{-3}$	M_w/M_n	M_z/M_w		$M_w \cdot 10^{-3}$	$M_v \cdot 10^{-3}$	M_w/M_n	M_z/M_w
282	1.65	—	—	286	—	1.52	1.29
192	1.25	1.15	—	193	—	1.30	1.15
172	1.25	1.20	—	170	—	1.25	1.18
110	1.25	1.25	—	115	—	1.25	1.21
—	—	—	280	295	285	1.65	—
—	—	—	157	160	152	1.45	—
—	—	—	67	70	66	1.74	—

[a] M_z = z-average molecular mass.
[b] M_v = viscosity-average molecular mass.

CONCLUSIONS

It is clear that the separation of CN by SEC is a highly complex process. When determining cellulose MMP via nitrates, the chemical heterogeneity of the latter should be taken into account, as the presence of functional groups differing in polarity, in addition to ionogenic groups, affect the behaviour of CN in the solution. The polyelectrolyte effects on electroneutral packings during SEC may be much less strong, but nevertheless, if they are disregarded, they would lead to incorrect molecular mass data and a distorted molecular mass distribution.

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