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Distribution analysis of cellulose acetate phthalate by ion-exclusion-moderated size exclusion chromatography

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Abstract

Ion-exclusion is the electrostatic repulsive interaction between a charged polymer and charges of the same sign on the surface of a column packing. Controlled ion-exclusion allows compensation of hydrophobic adsorption in size exclusion chromatography of negatively charged cellulose acetate phthalate (CAP) polymers in acetone/water/LiCl (80/20) as a mobile phase. Properly selected low-ionic-strength conditions provide correct separation in size-exclusion mode also in binary solvent mixtures. Possible interfering effects related to light scattering at low-salt conditions are shown to be negligible if on-line concentration/light scattering detection is used. The absence of these interferences is easily checked by a comparison of experiments at two different low-salt concentrations. Molecular weight averages and distributions identical within the experimental error are obtained when both salt concentrations are properly selected.

Keywords: Ion-exclusion; Size-exclusion chromatography; Dual RI-LALLS detection; Cellulose acetate phthalate

1. Introduction

Cellulose acetate phthalate (CAP) belongs (Wade & Weller, 1994) to the group of enteric coating materials resistant to gastric fluid but soluble in the intestine. Commercial products are characterized only by solution viscosity; the knowledge of their molecular weights and molecular weight distributions is desirable as there is a close relation between these parameters and coating technology performance.

Conventional size-exclusion chromatography (SEC) of this polymer with single refractive-index (RI) detection appears difficult if not impossible. No calibration standards are available and universal calibration approach is hindered by their polyelectrolyte character. A high salt content in the mobile phase necessary to suppress the polyelectrolyte effect usually introduces adsorption due to the onset of hydrophobic interaction. A key issue is thus to find a proper column packing — mobile phase combination that allows correct SEC behavior free of detrimental adsorption effects. However, good solvents of these polymers are found (Wade & Weller, 1994) only within the group of ketones, esters, ether alcohols, cyclic ethers and certain solvent mixtures like acetone/methanol or ethanol, acetone/methylene chloride, ethyl acetate/isopropyl alcohol and benzene/methanol. Also, CAP becomes soluble in certain buffered aqueous solvents at pH ≥ 6. Solvent requirements thus considerably limit the application of polymer-based SEC columns. Even worse, the solvent choice is also limited by a requirement of sufficiently large dn/dc values to avoid loss of detection sensitivity.

Almost all common column packings bear a negative surface charge (Dubin, 1988; Kopaciewitz & Regnier, 1982). Ion-exclusion is then observed for polyanions in aqueous mobile phases in the absence of a simple electrolyte, especially at neutral or high pH. An early elution of polyanions, due to this ion-exclusion effect, should decrease in other polar solvents than water but persist to some degree. Most of the SEC work to date was focused on identification and elimination of ion-exclusion and polyelectrolyte effects to be able to use columns in the pure SEC mode. As an exception, Kopaciewitz and Regnier (1982) suggested using the ion-exclusion effect to enhance selectivity in the SEC of proteins and provided the evidence that this unwanted interaction may be useful if properly handled. Potschka (1987, 1988, 1991a,b, 1993) has shown in his studies that both the effective hydrodynamic shape of a

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solute and the interfacial pore wall effect must be accounted for in the case of SEC of polyelectrolytes using packings of the same charge. In general, separation is governed by some overall radius R

$$R = R_{\mathrm{SEC}} + R_{\mathrm{IF}} = R_{\mathrm{SEC}} + \kappa^{-1}\bar{a} \quad (1)$$

where $\bar{a}$ is the average electrostatic repulsion distance in equilibrium in multiples of Debye length κ^{-1}, R_{SEC} the rotationally averaged mean radius of the solute and R_{IF} is the interfacial contribution to the total solute radius R. Actually, R_{IF} is introduced to account for the reduced pore size under low-salt conditions. For spherical particles like proteins, R_{SEC} should be simply the sphere radius but for the case of the coiled charged polymer, it could be a complex function of ionic strength including coil expansion due to the polyelectrolyte effect. The conventional universal calibration approach then assumes suppressed coil expansion and negligible R_{IF}, i.e. high ionic strength conditions, and

$$R_{\mathrm{SEC}} \sim \{[\eta]M\}^{1/3} \quad (2)$$

where $[\eta]$ is the intrinsic viscosity and M is the molecular weight of a macromolecule in bulk solution. Hydrophobic interaction (attractive) forces in aqueous media operate at long separation distance and electrostatic repulsion is just a convenient long-range force suitable for their compensation to obtain the net repulsion defining particle solution stability (van Oss, 1994). The same should apply when some hydrophobic interaction between the surface of packing and a solute takes place.

Assuming that correct SEC separation according to R (Eq. (1)) can be established at a low ionic strength to prevent solute-packing hydrophobic interaction, the knowledge of molecular weight as a function of R (i.e. as a function of elution volume) should be sufficient in this case to perform distribution analysis. This can be conveniently accomplished using RI and low-angle laser-light scattering (LALLS) or multiangle laser-light scattering (MALLS) detection. The only requirement is that the pore diameter of the column packing must be sufficiently large, $R_{\mathrm{pore}} \gg R$, to avoid loss of resolution due to total exclusion of the largest coils of the sample from the pores. This technique was shown recently (Porsch, Hillang, Karlsson & Sundelöf, 2000) to be applicable using single solvent/salt as a mobile phase in the case of negatively charged Eudragit® polymers, otherwise difficult to analyze at high ionic strengths due to their irreversible adsorption on the column packing.

This approach will be used in this paper to determine molecular weight distributions of negatively charged CAP using wide-pore silica-based diol SEC packing and a binary solvent mixture. The mobile phase will be a mixed solvent acetone–water (80/20) containing small amounts of LiCl as a modifier of charge interactions.

2. Experimental

2.1. Materials

CAP samples were commercial products of Eastman Chemical Company (Kingsport, Tennessee, USA) and of Wako Pure Chemical Industries Ltd. (Chuo-Ku, Osaka, Japan).

Analytical reagent grade acetone (Lachema, Neratovice, Czech Republic) and anhydrous lithium chloride (Fluka Biochemika AG, Buchs, Switzerland) were used as received.

Water was from a Millipore Milli-$\mathrm{Q}^{\mathrm{UF}}_{\mathrm{PLUS}}$ ultrapure water purification unit.

2.2. Chromatography

Modular chromatograph consisted of a Constametric® 3200 MS pump (Thermo Separation Products, Riviera Beach, Florida), a Pharmacia injection valve V-7 with 100 μl loop (Pharmacia and Upjohn, Uppsala, Sweden), a Chromatix KMX-6 LALLS detector (LDC/Milton Roy, Sunnyvale, CA) and an R-401 differential refractometer (Waters Assoc., Milford, MA) connected through a Black Star (Huntingdon, UK) 2308 A/D converter to an IBM-compatible computer. On-line RI-LALLS arrangement allows the simultaneous determination of M and c at any elution volume ('slice').The following relation is valid for Rayleigh scattering from polydisperse polymer/solvent system at low angle (6–7°)

$$(K^{*}c)/R_{\Theta} = 1/M_{\mathrm{W}} + 2A_{2}c \quad (3)$$

where c is the concentration of scattering species, R_{Θ} the excess Rayleigh scattering factor, M_{w} the weight average molecular weight of scattering species and A_2 is the second virial coefficient. $K^{*} = (2\pi n^{2}/N\lambda^{4})(\mathrm{d}n/\mathrm{d}c)^{2}$ where n is the refractive index of the solvent, λ is the wavelength in vacuo (633 nm), N is the Avogadro number and dn/dc is the refractive index increment of the scattering species in the solvent used. If correct separation takes place, the polymer seen at a slice is assumed to be monodisperse. Angular dependence of the scattered light is omitted at the low angle used. Polydispersity and column band broadening dilutes the sample considerably; hence, the term $A_{2}c$ may be neglected if the concentration of the injected solution is low enough. Conventional calibration log M vs. elution volume (V_{e}) is thus directly obtained. A home-made software (M. Netopilík, Institute of Macromolecular Chemistry) allows on-line data accumulation and all calculations of molecular weight distributions and their averages.

Dialyzed dn/dc values of CAP generally needed in mixed solvents were calculated from the refractometer data using the equation following from the application of Hummel–Dreyer approach (Hummel & Dreyer, 1962)

$$\mathrm{d}n/\mathrm{d}c = (\mathrm{d}n/\mathrm{d}c)_{\mathrm{s}}(\Delta c_{\mathrm{s}}/\Delta c)(A/A_{\mathrm{s}})(n/n_{\mathrm{s}}) \quad (4)$$

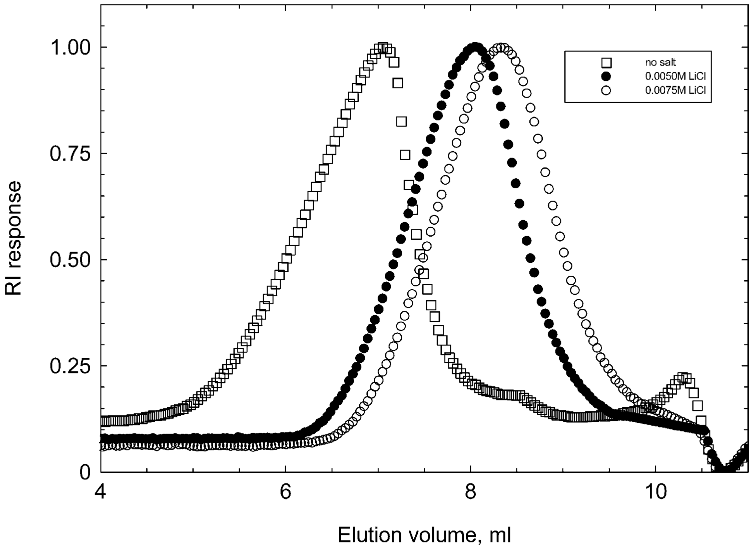

Fig. 1. Refractometric records of LiCl moderated ion-exclusion of CAP Wako in acetone/water 8/2.

where Δc is an injected concentration difference against a pure solvent, A is a corresponding peak area and the term n/n_s reflects the signal dependence of the deflection-type refractometer on the refractive index (Wyatt, 1993) of the solvent used for analysis and external calibration. The values without subscripts relate to CAP and those with subscripts refer here to sodium chloride injected as an external standard in 0.1 M NaCl as a mobile phase under identical conditions.

Two 250 mm long stainless-steel columns, 6 mm I.D., (supplied by Tessek, Prague, Czech Republic) packed with diol-modified LiChrospher 1000 and 4000 (Merck, Darmstadt, Germany) packings prepared according to a described procedure (Porsch, 1993) were connected in series.

LiCl-containing mobile phases were prepared by weighing and filtered with 0.2 μm Millex-FG 25 mm diameter filters (Millipore S.A., Molsheim, France).

Polymer solutions in mobile phases were prepared by weighing and filtered with Millex-HV$_{13}$, 0.45 μm filters (Millipore, Bedford, MA) when injected.

3. Results and discussion

A typical ion-exclusion-moderated SEC behavior of both polymers studied is shown in Fig. 1 for CAP Wako in a salt-free, 0.005 and 0.0075 M acetone/H_2O = 8/2 as a mobile phase. An early elution and increased asymmetry of the peak obtained in pure solvent mixture indicates elution near the exclusion limit of the column set used, indicating large R (Eq. (1)) values. Average pore sizes of LiChrospher packings used are 100 and 400 nm; hence, charge interactions operating over these distances here are confirmed. Let us note for comparison that the Debye length $1/\kappa$ decreases from ~1000 nm in pure water to ~10 nm in 0.001 M uni-univalent electrolyte solution (van Oss, 1994). Addition of a small amount of salt decreases the ionic repulsion distance and the higher is the LiCl content in the mobile phase the higher is the shift of polymer peak along the elution volume axis. The observed shift and better symmetry of peaks compared with salt-free conditions indicate correct SEC separation. If the salt content in the mobile phase was increased above 0.01 M a partial adsorption of both polymers studied was observed during three or four first injections until a constant peak area was obtained. This indicates suppression of the electrostatic repulsion distance below the operational distance of attractive forces originating from a low amount of active adsorption sites of the column packing. A further increase in salt concentration above 0.07 M resulted in a complete sample adsorption due to the hydrophobic effect. Thus, salt concentrations as in Fig. 1 were assumed to be optimum ones.

The use of light-scattering detection for polyelectrolytes deserves attention especially at low-salt conditions and in the case of a copolymer as the apparent molecular weights, different from true ones, may be obtained. The related issues may be summarized as follows: (i) Heterogeneity in chemical composition of copolymers may cause a systematic error in molecular weight determined by light scattering (Benoit & Froelich, 1972) as the refractive index increment of a copolymer cannot be assumed to be constant as in the case of homopolymers. (ii) An error may be introduced due to Donnan equilibrium within the coil in the case of salt containing eluent. In general, this error is found to be smaller than other errors of the light-scattering technique (Nagasawa & Takahashi, 1972). (iii) Light scattering intensity from an ionized polyelectrolyte dissolved at extremely low or no salt content is due to low osmotic compressibility of polyelectrolyte coils, lower than its scattering intensity in the non-ionized state. (iv) Preferential adsorption of one component of a two-component solvent mixture may take place within the polymer coil when this component has a

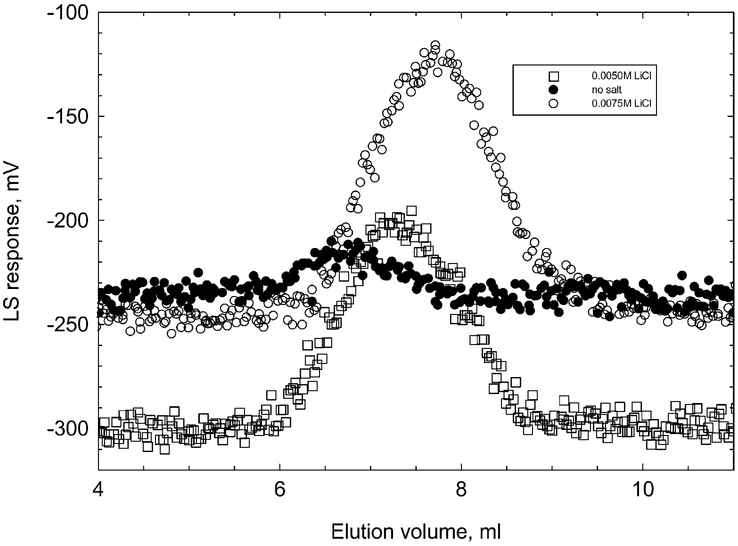

Fig. 2. Scattering intensity of CAP Eastman in acetone/water 8/2 as a function of LiCl addition.

greater affinity to the dissolved polymer. Fortunately, it has been shown (Cassasa & Eisenberg, 1964) that replacement of the refractive index increment of a polymer studied with the value obtained after dialysis of its solution against pure solvent is sufficient in this case to avoid error in molecular weight. In the previous paper (Porsch et al., 2000), we have summarized the relevant literature showing that the effects (i) are in general negligible in the case of statistical copolymers and (ii) and (iii) become negligible already at quite low salt concentrations. As (ii) and (iii) are dependent on salt concentration, we suggested that a comparison of two measurements at sufficiently low but substantially different salt concentrations conveniently prove the absence of this kind of bias.

Fig. 2 shows a substantially lower scattering intensity in pure acetone/water mixture and indicates pronounced quenching of light scattering due to the presence of unscreened long-range electrostatic ordering in the absence of salt. Visual comparison of peaks at both widely different concentrations indicates substantial increase in scattering intensity and no visible difference in the peak area; only a salt-concentration-dependent shift of these peaks along the elution volume axis (corresponding to that in Fig. 1) is observed. A relevant conclusion is that electrostatic long-range ordering is sufficiently screened already at the lower salt concentration used here.

Variation of d*n*/d*c* operating in light scattering experiment due to preferential sorption must be expected in all

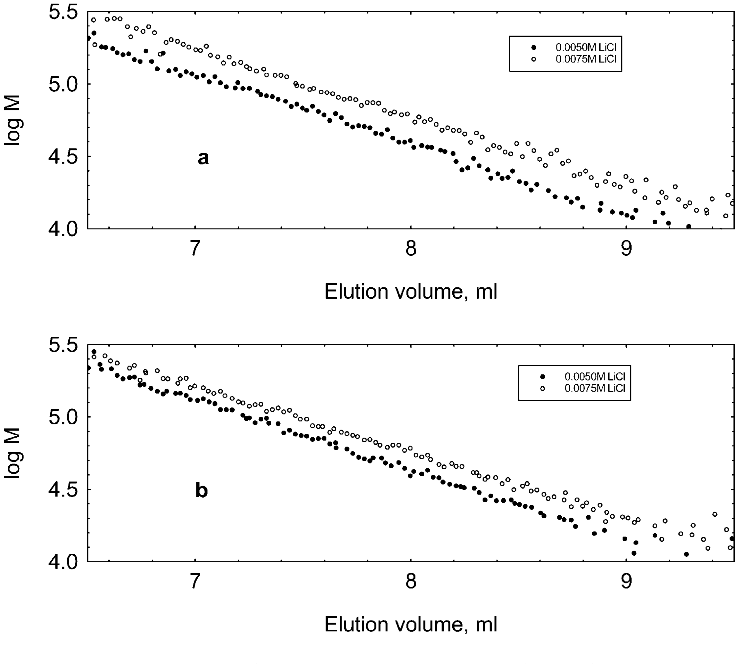

Fig. 3. Calibrations log $M = f(V_e)$ of CAP Eastman (a) and Wako (b) as a function of salt addition.

Table 1
CAP molecular weights and polydispersity indices determined in acetone/water 8/2 containing different amount of LiCl

CAP sample	LiCl content (M)			
	0.005		0.0075	
	M_w	M_w/M_n	M_w	M_w/M_a
Wako	56,900	1.5	53 300	1.6
Eastman	54,400	1.6	54 700	1.7

experiments in binary solvents when solvent components have different refractive indices especially in solvent/non-solvent and co-solvent mixtures. The Hummel–Dreyer approach (Hummel & Dreyer, 1962) already described in 1962 for studies of binding of low-molecular-weight solutes to proteins, frequently referred (Korpela & Himanen, 1988) to as dynamic dialysis equilibrium, can be applied here. When the preferential sorption effect exists, the polymer solution establishes an osmotic equilibrium between polymer coils and a solvent outside of coils. When a solvent component having higher refractive index is preferentially adsorbed by a polymer, its d*n*/d*c* value should increase relatively to the original solvent composition. At the same time, a certain deficit of this (preferentially adsorbed) component exists in the solvent outside of the coils. SEC column then separates polymer coils in osmotic equilibrium with the mobile phase from a deficit peak of solvent. The original Hummel–Dreyer technique uses negative peak (or positive peak — depending on the refractive indices of the solvent components in question) to quantify the amount of bonded solute to a protein molecule. It follows from its principle that d*n*/d*c* value that operates in this experiment is a dialyzed one. Assuming that the correct mass concentration of a polymer injected is known, Eq. (4) can be used to calculate dialyzed d*n*/d*c* value provided that the refractometer is properly calibrated. Let us note that this approach is free of well-known difficulties related to a partial permeability of dialysis membranes frequently found in a classical dialysis experiment. The values $dn/dc = 0.121$ and 0.111 were obtained for CAP Eastman and Wako, respectively, and may be compared with $dn/dc = 0.109$, conventionally determined for both samples by differential refractometry of non-dialyzed solutions. An increase of 11% seen in the case of CAP Eastman certainly exceeds the error of both determinations and indicates that acetone is preferentially adsorbed within coil domains. On the other hand, dialyzed and non-dialyzed d*n*/d*c* of CAP Wako agree within the error of determination. The relevant conclusion is that these polymers differ to some degree in chemical composition, most likely in the content of phthalate groups and these groups are the acetone sorption sites. The increased number of phthalate groups per coil means that also the number of carboxyl groups per coil is higher, i.e. CAP Eastman should exhibit slightly higher charge density when compared to CAP Wako. This is corroborated by a comparison of shifts of $\log M = f(V_e)$ calibrations along the elution volume axis when the salt concentration is varied for both samples (Fig. 3). R_{SEC} in Eq. (1) should be a function of polyelectrolyte expansion/contraction when low salt content changes and larger variations may be expected in the case of higher charge density. This is what is seen in Fig. 3 for CAP Eastman.

The log *M* vs. elution volume calibration curves actually

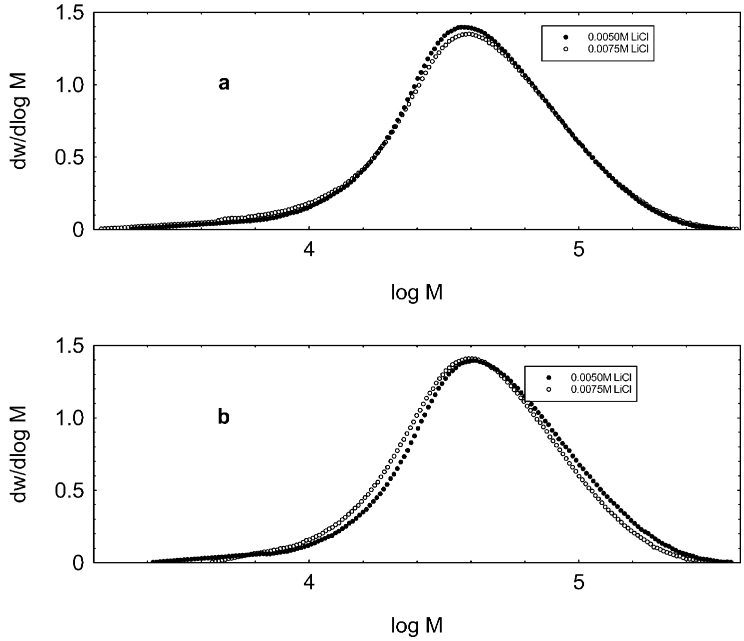

Fig. 4. Molecular weight distributions of CAP Eastman (a) and Wako (b) determined at different salt concentrations.

contain M values calculated for the concentration range between zero and that related to the maximum in the RI curve, assuming a negligible A_2c term in Eq. (3). Their correct, approximately linear shape at both salt contents used thus confirms that injected concentrations are low enough to fulfill this requirement and indicates the absence of any other concentration-dependent bias. Let us note that the dilution factor due to the SEC process should be around 10 in our case.

Table 1 shows that molecular weights and polydispersity indices of both polymers are identical in the presence of 0.005 and 0.0075 M LiCl within an experimental error. The absence of any salt concentration-dependent data bias (adequate electrostatic screening) is thus again confirmed at $c_{\text{salt}} \geq 0.005$ M. The values in Table 1 were calculated using 'dialyzed' dn/dc values; with conventionally determined dn/dc, M_w of the Wako sample would not change above the error of measurement but an increase from average $M = 54,500$ from both concentrations would go up to $M = 67,600$, i.e. by 24%. Table 1 shows that both polymers are almost undistinguishable as far as the molecular weight and polydispersity indices are concerned. This is seen as well from a comparison of molecular weight distributions of both samples determined again at both salt concentrations (Fig. 4) as a final proof of absence of any bias due to the use of quite low salt concentrations.

Only a subtle difference between Wako and Eastman distributions is seen, the latter being slightly more extended to lower molecular weights. It can be concluded that both polymers are surprisingly similar with regard to molecular weight distributions and differ a little in chemical composition in terms of the content of phthalate groups.

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