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Ion-exclusion controlled size-exclusion chromatography of methacrylic acid–methyl methacrylate copolymers

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Abstract

Controlled ion-exclusion allows compensation of hydrophobic adsorption in size-exclusion chromatography of negatively charged methacrylic acid–methyl methacrylate (Eudragit) polymers using methanol as a mobile phase. Properly selected low-ionic-strength conditions below 5 mM LiCl provide correct separation in the size-exclusion mode. Possible disturbing effects, mainly related to light scattering, under low-salt conditions are discussed and shown to be negligible if on-line concentration–light scattering detection is used. The absence of these disturbances is checked by a comparison of experiments performed in methanol containing 1.25 mM and 2.5 mM LiCl. Molecular mass averages and distributions identical within the experimental error are obtained. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

An Eudragit group of pharmaceutical excipients [1] represents copolymers of methacrylic acid with different acrylic or methacrylic esters. They are primarily used in oral capsule and tablet formulations as film coating agents. Eudragit L and S types are used as enteric coating agents since they are resistant to gastric fluid, e.g., Eudragit L100 and S100 are soluble at pH>6 and pH>7, respectively. Commercial products are characterized only by solution viscosity; the knowledge of their molecular masses and molecular mass distributions is desirable as there

is a close relation between these parameters and coating technology performance.

Conventional size-exclusion chromatography (SEC) of these copolymers 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, 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 for these polymers are found only within the group of acetone, alcohols, dichloromethane and

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ethyl acetate. Solvent requirements thus considerably limit the application of polymer-based SEC columns. Furthermore, the solvent choice is also limited by a requirement for sufficiently large values of the refractive index increment, dn/dc , to avoid loss of detection sensitivity.

Almost all common column packings bear a negative surface charge [2,3]. 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 polar solvents other than water but persist to some degree. Most of the SEC work to date has focused on identification and elimination of ion-exclusion and polyelectrolyte effects in order to be able to use columns in the pure SEC mode. As an exception, Kopaciewicz and Regnier [3] suggested to 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 has shown in his studies [4–8] that both the effective hydrodynamic shape of a solute and an 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

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

where \bar{a} is the average electrostatic repulsion distance in equilibrium in multiples of Debye length κ^{-1} , R_{SEC} is the rotationally averaged mean radius of the solute and R_{IF} is the interfacial contribution to the total solute radius R . In fact, 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 in the case of the coiled polyelectrolyte, it could be a complex function of ionic strength. The conventional universal calibration approach assumes negligible R_{IF} , i.e., high ionic strength conditions, and

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

where $[\eta]$ is the intrinsic viscosity and M is the molecular mass of a macromolecule in bulk solution. Potschka also pointed out [7] that electrostatic repulsion may be a very effective means of prevent-

ing adsorption and thus of establishing pure SEC conditions at low ionic strengths. Lifshitz–van der Waals (attractive) forces in aqueous media operate at relatively long separation distances and hydrophobic interaction may also extend up to several nanometers [9]. Electrostatic repulsion is just a convenient long-range force suitable for their compensation to obtain the net repulsion defining solution stability [9]. The same should apply when some hydrophobic interaction between the surface of packing and a solute takes place.

Assuming that the correct SEC separation according to R (Eq. (1)) can be established at a low ionic strength to prevent solute–packing attractive interaction, the knowledge of molecular mass 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 multi-angle laser light-scattering (MALLS) detection. The only requirement is that the pore diameter of the column packing must be sufficiently large, $R_{\text{pore}} \gg R$, to avoid loss of resolution due to total exclusion of the largest coils of the sample from the pores. This technique should be generally applicable to all negatively charged polymers otherwise difficult to analyze at high ionic strengths due to their irreversible adsorption to the column packing.

This approach is used in this paper to determine molecular mass distributions of negatively charged Eudragit polymers using wide-pore silica based diol-modified SEC packing. The mobile phase is methanol containing small amounts of LiCl as a modifier of charge interactions.

2. Experimental

2.1. Materials

Eudragit L100 (methacrylic acid–methyl methacrylate, ratio 1:1 [1]) and S100 (methacrylic acid–methyl methacrylate, ratio 1:2 [1]) were commercial products of Röhm-Pharma, Weiterstadt, Germany, lot nos. 016048 and 01604901, respectively. Analytical reagent grade methanol (Lachema, Neratovice, Czech Republic) and anhydrous lithium

chloride (Fluka, Buchs, Switzerland) were used as obtained.

2.2. Chromatography

Modular chromatograph consisted of a Constametric 3200 MS pump (Thermo Separation Products, Riviera Beach, FL, USA), a Pharmacia injection valve V-7 with 100- μ l loop (Pharmacia and Upjohn, Uppsala, Sweden), a Chromatix KMX-6 LALLS detector (LDC/Milton Roy, Sunnyvale, CA, USA) and an R-401 differential refractometer (Waters, Milford, MA, USA) connected through a Black Star (Huntingdon, UK) 2308 A/D converter to an IBM-compatible computer. An 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 ($6\text{--}7^\circ$)

$$(K^*c)/R_\Theta = 1/M_w + 2A_2c \quad (3)$$

where c is the concentration of scattering species, R_Θ is the excess Rayleigh scattering factor, M_w is the weight-average molecular mass of scattering species and A_2 is the second virial coefficient. $K^* = (2\pi n^2 / N\lambda^4)\nu^2$ where n is the refractive index of the solvent, λ is the wavelength in vacuo (633 nm), N is the Avogadro number and ν is the RI 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. The 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_2c may be neglected if the concentration of the injected solution is low enough. Conventional calibration of $\log M$ vs. elution volume (V_e) is thus directly obtained. Laboratory-written software (M. Netopílk, Institute of Macromolecular Chemistry) allows on-line data accumulation and all calculations of molecular mass distributions and their averages.

Two stainless steel columns (250 \times 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 recently described procedure [10] were connected in series.

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

Polymer solutions in mobile phases were prepared by weighing and filtering with Millex-HV₁₃, 0.45- μ m filters (Millipore, Bedford, MA, USA) when injected.

3. Results and discussion

The typical ion-exclusion-moderated SEC behavior of both polymers studied is illustrated in Fig. 1 for Eudragit S in a salt-free, and in 1.25 mM and 2.5 mM LiCl in methanol as a mobile phase. The sharper peak obtained in pure methanol indicates elution near to the exclusion limit of the column set used indicating large R (Eq. (1)) values. The average pore sizes of LiChrospher packings used are 100 and 400 nm; hence, charge interactions are confirmed to operate in methanol over these distances. Let us note for comparison that the Debye length $1/\kappa$ decreases from \sim 1000 nm in pure water to \sim 10 nm in 1 mM uni-univalent electrolyte solution [9] and does not decrease considerably in methanol. The addition of small amount of salt decreases ionic repulsion distance and the higher the LiCl content in the mobile phase the higher the shift of the polymer peak along the elution volume axis. The observed shift and broadening of peaks as compared with salt-free conditions indicate correct SEC separation. If the salt content in the mobile phase was increased above 5 mM an irreversible adsorption of both the studied polymers was observed. This indicates suppression of electrostatic repulsion distance below the operational distance of attractive forces in our system.

Fig. 2 indicates that the use of dual RI–LALLS detection allows to perform correct SEC separation under the low-salt conditions used; an approximately linear $\log M$ vs. elution volume 'calibration' (valid for this salt content only) is obtained. A behavior analogous to that in Fig. 2 is observed for both samples in salt-containing eluents.

The use of light-scattering detection for polyelectrolytes deserves attention especially at low-salt conditions and in the case of a copolymer as the

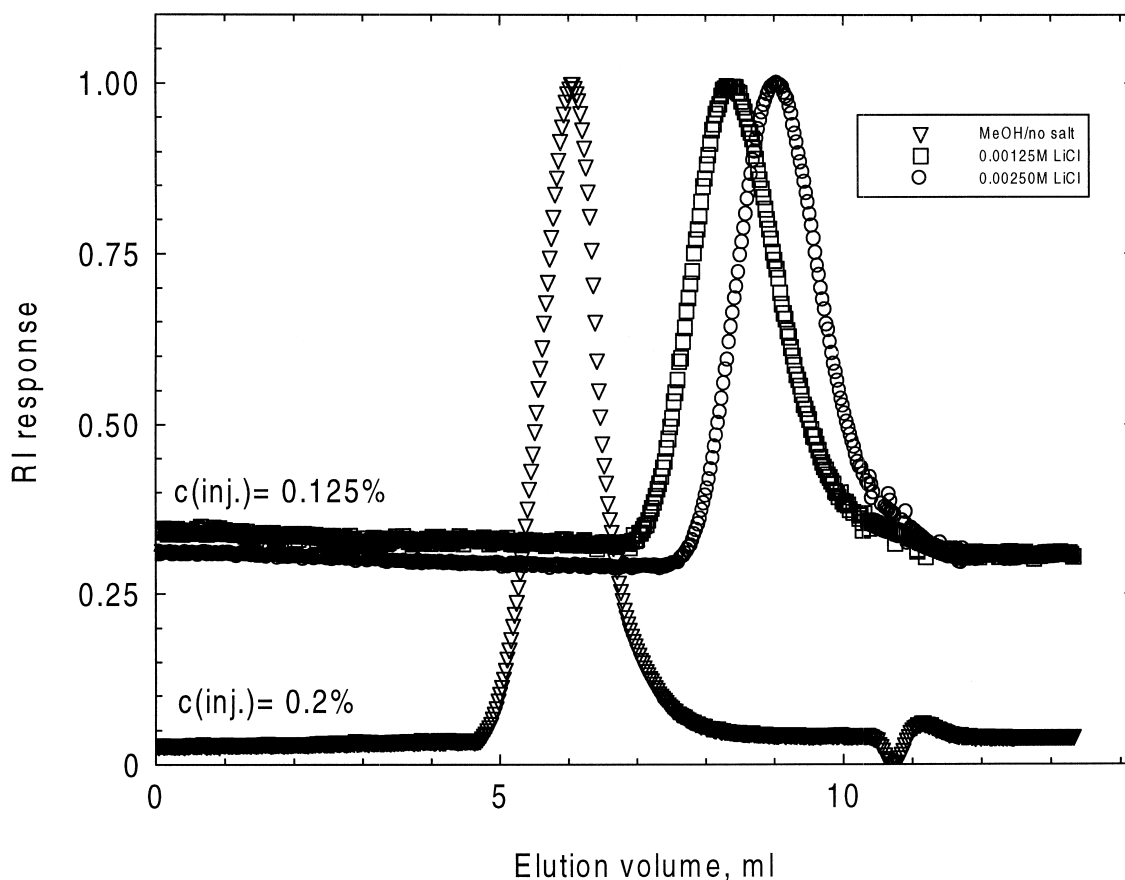


Fig. 1. LiCl moderated ion-exclusion of Eudragit S100 in methanol.

apparent molecular masses, different from true ones, may be obtained.

It has been known for many years [11] that the light scattering intensity from an ionized polyelectrolyte dissolved at very low or no salt content is due to low osmotic compressibility of polyelectrolyte coils smaller than its scattering intensity in the nonionized state. Also, strange concentration and angular dependences of the scattered intensity are obtained. There is a vast amount of recent static and dynamic light scattering work in this field; a detailed discussion of all complex phenomena observed in aqueous solutions is beyond the scope of this paper. Only limited references and conclusions relevant to our case of LALLS detection related to the reliability of molecular masses measured will be presented here. A typical feature of these experiments at low salt concentrations [12–15] is the observation of a

peak in the angular dependent light scattering intensity due to intermolecular long-range electrostatic forces ('Bragg peak'). A very low amount of salt around $10^{-4} M$ is usually sufficient [14] to cancel this peak. Nevertheless, a non-linear curve with negative initial slope which gradually changes to a straight line with a positive slope can be observed [12] on addition of more salt. Fortunately, it has been found that non-linear angular extrapolations of normalized reciprocal scattered intensity obtained at different salt additions have a common intercept [12] and the same was observed for non-linear extrapolations against polymer concentration [16]. This means that the final measured molecular mass is a constant independent of the salt content. These observations indicate that the effect of long-range electrostatic interactions and coil expansion (expressed as an increase in R and large second and higher virial

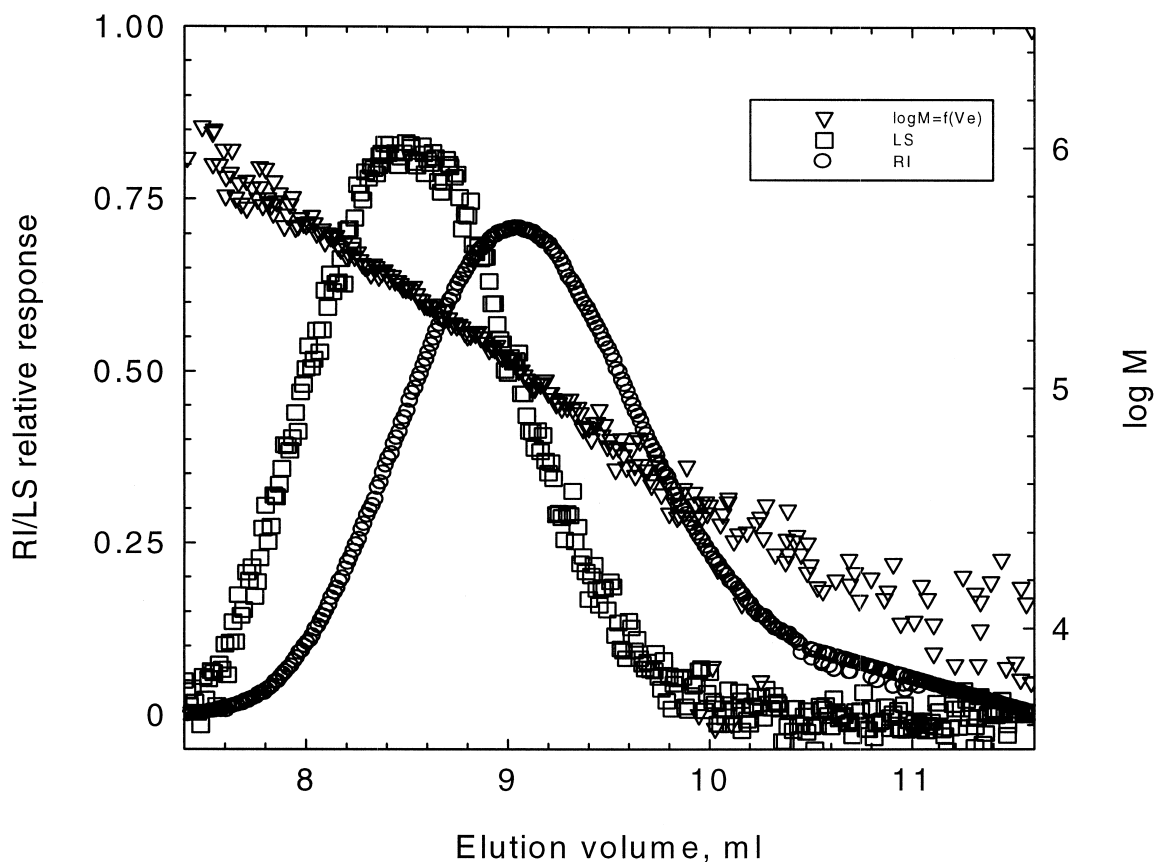


Fig. 2. SEC-LALLS of Eudragit S100 in methanol containing 2.5 mM LiCl.

coefficients) should disappear at sufficiently low polymer concentrations in the presence of a low amount of a simple salt. Nevertheless, increased difficulties as compared with high-salt conditions due to more pronounced curvatures of angular and concentration dependences should be expected. The use of LALLS detection seems preferable in this respect. Some criteria [13,17–19] relating polymer concentration to the salt concentration were formulated to define the range where single polyion behavior should be seen: For quaternized poly(2-vinylpyridine), the concentration in water of the order of 10^{-4} g/ml, the requested salt concentration should be above 4 mM [13]. For sodium poly(styrenesulfonate) at a concentration of 0.025 g/ml, at least 10 mM of salt should be added [17]. Let us note that the total ionic strength of the solutions is higher due to a significant contribution of polyelec-

trolyte counterions [18]. Both these polymers are highly charged strong polyelectrolytes. In the case of weak polyelectrolyte, the electrostatic effects should decrease with decreasing fractional ionization [18] and also with decreasing permittivity of the solvent [19] if a solvent other than water is used.

It is well known that heterogeneity in chemical composition of copolymers may cause a systematic error in molecular mass determined by light scattering [20]. The RI increment of a copolymer cannot be assumed to be a constant as in the case of homopolymers and apparent molecular masses are in general obtained as a function of the RI of the solvent used. The decisive parameter [21] determining a bias of true molecular mass of a copolymer is a difference $\nu_A - \nu_B$ where ν_A and ν_B denote the RI increments of the neat A and B homopolymers, respectively. It was shown that the bias [20,21]

should be low if ν_A , ν_B are high and of the same sign and should approach zero if ν_A and ν_B approach each other. Also, the coil size (Eq. (1)) is a function of composition in the case of a copolymer. This gives rise to ‘slice’ heterogeneity in molecular masses, i.e., a mixture of copolymers having different molecular masses but the same R value is eluted at a fixed elution volume. It was shown in a recent paper [22] that this effect of chemical heterogeneity on the evaluation of molecular masses and distributions from SEC data is negligible when $\nu_A = \nu_B$ for statistical copolymers, provided the dual RI–LS detection is used. It also remains quite small in the case of statistical and conversional heterogeneity unless ν_A and ν_B differ considerably. In the realistic range of $0.05 < \nu \text{ (ml/g)} < 0.2$, the molecular masses determined are essentially correct.

The RI increment in Eq. (3) should be, strictly speaking, the value determined after dialysis of a

polymer solution against pure binary solvent containing salt to account for the Donnan equilibrium [11]. When the dialysis is omitted, an error may be introduced and the measured molecular mass becomes apparent. In general, this error is found to be smaller than other errors of the light-scattering technique. Some exceptions exist only in the case of salt-containing aqueous solutions of highly charged strong polyelectrolytes like polyphosphates [11]. On the other hand, the Donnan effect is fully negligible in protein solutions containing salt [23]. As another example, no change in ν before and after dialysis of aqueous solutions of hyaluronic acid within the salt concentration range 2–100 mM NaCl was observed [24].

Summarizing, all the above described charge effects observed in aqueous solutions should diminish with decreasing charge density when a less polar solvent is used as a mobile phase instead of water;

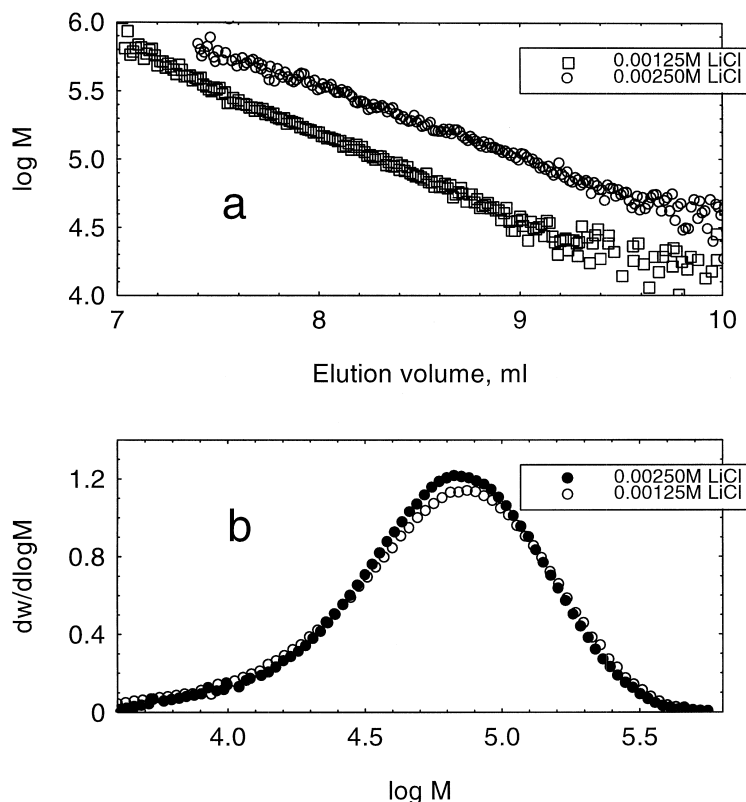


Fig. 3. $\log M$ vs. V_e (a) and molecular mass distributions (b) of Eudragit L100 as a function of salt addition.

hence, for relative low-charge density weak polyelectrolytes in methanol studied in this paper can be safely expected to be negligible. Also, the difference $\nu_A - \nu_B$ should be quite small when both monomers are alike. Accordingly, the RI increments of Eudragit L100 and S100 differing substantially in the content of both comonomers and, at the same time, in the content of charged groups, were determined in methanol containing 1.25 mM of LiCl as identical within the experimental error as $\nu = 0.124 \pm 0.004$ and $\nu = 0.121 \pm 0.004$, respectively.

The $\log M$ vs. elution volume calibration curve actually contains M values calculated for the concentration range between zero and that related to the maximum of RI curve, assuming the A_2c term in Eq. (3) is negligible. The quenching of light scattering due to the long-range ordering of the polyelectrolyte coils at fixed low salt concentration, if present at high polymer concentration, should lead to lower M

values. No distortion of the calibration curve thus indicates that the salt content is sufficient to suppress this effect at least up to the polymer concentration at the maximum of the RI curve. Let us note that the dilution factor due to the SEC process should be around ten.

Long-range ordering at low salt concentrations should rapidly disappear when the salt content increases at fixed polymer concentration. A simple test of its absence is possible if two mobile phases differing in the salt content by a factor of two at a constant polymer concentration are used in the SEC experiment. The $\log M = f(V_e)$ calibrations are shown in Figs. 3a and 4a for Eudragit S100 and L100, respectively. In agreement with Eq. (1), only a parallel shift along the V_e axis is seen, without any change in shape, thus confirming no effect of long-range electrostatic interaction at both the salt contents used. The corresponding M_w and M_w/M_n

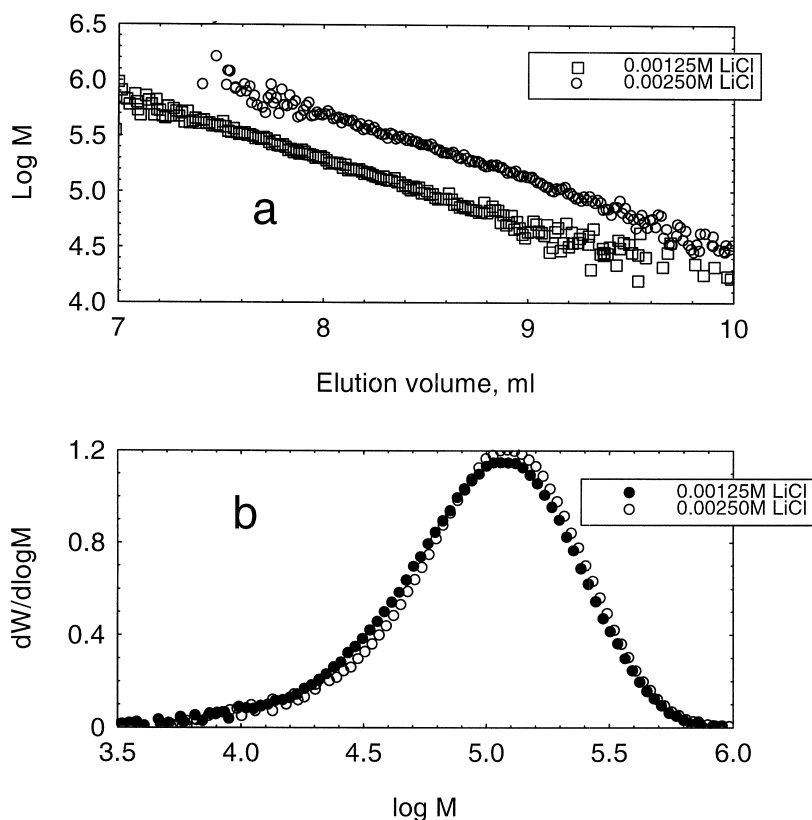


Fig. 4. $\log M$ vs. V_e (a) and molecular mass distributions (b) of Eudragit S100 as a function of salt addition.

Table 1
Eudragit molecular masses and polydispersity indices determined in methanol containing different amount of LiCl

Eudragit sample	LiCl content (mM)					
	0		1.25		2.5	
	$(M_w)_{app.}$	$(M_w/M_n)_{app.}$	M_w	M_w/M_n	M_w	M_w/M_n
L100	31 300	1.6	114 800	2.1	115 300	1.9
S100	42 500	1.3	132 000	2.3	139 100	2.2

values (M_n =number-average molecular mass) are summarized in Table 1.

It can be seen that molecular masses and polydispersity indices are identical in the presence of 1.25 mM and 2.5 mM LiCl within an experimental error. The adequate electrostatic screening is thus confirmed at $c_s \geq 1.25$ mM if the injected polymer concentration $c_p \leq 0.125\%$. On the other hand, substantially lower apparent values of M_w in the absence of salt indicate quenching of light scattering due to the presence of unscreened long-range electrostatic ordering. Also, lower values of M_w/M_n agree with the observation of elution in the vicinity of the exclusion limit of the column set used (Fig. 1).

Both polymers analyzed also differed in charge density due to different ratios of the charged and uncharged monomers used in their polymerization. Their statistical and compositional heterogeneity, if significant, should be paralleled by some charge heterogeneity. A charge distribution effect, if pronounced, should change R_{SEC} in Eq. (1), vary with the elution volume and depend on c_s in the mobile phase. The Donnan effect mentioned, if significant, should also vary with c_s when the polyelectrolyte concentration is kept constant. The molecular mass distributions of both Eudragit L100 and S100 in Figs. 3b and 4b, respectively, determined at salt contents differing by a factor of two coincide within an experimental error. No change in molecular mass averages and distributions with c_s thus means that these effects are also negligible within an experimental error.

4. Conclusions

A novel SEC approach is proposed for distribution analysis of Eudragit polymers exhibiting irreversible

adsorption due to significant attractive interactions at salt concentrations above 5 mM in the mobile phase. An on-line combination of RI–LALLS detectors can be successfully used for SEC at the ionic strength low enough to prevent adsorption but allowing correct SEC behavior provided that wide-pore packing is used. To suggest that all side effects of low salt contents on the experimental data are absent, experiments at several salt concentrations (all of them low enough) are recommended.

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