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Molar mass characterization of cationic methyl methacrylate–ethyl acrylate copolymers using size-exclusion chromatography with online multi-angle light scattering and refractometric detection

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Abstract

Size-exclusion chromatography (SEC) combined with online multi-angle light scattering (MALS) and refractometric (RI) detection has been employed for the molar mass characterisation of water-insoluble cationic methyl methacrylate–ethyl acrylate copolymers (Eudragit RS and RL). Due to their positive charge, cationic polymers are particularly difficult to separate on a SEC column, in worst cases being completely adsorbed on the oppositely charged packing material. This work has examined how a careful addition of salt (LiCl) to the copolymer solution in ethanol decreases the electrostatic interactions, clearly seen as a decrease in elution volume from the SEC column as well as an improved recovery. At a certain level of ionic strength, typically about 50 m*M*, the copolymer recovery from the SEC column reached 100% and molar mass distributions corresponding to the complete sample could be obtained. The combined MALS/RI detection gives the opportunity to measure the absolute molar mass independent of recovery and retention. Thus, in this study, it turned out to be a favourable tool for tracing the changes in elution behaviour of the charged copolymer as the ionic strength was increased.
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1. Introduction

Cationic methyl methacrylate–ethyl acrylate copolymers (Eudragit RL, RS) belong to a group of pharmaceutical excipients primarily used as controlled release film coating agents in oral capsule and tablet formulations [1]. Eudragit RL containing 10% of quaternary ammonium groups forms films freely permeable to water, whereas films formed from Eudragit RS containing 5% of quaternary ammonium groups are only slightly water permeable. Both polymers are water insoluble and film coatings prepared from them give a pH-independent release of active substance. Commercial products are characterized only by solution viscosity; the knowledge of their molar masses and molar mass distributions is desirable as there might be a close relation between these parameters and coating technology performance as well as film permeability.

Conventional size-exclusion chromatography (SEC) of these copolymers with single refractive index (RI) detection appears difficult if not im-

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possible. No calibration standards are available and the universal calibration approach is hindered by their polyelectrolyte character [2]. Since almost all common column packings bear a negative surface charge [3,4], cationic polymers appear to be the most difficult polymers to analyse by SEC as charge attraction may lead to an excessive retention or even complete adsorption. Fairly high salt contents in the mobile phase necessary to suppress the charge attraction in this case also reduce the polyelectrolyte effect to some degree and may, if increased further, cancel the polyelectrolyte effect completely. However, unwanted adsorption to the column packing material might appear due to the onset of hydrophobic solute–solvent interaction. A key issue is thus to find a proper column packing–mobile phase combination that allows correct SEC behaviour free of detrimental adsorption effects. Unfortunately, good solvents of these copolymers are found only within the group of acetone and alcohols. Solvent requirements thus considerably limit the application of polymer-based SEC columns. To make matters worse, the solvent choice is also limited by a requirement for a sufficiently large refractive index increment (dn/dc, i.e. the slope of the dependence of the refractive index, n, of the solution on the solute concentration, c) values to avoid loss of detection sensitivity.

The combination of light scattering and refractometric detection thus remains the only reasonable alternative here provided that proper column–mobile phase combination is found. The main advantage of this combination is that the elution profile shifts for polyelectrolytes when changing ionic strength are irrelevant as far as data evaluation is concerned [2]. In other words, true molar mass distributions are obtained even in the case when polyelectrolyte effect is not sufficiently suppressed, provided that some apparent size (in a general sense) governs the separation [5,6].

SEC with dual multi-angle light scattering (MALS) and refractometric detection is used in this paper to obtain correct molar mass distributions (MMDs) of two cationic Eudragit copolymers using a suitable polymer-based SEC packing. Ethanol (95%) containing various amount of LiCl is shown to be a favourable solvent which allows convenient manipulation and optimisation to remove unwanted sample-packing interactions as a necessary condition to maintain correct SEC behaviour.

2. Experimental

2.1. Materials

Two aqueous dispersions of cationic methyl methacrylate–ethyl acrylate copolymers with 10% (Eudragit RL) or 5% (Eudragit RS) of quaternary ammonium groups, respectively, were products of Röhm, Darmstadt, Germany. Sample solutions with a polymer concentration of 3 mg/ml were prepared by dissolving dried dispersions, i.e. the corresponding film, in the mobile phase. A specific amount of the dispersion was dried to the constant weight in the flask in which the sample solution was prepared. The dispersions were dried at room temperature by placing the flask on a ventilated table. The amount of dispersion was weighed before and after drying which made it possible to calculate the exact concentration of the sample solution. After adding solvent (mobile phase) to the film, the solutions were kept at room temperature under gentle stirring for at least 2 days using a magnetic stirrer. The sample solution was then injected directly on to the column without further preparation.

Five different mobile phases were prepared consisting of 95% ethanol (Finsprit, Kemetyl Haninge, Sweden) with varying amounts (1, 10, 50 100 or 150 mM) of LiCl (ACS reagent, Sigma, St Louis, MO, USA).

2.2. SEC–MALS/RI

The system consisted of a combination of two size-exclusion chromatography columns, Labio Biospher GMB 1000 and GMB 200, particle size 10 μm, 300 mm×7.5 mm (Labio, Prague, Czech Republic). The columns were connected online to a multi-angle light scattering detector (DAWN DSP, Wyatt Technology, Santa Barbara, CA, USA) and a refractive index detector (Optilab DSP, Wyatt Technology). The instrument wavelength was 633 nm for both detectors. ASTRA 4.73 software (Wyatt Technology) was used for analysis of the obtained RI and MALS chromatograms. Further, the instrumental set-

up contained a degasser (ERC-3110, Erma Optical Works, Tokyo, Japan), a LC pump (10ADvp, Shimadzu, Kyoto, Japan) and an autosampler (717+ autosampler, Waters, Milford, MA, USA) equipped with a 100-μl sample loop. A 0.2-μm in-line filter [Chemical resistant Regenerated Cellulose (RC) membrane, type 184, Sartorius, Göttingen, Germany] was placed after the pump prior to the autosampler. The analyses were carried out at room temperature (22 °C) and the flow-rate was set at 0.5 ml/min.

The use of SEC–MALS/RI provides the opportunity to determine the molar mass and radius of gyration in an absolute manner and for each fraction eluted from the column. The MALS measures the light scattering intensity at 18 different angles (22.5–147°) [7] and the RI the concentration of each slice. These parameters are converted into the molar mass using the well-known relationship:

$$Kc/R_{(\theta)} = 1/(M_w P_{(\theta)}) + 2A_2 c \quad (1)$$

where c is the concentration of the sample in each fraction, $R_{(\theta)}$ the excess Rayleigh ratio, $P_{(\theta)}$ is the form-factor and M_w the weight-average molar mass. A_2 is the second virial coefficient, the term $A_2 c$ can be neglected for dilute sample solutions. K is an optical constant, $K = 4\pi^2 n_0^2 (dn/dc)^2 \lambda_0^{-4} N_A^{-1}$, where n_0 is the refractive index of the solvent at the incident radiation wavelength, λ_0 is the incident radiation wavelength, N_A is Avogadro's number and (dn/dc) is the refractive index increment of the polymer in a specific solvent.

2.3. Measurement of (dn/dc)

The refractive index increment (dn/dc) was determined separately using the Optilab DSP. The (dn/dc) value was determined for both samples dissolved in 95% ethanol with 10 m*M* LiCl and in 95% ethanol with 50 m*M* LiCl. The samples, in six different concentrations, were injected directly into the refractive index detector. Measurements were carried out with a flow-rate of 0.5 ml/min, a 1-ml polyether ether ketone (PEEK) sample loop (Rheodyne, Cotati, CA, USA) was used, the detector wavelength was 633 nm and the detector temperature was set at 40 °C. The (dn/dc) value was calculated using the DNDC software (Wyatt Technology) and was found to be approximately the same despite differences in the LiCl content, 0.116 ml/g (10 m*M* LiCl) and 0.111 ml/g (50 m*M* LiCl).

The (dn/dc) value is important not only for the molar mass calculations but also for calculation of the recovery. The concentration is calculated for each slice of the peak by dividing the change in refractive index for each slice by the known refractive index increment (dn/dc). The result is the change in concentration of that slice, which equals the concentration of the slice since the baseline represents pure solvent. The sum of the concentrations obtained represents the peak concentration. The obtained mass is then compared to the known injected amount. The recovery is given in percent.

3. Results and discussion

Preliminary screening of various mobile phases was carried out using only one Labio Biospher 1000 column to speed up the experiments and preliminarily judge which various interactions can be expected in the system considered. Ethanol (95%) was found to be a suitable solvent for dissolving the polymethacrylates. Having a column carrying very low but noticeable negative charge which gives rise to ion-exclusion in water as well as in methanol in the case of negatively charged polymers [6], then a long range attractive electrostatic force has to be expected here for a polymer carrying positive charge unless the charge attraction is sufficiently suppressed. It follows from Fig. 1 that such an attractive force in the absence of salt leads to the situation where only a minor part of the sample is eluted and even the majority of that part elutes later than an unretained solute (the total permeation volume, V_{tot}, as indicated in Fig. 1 by an arrow). The small amount of the sample eluted before V_{tot} recorded by the RI unit gives almost no signal on MALS, hence, its molar mass should be very low. A stepwise addition of LiCl to 95% ethanol as mobile phase gradually screened the attractive interaction and improved the sample elution. At this point, it became clear that the samples have a molar mass distribution extending down to the oligomeric range where the single column used does not have sufficient resolution. Hence, a Labio Biospher 200 column having res-

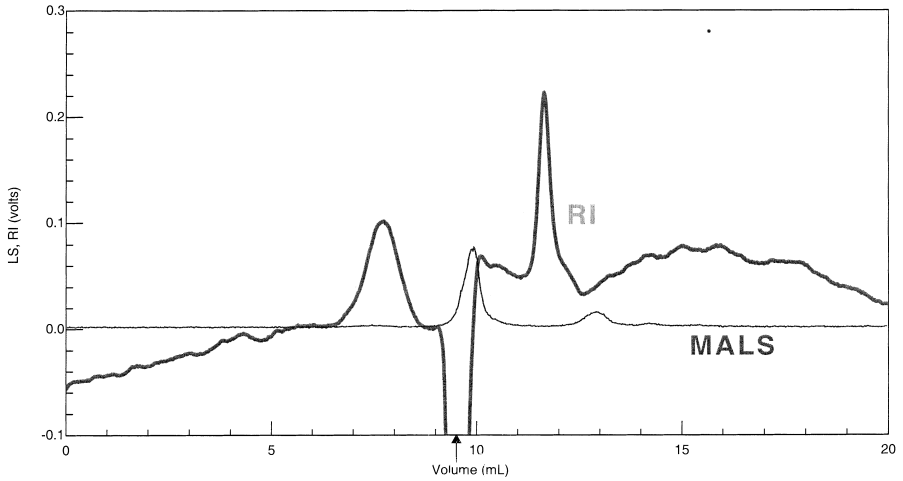

Fig. 1. Chromatographic behaviour of Eudragit RS (5% quaternary ammonium groups) on a single Labio Biospher GMB 1000 column in 95% ethanol without salt. The dried dispersion Eudragit RS was dissolved in the mobile phase and 0.20 mg was injected.

olution down to this range has been added and this combination of two Labio GMB 1000 and GMB 200 columns was further used throughout this study.

Refractometer signals obtained for Eudragit RS (5% quaternary ammonium groups) when the LiCl concentration in 95% ethanol was varied from 1 to 150 m*M* are depicted in Fig. 2. It is immediately seen from an increase in the peak area as a function of LiCl concentration in the mobile phase that a gradual suppression of the attractive interaction takes place. Some part of the sample is seen to be still adsorbed at LiCl concentrations of 1 and 10 m*M*. Starting from 50 m*M* LiCl, the eluted peaks almost coincide (small variations in peak areas of C–E reflect small variations in sample concentrations close to 3 mg/ml) within experimental error and are eluted well before the total permeation volume. No shift in peaks C–E along the elution volume axis indicates the total absence of unwanted interactions here. The same set of experiments was carried out

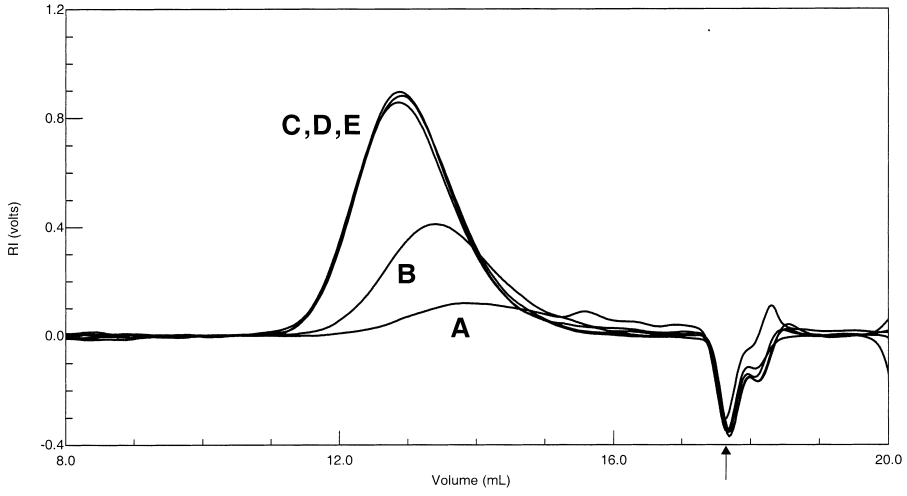

Fig. 2. RI chromatograms of Eudragit RS (5% of quaternary ammonium groups) in 95% ethanol with 1, 10, 50, 100 and 150 m*M* LiCl (A–E). The injected amount was 0.30, 0.30, 0.30, 0.29 and 0.30 mg, respectively. The samples were dissolved in the mobile phase.

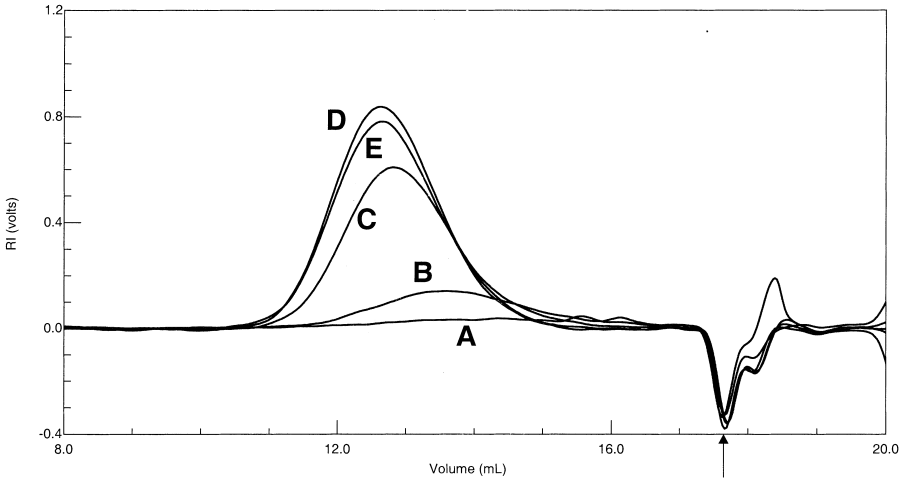

Fig. 3. RI chromatograms of Eudragit RL (10% of quaternary ammonium groups) in 95% ethanol with 1, 10, 50, 100 and 150 m*M* LiCl (A–E). All samples were dissolved in the mobile phase and the injected amount was 0.30, 0.30, 0.27, 0.32 and 0.29 mg, respectively.

with Eudragit RL (10% of quaternary ammonium groups) and is displayed in Fig. 3. An analogous behaviour to the previous sample is observed with the exception of higher concentrations of LiCl needed to elute the sample completely. Only curves D and E do not shift along the elution axis and almost coincide here. These results indicate that both samples should have certain charge distributions. The less charged coils seem to be eluted first at a low salt content and more charged ones remain adsorbed. Coincidence and almost no shift in peaks between 50 and 150 m*M* (100 and 150 m*M*) of LiCl for 5% (10%) of charge confirm sufficient suppression of both solute–packing charge attraction forces as well as of the polyelectrolyte effect itself. Returning to Fig. 1, it can be concluded that a small peak eluted in the SEC window probably belongs to an uncharged sample component having very low molar mass.

In general, increased salt content in the mobile phase may lead to the onset of hydrophobic polymer–packing attraction which should also result in reduced sample recovery. Fig. 4 shows that this is not the case here. Practically 100% recovery is obtained for both samples at optimised salt concentrations and confirms the absence of any unwanted interaction, i.e. the correct elution in the SEC mode.

The use of light scattering detection in the case of copolymers and of polyelectrolytes deserves attention as an apparent molar mass, different from the true value, may be obtained. It is well known that heterogeneity in chemical composition of copolymers may cause a systematic error in molar mass determined by light scattering [8]. The refractive index increment of a copolymer cannot be assumed to be a constant as in the case of homopolymers and apparent molar masses are in general obtained as a function of the refractive index of the solvent used. The decisive parameter [9] determining a bias of true molar mass of a copolymer is a difference $\nu_A - \nu_B$ where ν_A and ν_B denote the refractive index increments of the neat A and B homopolymers, respec-

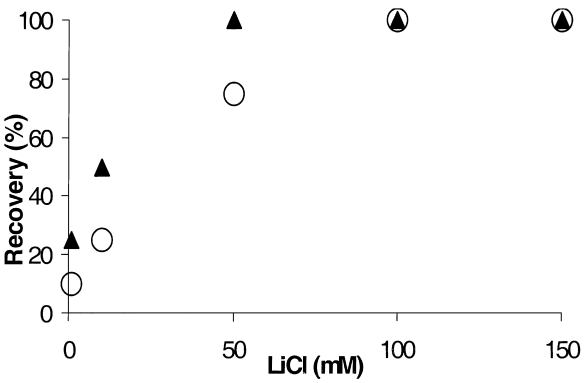

Fig. 4. The recovery of Eudragit RS (▲, 5% of quaternary ammonium groups) and RS (○, 10% of quaternary ammonium groups) as a function of LiCl content in 95% ethanol.

tively. It was shown that the bias [8,9] 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 is a function of composition in the case of a copolymer. This gives rise to ''slice'' heterogeneity in molar mass, i.e. a mixture of copolymers having different molar masses but the same hydrodynamic size is eluted at a fixed elution volume. It was shown in a recent paper [10] that this effect of chemical heterogeneity on the evaluation of molar mass and distributions from SEC data is negligible in the case of $\nu_A = \nu_B$ for statistical copolymers, provided that combined refractometric–light scattering detection is used. It remains quite small also in the case of statistical and conversional heterogeneity unless ν_A and ν_B differ considerably. In the realistic range of $0.05 < \nu\ (\mathrm{ml/g}) < 0.2$, the molar masses determined are essentially correct. The refractive index increment in Eq. (1) 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 molar 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]. In addition, the difference $\nu_A - \nu_B$ should be quite small when both monomers are chemically similar.

The molar mass against the elution volume obtained for Eudragit RS (5% of quaternary ammonium groups) is displayed with corresponding MALS signals (A–E) for various content of LiCl in mobile phase in Fig. 5. It is seen that all calibrations match each other irrespective of the sample recovery and are linear with the exception of both ends where a small error in the baseline selection of both RI and MALS signals introduces (if visible) a curvature and, of course, the lower the recovery, the higher the data scatter. The ability to obtain true and recovery independent concentrations of slices that are calculated from a known (dn/dc) value and a calibration

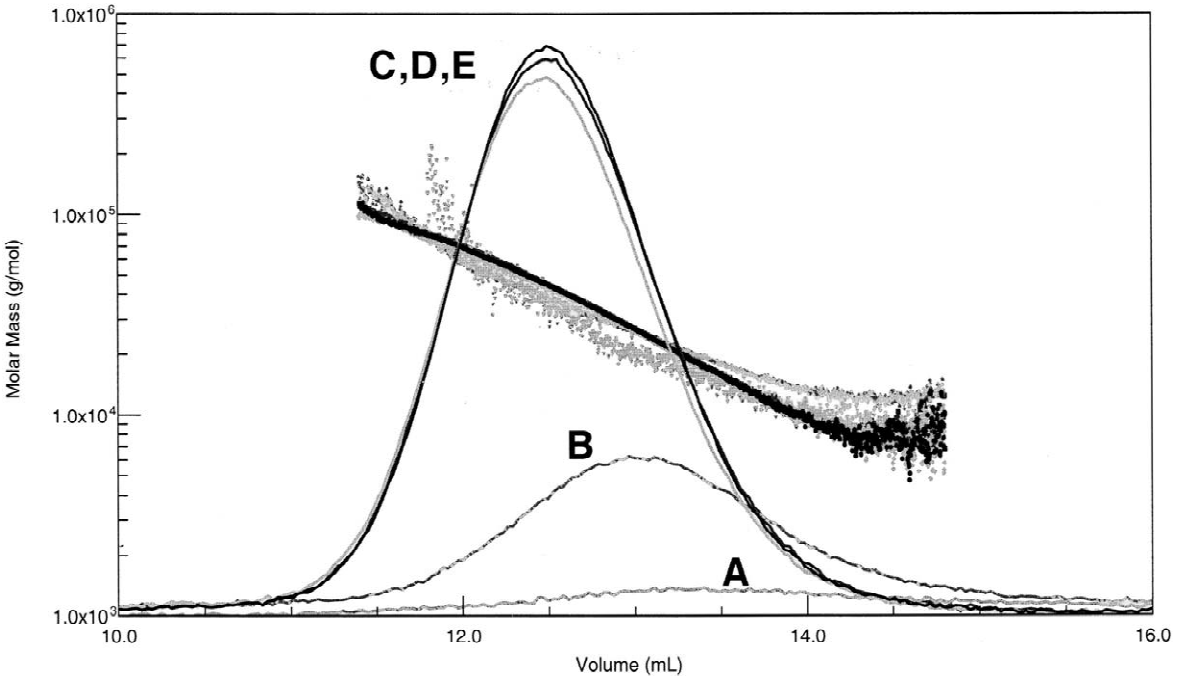

Fig. 5. The calculated dependencies of molar mass against elution volume for Eudragit RS (5% of quaternary ammonium groups) as a function of content of LiCl (1, 10, 50, 100 and 150 mM) in mobile phase (A–E). The corresponding MALS (90° detector) chromatograms are presented in the background.

constant of the RI unit and that in turn are used to calculate the molar mass of a given slice, allows to conclude that a correct SEC mechanism takes place even if the recovery is as low as 10%. In another words, any part of the sample eluted in SEC separation window is separated according to size. Hence, if 100% recovery is reached, the whole sample is separated according to size, i.e. all side solute–packing interactions are sufficiently suppressed. Accordingly, MALS peaks for LiCl contents between 50 and 150 m*M* (C–E), where 100% recovery was obtained (Fig. 4), coincide within the experimental error. Again, these results confirm that the polymers investigated should exhibit some charge distribution, the coils having the lowest charge are eluted according to size already at the lowest LiCl concentration.

An analogous plot of data obtained for Eudragit RL (10% of quaternary ammonium groups) is presented in Fig. 6. It is seen that all features observed in Fig. 5 are also preserved here. The only significant difference is that this sample being on average more charged requires a higher salt content in the mobile phase to see the beginning of elution of a detectable amount and to reach 100% recovery (curves D, E), compared to the previous one. Hence, there is no doubt that both samples have a heterogeneous charge density. A LiCl gradient elution should thus allow a separation according to charge density provided that SEC separation mode is suppressed, e.g. using a very wide or a very narrow pore column and having a suitable concentration detection.

Molar mass distributions obtained for both Eudragits at different ionic strengths are presented in Figs. 7 and 8. The distributions are similar in shape for both samples and clearly show that at low ionic strength (Figs. 7, curves A, B and 8, curves B, C), there is an underestimation of the molar mass distribution due to a preferential elution of low molar mass components. A relevant conclusion might be that the larger coils exhibit stronger charge attraction to the column packing due to the fact that the charge of both polymers increases with their molar mass.

The optimum concentration of LiCl where true distributions may be obtained for Eudragit RS fol-

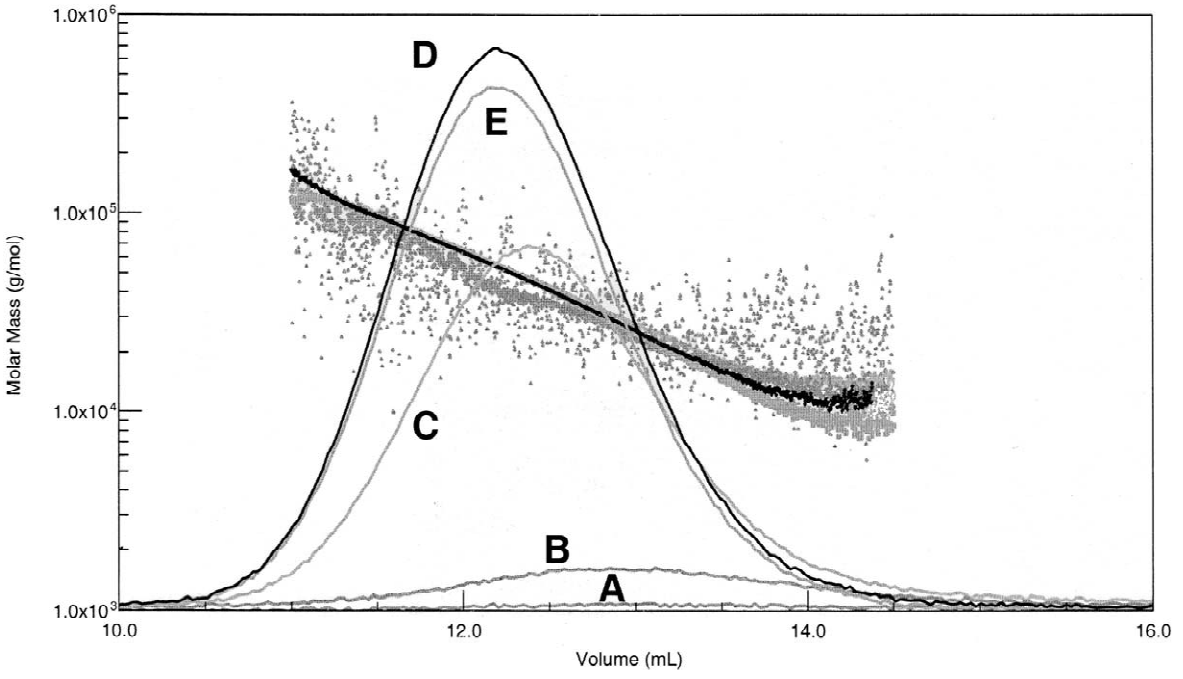

Fig. 6. The calculated dependencies of molar mass against elution volume for Eudragit RL (10% of quaternary ammonium groups) as a function of content of LiCl (1, 10, 50, 100 and 150 m*M*) in mobile phase (A–E). The corresponding MALS (90° detector) chromatograms are presented in the background.

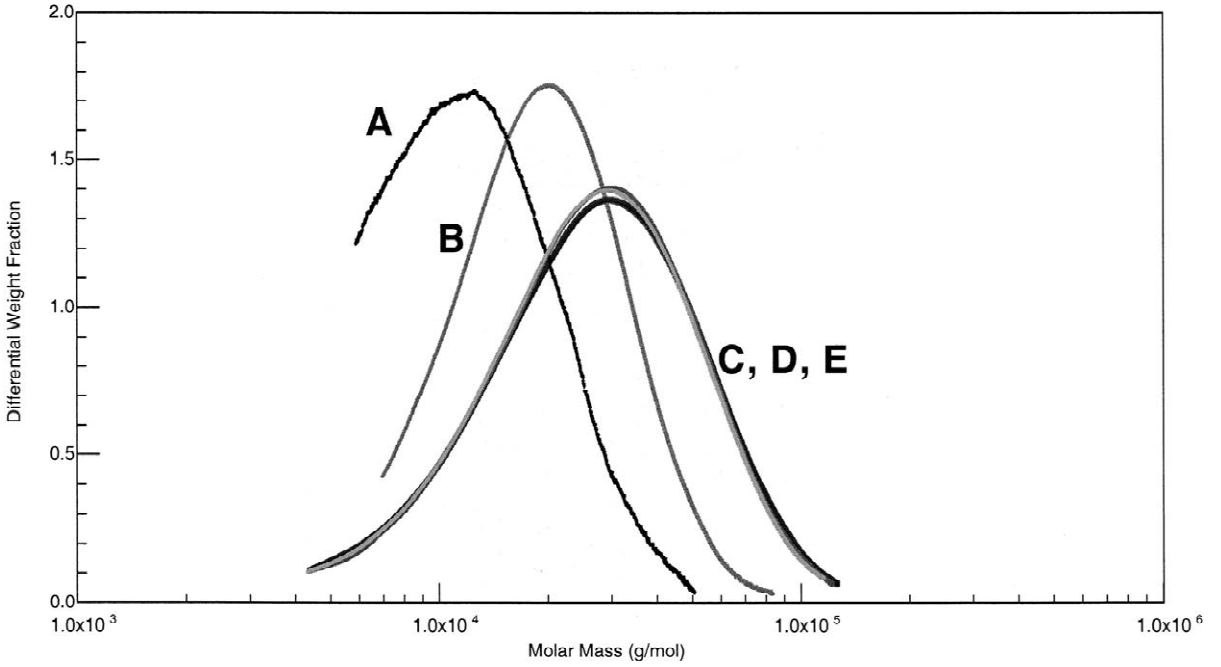

Fig. 7. The molar mass distributions of Eudragit RS (5% of quaternary ammonium groups) as a function of content of LiCl (1, 10, 50, 100 and 150 m*M*) in mobile phase (A–E).

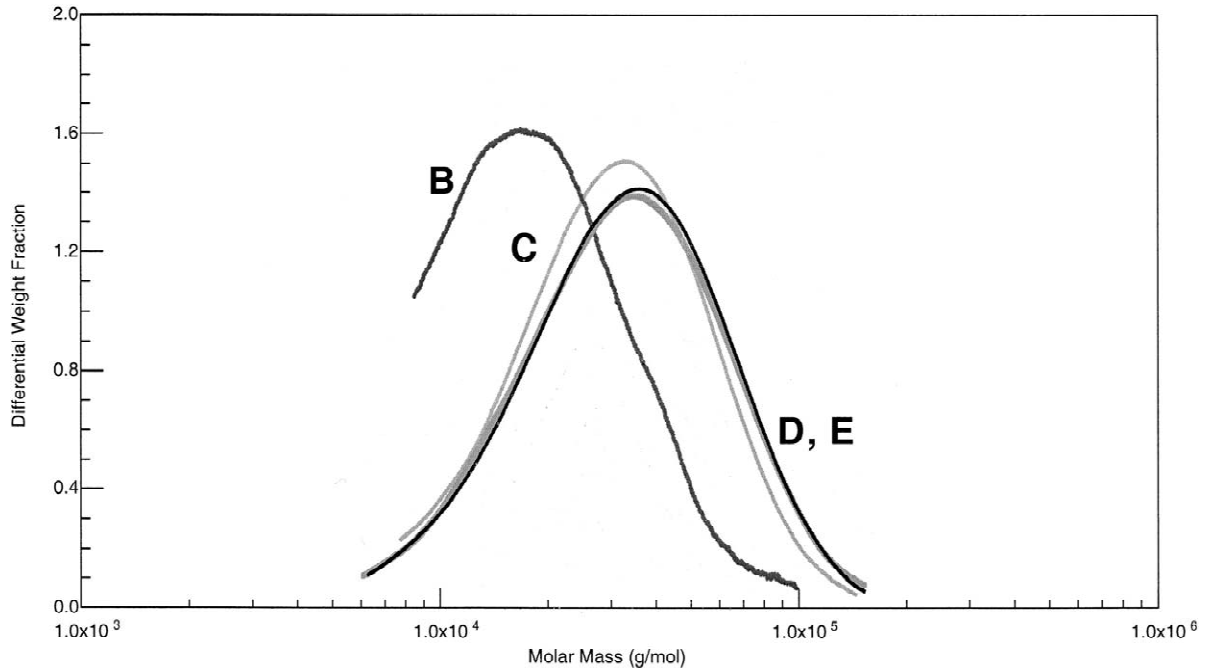

Fig. 8. The molar mass distributions of Eudragit RL (10% of quaternary ammonium groups) as a function of content of LiCl (1, 10, 50, 100 and 150 m*M*) in mobile phase (A–E).

lows from Fig. 7 to be 50 m*M* of LiCl and from Fig. 8 for Eudragit RL is 100 m*M* of LiCl. Also, the LiCl concentration can be increased up to 150 m*M* of LiCl in both cases without any onset of hydrophobic interaction. The corresponding weight-average molar mass M_w obtained under these preferred conditions is 39 000 g/mol for Eudragit RS and 32 000 g/mol for Eudragit RL. The polydispersity, defined as the ratio of M_w and M_n, the number-average molar mass, is about 1.5 for both samples.

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