Molar mass characterization of hydrophilic copolymers, 2 Size exclusion chromatography of cationic (meth)acrylate copolymers

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Abstract: A robust and reproducible method for the molar mass analysis of cationic copolymers based on dimethylaminoethyl methacrylate or trimethylammonioethyl methacrylate and different (meth)acrylates has been developed. Size exclusion chromatography (SEC) using a novel polyester-based packing as the stationary phase and dimethylacetamide (DMAC) as the mobile phase yields highly accurate results for copolymers with an amino comonomer content up to 50 wt.-%. To suppress the different polar and ionic interactions between sample molecules, stationary phase and eluent, DMAC was modified with LiBr and tris(hydroxymethyl-amino)methane (TRIS). Calibrating the SEC system with poly(methyl methacrylate) of narrow polydispersity, molar masses were obtained that are in good agreement with viscosity data. Reproducibility and robustness of the novel method were proven by running samples for an extended period of two weeks.

Introduction

Hydrophilic copolymers are extremely important precursors for surfactants, dispersing agents and drug carriers in the chemical and pharmaceutical industries. In particular, they are used as binders and coating materials. Very frequently these polymers contain hydrophilic and hydrophobic segments. In addition to the molar mass distribution they frequently exhibit a chemical composition distribution.

Cationic aminoalkyl methacrylate containing copolymers belong to a group of pharmaceutical excipients that are primarily used as controlled release film coating agents in oral capsule and tablet formulations [1]. Their aminoalkyl methacrylate content is up to 50 wt.-% making them water soluble in some cases and water insoluble in other cases.

Due to the large variety in chemical composition and molar mass, up to now there is no reliable and robust size exclusion chromatography (SEC) method for the molar mass characterization of such copolymers. Experimental difficulties in SEC arise from the presence of strongly polar functional groups at the polymer chains. Such very

polar macromolecules tend to form associates with themselves and with solvent molecules. With regard to SEC, intermolecular electrostatic interactions (ion exchange, ion exclusion, ion inclusion) between the macromolecules and the stationary phase may occur. Other non-size-exclusion effects encountered in aqueous SEC are intramolecular electrostatic interactions and adsorption due to hydrogen bonding and hydrophobic interactions between the polymer and the stationary phase [2-6]. For the suppression of non-size-exclusion effects, measures have been proposed including variation of the pH of the mobile phase, addition of an electrolyte to the mobile phase, addition of organic modifiers to the mobile phase, and variation of the pore size of the stationary phase [7].

Although in selected cases well reproducible results have been obtained, there are up to now only few systematic investigations on the SEC optimization of ionic and water soluble synthetic copolymers. More or less systematic investigations have only been conducted for polystyrene sulfonate and for biopolymers (proteins, peptides, sugars) [8-11]. In a recent paper, the molar mass analysis of cationic methyl methacrylate-acrylate copolymers by SEC in ethanol/LiCl has been described [12]. The measurements had to be conducted with multiangle laser-light scattering detection, which is not available in most standard laboratories.

In the first part of this publication we described a robust and reproducible method for the molar mass analysis of neutral and anionic copolymers based on methacrylic acid and different (meth)acrylates [13]. These copolymers are produced by Röhm, Germany, under the trade name EUDRAGIT®. Size exclusion chromatography using a novel polyester-based packing as the stationary phase and dimethylacetamide (DMAC) as the mobile phase yielded highly accurate results for copolymers with a methacrylic acid content up to 50 wt.-%. To suppress the different polar and ionic interactions between sample molecules, stationary phase and eluent, DMAC was modified with LiBr and acetic acid. The reproducibility and the robustness of the novel method were proven by running similar samples in three different laboratories and for an extended period of two weeks. The use of DMAC with lithium salts has also been described by other authors, in particular for the analysis of cellulose [14-16].

In this part of the publication we want to present a robust SEC method for cationic copolymers of the EUDRAGIT® type. This method shall serve as a means of characterization that can be used by producers as well as users of such copolymers.

Results and discussion

The hydrophilic cationic copolymers EUDRAGIT® under investigation were based on dimethylaminoethyl methacrylate or trimethylammonioethyl methacrylate chloride and different (meth)acrylic esters. The content of the amino comonomers ranged up to 50 wt.-%. The compositions of the copolymers and their viscosities are summarized in Tab. 1. There are three different sample sets with samples A, F, and K being the parent copolymers. Samples B - E and G - J have the same chemical compositions as samples A and F, respectively, but different molar masses.

It was found in a preliminary screening that all samples are properly soluble in DMAC. In ref. [13] it was shown that anionic copolymers can be separated by SEC using a novel polyester-based packing (GRAM) as the stationary phase and dimethylacetamide (DMAC) as the mobile phase. The mobile phase was modified with LiBr and acetic acid to screen polar and ionic interactions. In addition to tests with the GRAM column, conventional styrene-divinylbenzene (SDV) and hydroxyethyl

methacrylate (HEMA) based stationary phases were tested. These stationary phases resulted in distorted and poorly reproducible chromatograms that indicated that proper SEC measurements could not be conducted.

Tab. 1. Chemical compositions and viscosity numbers of the cationic EUDRAGIT[®] copolymers; viscosity numbers were obtained by solution viscometry

Sample	Name	DMAEM ^{a)} content in wt.-%	Ester content ^{b)} in wt.-%	Viscosity ^{c)} number in mL/g
A	E 100	50	50	28.0
B	E 100 (modified, EA)	50	50	27.3
C	E 100 (modified, EB)	50	50	14.7
D	E 100 (modified, EC)	50	50	45.3
E	E 100 (modified, ED)	50	50	56.0
F	RL 100	10 (chloride)	90	26.9
G	RL 100 (modified, RLE)	10 (chloride)	90	25.9
H	RL 100 (modified, RLF)	10 (chloride)	90	13.7
I	RL 100 (modified, RLG)	10 (chloride)	90	64.3
J	RL 100 (modified, RLH)	10 (chloride)	90	38.9
K	RS 100	5 (chloride)	95	26.2

^{a)} DMAEM: Dimethylaminoethyl methacrylate; chloride: trimethylammonioethyl methacrylate chloride. ^{b)} Esters are methyl methacrylate, methyl acrylate, and ethyl acrylate. ^{c)} Measurements conducted in chloroform at 25°C; sample concentrations 5 - 6 mg/mL, Ubbelohde capillary viscometer, capillary Type I (Schott)

To test the suitability of the GRAM/DMAC system for SEC measurements of cationic copolymers, the chromatographic behaviour of different samples was investigated. As is shown in Fig. 1, sample A gives a SEC-like elution profile with a second small peak eluting before the main peak. Different from sample A, containing 50 wt.-% of DMAEM, samples F and K do not provide proper elution profiles. These samples contain 10 and 5 wt.-% of trimethylammonioethyl methacrylate chloride. From the chromatograms shown in Fig. 1b and c it is even not clear if the samples do elute or if they just cause a baseline deformation.

Previous investigations on anionic copolymers showed that LiBr can be used effectively to screen polar and ionic interactions between the molecules themselves, and the molecules and the stationary phase. In a second set of experiments the effect of the addition of LiBr to DMAC was investigated for the cationic copolymers. In agreement with previous results a mobile phase composition of DMAC + 5 g/L LiBr was used. As is shown in Figs. 2 and 3, uniform SEC elution profiles were obtained for a number of samples under these chromatographic conditions.

Fig. 1. Elution curves of copolymers; stationary phase: GRAM 3000 Å + 100 Å, mobile phase: DMAC, refractive index (RI) detector, column temperature: 60°C; samples: A (a), F (b), K (c)

In Figs. 2 and 3 dual injections of all samples are shown indicating the reproducibility of the measurements. As can be seen, the reproducibility for samples A, C, and E is very good while for samples F, H, I, and K it needs further improvement. Obviously, for these samples there are still residual unwanted interactions in the chromatographic system.

It is known that enthalpic interactions between quaternary ammonium groups and polar stationary phases can be suppressed by using an ion-pair reagent like tris-(hydroxymethylamino)methane (TRIS). In order to test the effect of the addition of TRIS to the mobile phase on the reproducibility of the measurements, tests were conducted with different LiBr to TRIS ratios in the mobile phase. It was also tested if TRIS alone could be used as modifier for the mobile phase. It would be a significant advantage if one could avoid using LiBr because of its corrosive properties.

The effect of the addition of TRIS to DMAC is presented in Fig. 4. For comparison, sample A with 50 wt.-% of DMAEM, and samples F and K with 10 and 5 wt.-% of trimethylammonioethyl methacrylate chloride, respectively, were investigated. As is clear from the chromatograms, the effect of mobile phase modifications is different for different samples. For sample A, proper elution profiles are obtained in mobile phases with and without LiBr, see Fig. 4. There is a shift in the peak maximum towards lower elution volumes when the mobile phase does not contain LiBr. This can be attributed to changes in the hydrodynamic volume as a function of mobile phase composition.

Different from sample A, the other samples do not provide typical SEC elution profiles when only TRIS is used as modifier. This strongly indicates that LiBr must be used for effective screening of unwanted enthalpic interactions in the chromatographic system. Another reason for the unusual elution profile could be an unfavourable

refractive index difference between the mobile phase and the copolymers that decreases elution peak intensity and makes detection more difficult.

Fig. 2. SEC elution curves of copolymers; stationary phase: GRAM 3000 Å + 100 Å, mobile phase: DMAC + 5 g/L LiBr, detector: RI, column temperature: 60°C; samples: E (blue), A (red), C (green)

Fig. 3. SEC elution curves of copolymers; stationary phase: GRAM 3000 Å + 100 Å, mobile phase: DMAC + 5 g/L LiBr, detector: RI, column temperature: 60°C; samples: I (blue), F (red), K (black), H (green)

After further testing different LiBr to TRIS ratios in the mobile phase the optimum mobile phase composition was found to be DMAC + 2 g/L LiBr + 2 g/L TRIS. The excellent reproducibility of SEC measurements using this mobile phase for a number of samples analyzed with dual injections is shown in Fig. 5.

As is known in particular for very polar or ionic polymer samples, the chromatographic behaviour and the proper operation of the SEC mechanism depend significantly on the injected amount of sample. Therefore, the reproducibility of the SEC

procedure was investigated as a function of sample concentration. It has been found that stable and reproducible results were obtained for sample concentrations up to 7 g/L at an injection volume of 100 μ L. Under these conditions no changes in the elution volumes at peak maximum and in the peak areas were observed.

Fig. 4. Elution curves of copolymers A, F, and K. Stationary phase: GRAM precolumn + 3000 Å + 100 Å, mobile phase: DMAC + 2 g/L LiBr + 2 g/L TRIS (red), DMAC + 2 g/L TRIS (green), DMAC + 4 g/L TRIS (blue), detector: RI, column temp.: 60°C

Fig. 5. SEC elution curves of copolymers; stationary phase: GRAM precolumn + 3000 Å + 100 Å, mobile phase: DMAC + 2 g/L LiBr + 2 g/L TRIS, detector: RI, column temperature: 60°C; samples, a: A (red), B (black), C (green), D (grey), E (blue), b: F (red), G (black), H (green), I (blue), J (grey), K (pink)

Fig. 6. Molar mass distributions of copolymers before and after mobile phase change; chromatographic conditions see Fig. 5; samples: A (red and pink), F (green and light green), K (blue and light blue)

A further indication for the robustness and the reproducibility of the present chromatographic system was obtained when conducting the following experiment: within a period of 4 months the present column system was exposed to changing mobile phases of DMAC + LiBr + TRIS and DMAC + LiBr + acetic acid, i.e., changing from basic to acetic to basic eluent conditions. At the beginning and at the end of the mobile phase change the copolymer samples were measured and their molar masses calculated. As can be seen in Fig. 6 and Tab. 2, the data are in excellent agreement and document the good applicability of the chromatographic system.

A long-term test of the robustness of the method was conducted over a period of two weeks for the most representative samples. Samples A, F, K and a reference poly-(methyl methacrylate) (PMMA) were measured once a day by dual injections. The high stability of the molar mass determinations is obvious from Fig. 7. Over the indicated period of time, stable and reproducible results were obtained.

Tab. 3 summarizes the results of the reproducibility and stability tests. As can be seen, in all cases well reproducible results were obtained.

Tab. 2. Weight-average molar masses of the copolymers determined before and after mobile phase change; molar mass calculations based on PMMA calibration

Sample	M_w before change in g/mol	M_w after change in g/mol
A	52 000	50 000
B	51 000	51 000
C	23 000	22 000
D	100 000	97 000
E	131 000	128 000
F	35 000	34 000
G	34 000	34 000
H	12 000	12 000
I	136 000	141 000
J	62 000	64 000
K	32 000	33 000

Tab. 3. Weight-average molar masses and error statistics for reproducibility and stability tests

	Sample A	Sample F	Sample K
	46 500	30 400	29 800
	47 600	30 400	30 000
	47 400	30 700	29 600
	46 900	30 400	29 800
	47 500	31 100	29 900
	47 500	30 800	30 200
	47 000	31 400	29 700
	47 700	31 300	29 800
	45 400	31 200	30 100
	48 000	30 900	29 600
	47 900	31 100	29 600
	47 300	31 200	29 800
	47 700	31 100	29 700
	47 800	29 500	29 300
	47 700	31 300	29 400
Average M_w	47 300	30 900	29 800
Standard deviation	668	507	242

Fig. 7. Long-term stability of the method indicated by molar mass analyses of samples E (◆), F (■), K (●) and a reference PMMA (■) determined over a period of 16 days; stationary phase: 2 x GRAM linear XL, mobile phase: see Fig. 6, detector: RI

Tab. 4. Comparison of molar mass determinations of freshly prepared and 7 days old polymer solutions

	M_w in g/mol	M_n in g/mol	M_w/M_n
Reference PMMA			
fresh solution	131 700	53 600	2.47
old solution	130 700	52 300	2.50
Sample A			
fresh solution	47 200	17 700	2.72
old solution	47 500	18 100	2.62
Sample F			
fresh solution	30 900	10 800	2.88
old solution	31 500	11 600	2.71
Sample K			
fresh solution	29 800	10 900	2.75
old solution	29 600	10 200	2.91

In a last set of experiments, the stability of the polymer solutions for SEC analysis was investigated. It is known from previous experiments that extended exposition of copolymers to the SEC eluent can cause changes in the molar mass distribution. Therefore, freshly prepared polymer solutions were compared to polymer solutions that were kept for 7 days as prepared. As can be seen clearly in Tab. 4, within this period of time no changes in molar masses of the samples could be detected.

Unfortunately, the molar mass data presented here could not be verified by SEC-MALLS measurements. Different from the anionic copolymers investigated in ref. [13] the refractive index increments of the cationic copolymers are very low resulting in very noisy light scattering curves, which cannot be used for the calculation of molar mass distributions.

Experimental part

Chromatographic system

An Agilent 1100 Series HPLC system (Agilent Technologies) comprising a pump, an autosampler and a column oven was used. Due to the salt containing eluent a seal wash was installed. For data collection and processing the software package 'WinGPC-Software' (Polymer Standards Service, Mainz, Germany) was used. Molar mass calibration was based on a series of well-defined narrow-disperse poly(methyl methacrylate) calibration standards.

Columns: GRAM 3000 Å and GRAM 100 Å (Polymer Standards Service, Mainz, Germany), all of 300 x 8 mm i.d. and 10 µm average particle size. To condition new from-the-shelf GRAM columns, they were rinsed for 5 h with a flow rate of 0.2 mL/min with pure DMAC, then for 2 h with the mobile phase; subsequently the system was heated to 60°C and the columns were conditioned overnight with a flow rate of 0.1 mL/min. Prior to analysis the flow rate was elevated to the final value of 1.0 mL/min. In all cases a GRAM precolumn was used.

Mobile phase: *N,N*-Dimethylacetamide HPLC grade (Fluka and Schopp, Karlsruhe, Germany); LiBr 99+% (Acros, Belgium); tris(hydroxymethylamino)methane (TRIS) for analysis grade (Acros, Belgium).

Detector: Agilent Series 1100 RI detector.

Samples: The EUDRAGIT® samples were provided by Röhm GmbH, Darmstadt, Germany. The samples were dissolved in a concentration of 3 g/L overnight and filtered through a 1 µm filter prior to analysis.

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