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Development of a size exclusion chromatography method for the determination of molar mass for poloxamers

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Abstract

An aqueous size exclusion chromatography (SEC) method for determination of the molar mass of poloxamers 188 and 407 has been developed as an alternative to the pharmacopoeia methods. During the development work two different columns and several different eluent compositions were investigated. With a PL-aquagel-OH column, non-exclusion behaviour was obtained. A TSKgel column gave good separation of both poloxamers. The best separation was obtained with an eluent consisting of sodium chloride (0.01 M)–methanol (90:10, v/v) on the TSKgel column. The method was shown to be linear within the elution time of the two poloxamers and to have acceptable precision. The results from the SEC method was compared to results obtained using SEC with online multi angle light scattering detection (MALS) and to results obtained with matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-MS).

Keywords: Aqueous size exclusion chromatography; Poloxamers; Non-exclusion behaviour; Multi angle light scattering; Matrix-assisted laser desorption/ionisation mass spectrometry

1. Introduction

Poloxamers are water-soluble synthetic A–B–A type block copolymers of ethylene oxide and propylene oxide with the general structure shown in Fig. 1. The ethylene oxide part gives the poloxamer a hydrophilic character, while the propylene oxide part gives a lipophilic character. The poloxamers are used in the pharmaceutical industry as detergents, dispersing agents, emulsifying agents, gelling agents and solubilising agents [1,2]. By mixing two types of poloxamer, 188 and 407, a thermoreversible gelling system is obtained [3,4]. The two poloxamers differ in both composition and molar mass, as shown in Fig. 1. The actual composition of the different poloxamers varies and both poloxamers 188 and 407 contain molecules of different molar mass, as polymer samples usually do. This is called a molar mass distribution and is often described by calculating two molar mass averages, the number average molar mass ($\bar{M}_n$) and the mass average molar mass

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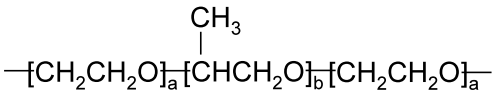

Fig. 1. General structure of poloxamers. For poloxamer 188 a is 75–85 and b 25–30 and for poloxamer 407 a is 95–105 and b 54–60.

($\bar{M}_m$) according to Eq. (1) (equations for calculating: (a) number average molar mass; and (b) mass average molar mass. (m_i is the mass of a polymer with i repeating units, N_i is the number of moles of a polymer with i repeating units and M_i is the molar mass of a polymer with i repeating units.)).

$$\text{(a)}\quad \bar{M}_n = \frac{\sum_{i=1}^{\infty} m_i}{\sum_{i=1}^{\infty} N_i} = \frac{\sum_{i=1}^{\infty} N_i \cdot M_i}{\sum_{i=1}^{\infty} N_i},$$
$$\text{(b)}\quad \bar{M}_m = \frac{\sum_{i=1}^{\infty} m_i \cdot M_i}{\sum_{i=1}^{\infty} m_i} = \frac{\sum_{i=1}^{\infty} N_i \cdot M_i^2}{\sum_{i=1}^{\infty} N_i \cdot M_i}. \quad (1)$$

The molar mass is an important property for predicting the end-use properties of a polymer and the pharmacopoeias contain a method for determination of the molar mass of poloxamers. In the pharmacopoeia methods the poloxamers are reacted with phthalic-anhydride in a pyridine solution, which is titrated with a sodium hydroxide solution [5,6]. This is a time-consuming method, which gives only one of the molar mass averages. Another drawback of the method is its lack of specificity, which can give an erroneous result if the sample which is analysed contains molecules able to react in the same way as the poloxamers. It will also overestimate the molar mass if the poloxamer molecules have too many unsaturated chain ends. Other means of determining the molar mass of polymers is by size exclusion chromatography (SEC). Here the molecules are separated according to their hydrodynamic volume, which depends on their molar mass, the solvent and the chemical composition of the polymer. By calibrating the column with known standards, the molar mass of an analyte can be calculated. If the analytes do not have the same relationship between molar mass and hydrodynamic volume as the standards used, the calculated molar mass will only be relative to the standards. An absolute molar mass can be determined by coupling a multi angle light scattering detector (MALS) with SEC or by using matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-MS).

An SEC method separates the molecules in a polymer sample according to size and allows the determination of both $\bar{M}_n$ and $\bar{M}_m$. As mentioned previously, these two give information about the molar mass distribution of the polymer, which is an essential parameter for characterising the polymer. If only one of the averages is determined you can not determine the molar mass distribution, whereby essential information about the polymer is missed. A method, such as SEC, that can determine the molar mass distribution is therefore a preferred method over such that only gives one of the averages, such as titration.

In using aqueous SEC the risk for non-exclusion behaviour is larger than using non-aqueous SEC. The non-exclusion behaviour of polyelectrolytes can be controlled by adding salt [7–9]. The exclusion behaviour of uncharged polymers, such as poly(ethylene oxide) (PEO), is also influenced by the ionic strength [10]. An aqueous SEC method, using a water–methanol (70:30, v/v) mobile phase, was developed for poloxamer 188. In spite of the uncharged nature of the poloxamers, adsorption to the stationary phase was observed and an organic system was the preferred one [11].

In this paper, an aqueous SEC method is developed for the determination of molar mass of poloxamers 188 and 407 as an alternative to the pharmacopoeia methods. The method is validated as regards linearity, range, precision and influence of sample concentration. The SEC result for the poloxamers is evaluated against the results obtained with titration, SEC-MALS and MALDI-MS.

2. Experimental

2.1. Materials

Two block copolymers of ethylene oxide and propylene oxide called poloxamers 188 and 407 (BASF, Germany) were used. Poly(ethylene glycol) (PEG) and PEO standards ranging between

106 and 145 000 g/mol (Waters, MA, USA) were used to calibrate the columns for the SEC method.

The following reagents were used to prepare the different eluent compositions: Ultrapure water with a resistance of >18 MΩ/cm, $NaNO_3$, analytical grade (Merck, Germany), NaH_2PO_4, analytical grade, and Na_2HPO_4, analytical grade (Merck, Germany), NaCl, analytical grade (Merck, Germany), methanol, gradient grade (Merck, Germany) and acetonitrile, gradient grade (Merck, Germany).

2.2. Size exclusion chromatography

The analyses were performed using a Shimadzu LC-10AD pump (Shimadzu, Japan), an HP 1100 injector (Hewlett-Packard, Germany) and a Waters 410 RI detector (Millipore, MA, USA) operating at 30 °C. Two different types of columns were tested: PL-aquagel-OH mixed (300 × 7.5 mm) (Polymer Laboratories Ltd., UK), and TSKgel 3000PW (300 × 7.5 mm) (Supelco Inc., PA, USA). The columns contained cross-linked hydrophilic copolymers of undisclosed composition. Data was collected and analysed using HP ChemStation (Hewlett-Packard, Germany) and PL Caliber® GPC/SEC software for the Hewlett-Packard LC ChemStation (Polymer Laboratories Ltd., UK).

Approximately, 10 mg of the standards and 20 mg of the samples were dissolved in 10 ml of eluent. When the samples had dissolved, the solution was filtered through a Millex-HV 0.45 μm filter unit. Standards and samples were injected with the same volume, 80 μl. A flow-rate of 0.8 ml/min was used for the PL-aquagel-OH column and one of 0.5 ml/min for the TSKgel column. The analyses were performed at room temperature. The columns were equilibrated overnight before use. All eluents were degassed before use.

2.3. Titration

The molar mass was determined according to NF and Ph Eur by titrating the poloxamers dissolved in a phthalic anhydride–pyridine solution with NaOH using phenolphthalein as an indicator [5,6]. Two sample preparations were analysed and the mean value reported.

2.4. SEC-MALS/RI

The separation system consisted of two size exclusion columns, TSK-gel G 3000 PW_{XL} 7.8 × 300 mm, particle size 10 μm, connected in series. The pump was a Shimadzu LC10AD liquid chromatography pump (Shimadzu Corp., Tokyo, Japan). The degasser used was a Gastorr 154 (Gastorr, Japan). The flow rate of the mobile phase was held at 0.50 ml/min. The polymer sample was injected on the column by a Perkin–Elmer 200 LC autosampler (Perkin–Elmer Corp., Norwalk, CT, USA) equipped with a 100 μl sample loop. The polymer concentration in solution was held at 3.0 and 1.5 mg/ml for poloxamers 188 and 407, respectively. The mobile phase was a mixture (90/10, v/v) of 0.010 M sodium chloride (p.a., Merck, Darmstadt, Germany)–methanol (gradient grade, Merck, Darmstadt, Germany) filtered with a 0.22 μm mixed cellulose ester filter GSWP (Millipore Corp., Bedford, MA, USA).

A stainless steel High Pressure Filter Holder, 25 mm (Millipore Corp., Bedford, MA, USA), with a 25 mm 0.025 μm VSWP filter (Millipore Corp., Bedford, MA, USA) was connected directly to the pump on-line. A small volume Precolumn Filter A315 (Upchurch Scientific Inc., Oakharbor, WA, USA) with a replaceable stainless steel frit A101x (pore size 2 μm) was employed in-line between the column and the detector. Its frit served as support for a post-column cellulose acetate filter (Sartorius Cellulose Acetate 111 11106-047 N, Sartorius AG, Goettingen, Germany) the pore size of which was 0.45 μm.

The light scattering photometer was a DAWN-EOS multi-angle light scattering (MALS) instrument (Wyatt Technology, Santa Barbara, CA, USA). Simultaneous concentration detection was performed using an Optilab DSP interferometric refractometer (Wyatt Technology, Santa Barbara, CA, USA). Both detectors used a wavelength of 690 nm. The angular dependence of the scattered light was extrapolated to zero angle using the linear Berry fit method.

The refractive index increment (dn/dc) was determined by the injection of six different concentrations of each of the Poloxamer samples into the refractometer. The (dn/dc)-value obtained for both poloxamers 188 and 407 was 0.133.

2.5. MALDI-MS

MALDI-MS was performed on a Voyager-DE STR (Applied Biosystems, Framingham, MA, USA) time-of-flight mass spectrometer equipped with an N_2 laser and a Hi-mass detector in linear mode. Since separation of individual peaks could not be obtained in reflector mode, the analysis was performed in linear mode only, with delayed extraction activated.

The matrix utilized was 2-(4-hydroxyphenylazo)benzoic acid (HABA). The poloxamer analytes and matrix were both dissolved in tetrahydrofuran, 4 and 20 g/l, respectively and vortexed. Analyte and matrix were then mixed 1:4 (v/v) and vortexed. Samples were then prepared by depositing 0.5 μl droplets on a stainless steel plate. This resulted in a thin homogeneous layer.

Spectra were accumulated from 200 laser shots. Since the signal faded after a few shots at the same location it was necessary to move the sample plate during acquisition. Each poloxamer were analysed in duplicate. All spectra were calibrated externally.

3. Results and discussion

The molar mass of poloxamers can be determined by a titration method described in the pharmacopoeias [5,6]. To find an alternative method that can be used for quality control an SEC-RI method was developed and validated. The results obtained with the SEC-RI method is only relative to the standards. The results obtained with that method is therefore compared to the results obtained with SEC-MALS and MALDI-MS.

3.1. SEC-RI

Eluents of different compositions were tested with the PL-aquagel-OH column and with the TSKgel column. Eluents consisting of ultrapure water, salt solutions, ultrapure water with organic modifier and salt solutions with organic modifier were used on the two different columns, which contained gels of cross-linked hydrophilic copolymers of undisclosed composition.

3.1.1. Ultrapure water

When ultrapure water was used as an eluent, both poloxamer samples were adsorbed to the column using the PL-aquagel-OH column. With the TSKgel column, a separation was achieved between poloxamers 188 and 407. Fig. 2 shows that the poloxamers elute close to each other, although they have quite different molar masses. This is explained by the different composition of the poloxamers, where 188 contains more of the hydrophilic part than 407, which will change the relationship between hydrodynamic volume and molar mass.

3.1.2. Salt solutions

The PL-aquagel-OH column was tested with different salt solutions as eluent. Both poloxamers were adsorbed to the column when the eluent consisted of sodium nitrate (0.01 M) solution or two different phosphate buffers, (pH 5, 0.2 M) or (pH 7, 0.01 M). The TSKgel column gave a separation between both poloxamers when a sodium chloride (0.01 M) solution was used as an eluent (Fig. 3). The separation of poloxamer 188 is unchanged compared to the ultrapure water, although poloxamer 407 using ultrapure water has a more pronounced shoulder at the low molar mass side (Fig. 2) than seen when a salt solution was used as eluent. Previously Volet and Lesec showed that the low molar mass components eluted earlier for an anionic polyelectrolyte when the ionic strength was too low [7].

3.1.3. Ultrapure water with organic modifier

Using ultrapure water as eluent was not successful in separating either poloxamer on the PL-aquagel-OH column. Therefore, was ultrapure water–methanol (50:50, v/v) used as an eluent. Fig. 4 shows that poloxamer 188 was successfully separated with this system, whereas only a small peak could be detected for poloxamer 407, although the same amount was injected of both

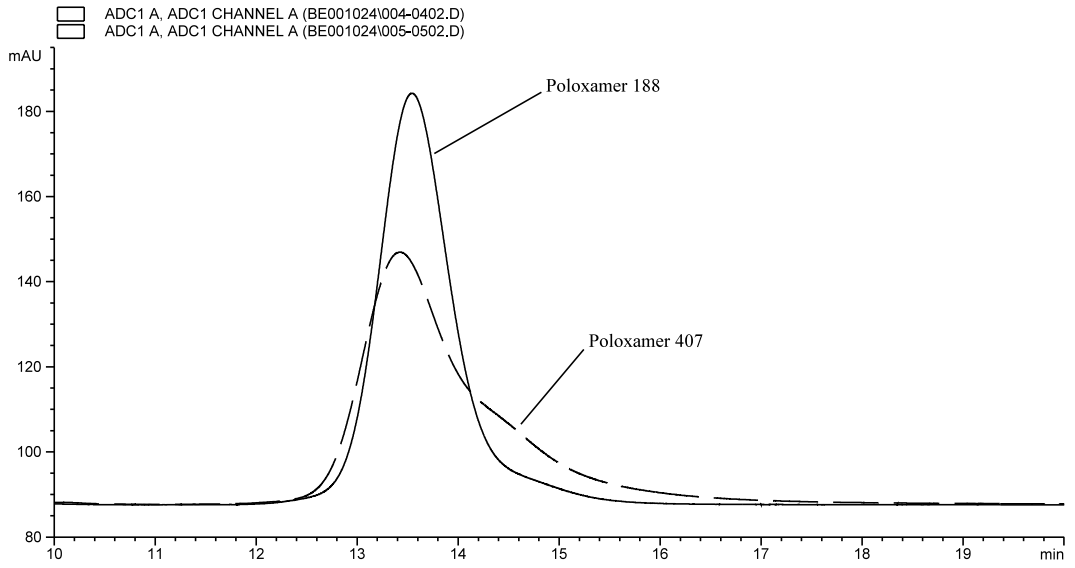

Fig. 2. Overlaid chromatograms of poloxamers 188 and 407 using ultrapure water as eluent with a TSKgel column.

poloxamers. In spite of the fact that the eluent contains 50% organic modifier, the adsorption of poloxamer 407 to the column material is strong.

3.1.4. Salt solutions with organic modifier

Different eluents consisting of salt solutions with organic modifier were tested with the PL-aquagel-OH column. When a mixture of sodium nitrate (0.2 M) phosphate buffer (pH 7, 0.01 M)–methanol (70:30, v/v) was tested, no poloxamer passed through the column. Fig. 5 shows that a mixture of sodium nitrate (0.01 M)–acetonitrile (80:20, v/v) was more successful, although most of poloxamer 407 was adsorbed to the column. The TSKgel column was able to separate poloxamers 188 and 407 using a mixture of sodium chloride

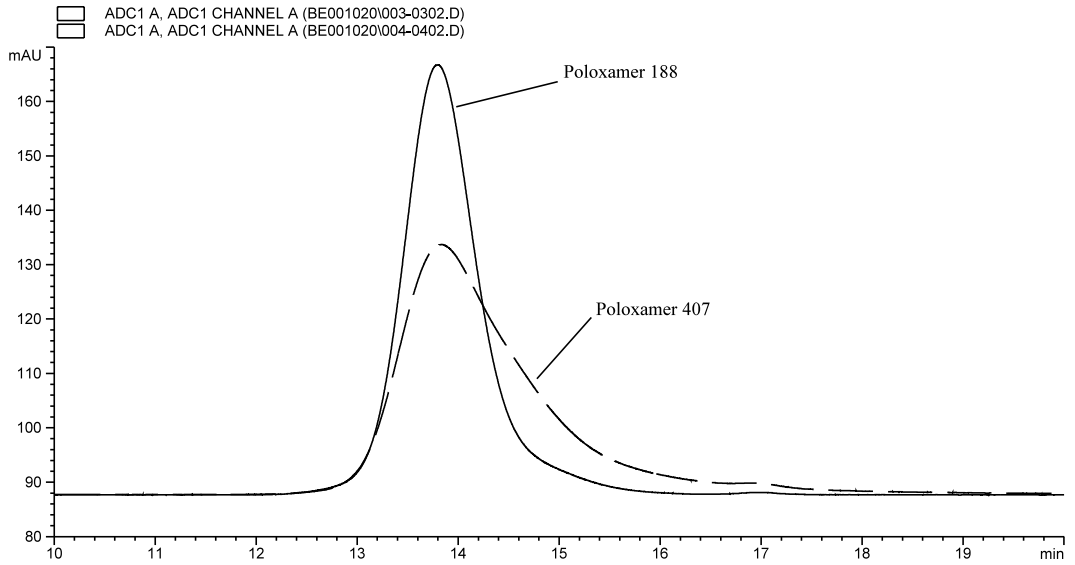

Fig. 3. Overlaid chromatograms of poloxamers 188 and 407 using sodium chloride (0.01 M) solution as eluent with a TSKgel column.

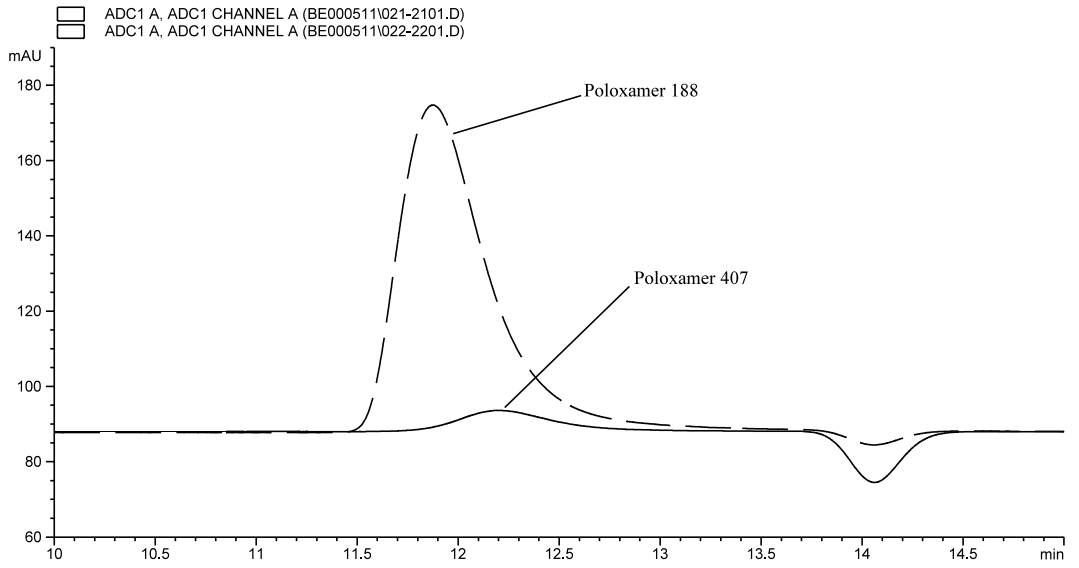

Fig. 4. Overlaid chromatograms of poloxamers 188 and 407 using ultrapure water–methanol (50:50, v/v) as eluent with a PL-aquagel-OH column.

(0.01 M)–methanol (90:10, v/v) (Fig. 6). Using the latter eluent, the poloxamer 407 peak was more separated from the poloxamer 188 peak than in the previous experiments. With this system both poloxamers 188 and 407 gave good peak shapes. Poloxamer 407 gave a typical distribution profile, where a bimodal molar mass distribution is revealed. This is in contrast to poloxamer 188, which has a rather narrow unimodal distribution. The mixture of sodium chloride (0.01 M)–methanol (90:10, v/v) using the TSKgel column was found to be the best system for determining the

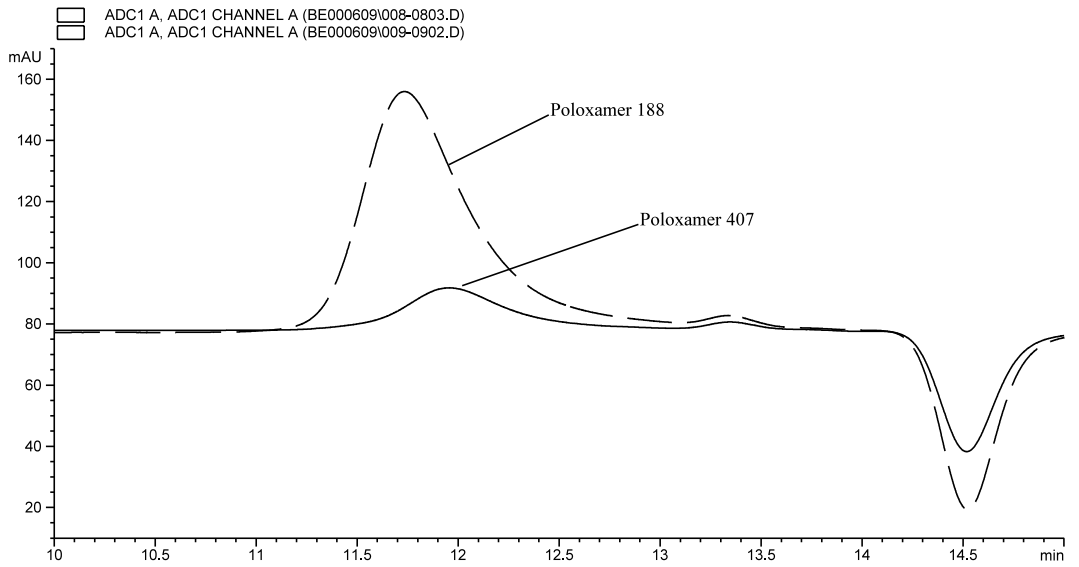

Fig. 5. Overlaid chromatograms of poloxamers 188 and 407 using sodium nitrate (0.01 M)–acetonitrile (80:20, v/v) as eluent with a PL-aquagel-OH column.

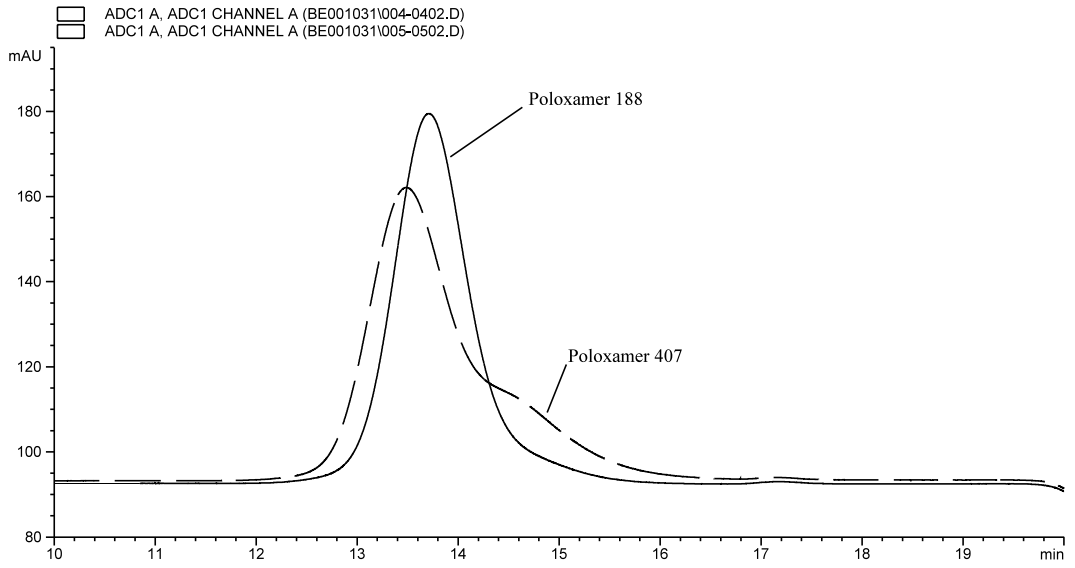

Fig. 6. Overlaid chromatograms of poloxamers 188 and 407 using sodium chloride (0.01 M)–methanol (90:10, v/v) as eluent with a TSKgel column.

molar mass of poloxamers 188 and 407. The bimodal distribution of poloxamer 407 has been discussed by Gallet et al. [12]. They found that the low molar mass distribution consisted of a diblock copolymer of ethylene oxide and propylene oxide. The diblock structure is explained by the chain transfer mechanism that can occur during polymerisation of propylene oxide, which results in an unsaturated chain end [13].

The result obtained with the proposed method is compared to the result obtained by titration in Table 1. The difference in molar mass between the two poloxamers determined with SEC is smaller than that obtained using titration. The result obtained by SEC is only relative to the standards, and the separation of the two poloxamers will differ as they have different chemical compositions. It is possible to distinguish the two poloxamers from each other as they have different shape of their molar mass distribution.

The linearity, precision and influence of the sample concentration of the proposed method was investigate to evaluate the performance of the method.

3.1.5. Linearity

In the method PEG/PEO standards with the following molar masses were used for calibration: 106, 194, 600, 970, 1500, 6240, 12 000, 23 000, 44 400 and 145 000 g/mol. Fig. 7 shows a typical calibration curve with all standards injected twice. The equation of the standard curve is $y = -0.3781x + 9.0715$ with a correlation coefficient (r) of 0.999.

3.1.6. Precision

System precision was determined by making six replicate injections of PEG/PEO standard 600 g/mol, 23 000 g/mol and poloxamer 188. Table 2 shows that the relative standard deviation (RSD) for the retention time varies between 0 and 0.04%,

Table 1
Molar mass determined by titration and by SEC relative to PEG/PEO standards for poloxamers 188 and 407

Sample	$\bar{M}_n$ titration (g/mol)	$\bar{M}_n$ SEC (g/mol)	$\bar{M}_m$ SEC (g/mol)
Poloxamer 188	9020	6900	8000
Poloxamer 407	12 900	6200	8600

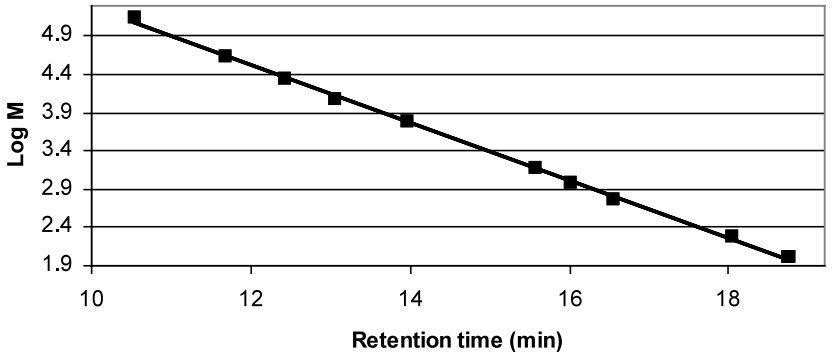

Fig. 7. A calibration curve of PEG/PEO standards in the range 106–145 000 g/mol.

while the RSD for the peak area varies between 1.0 and 2.9%. The molar mass of poloxamer 188 was calculated and the RSD for $\bar{M}_m$ was 0.65%, while the RSD for $\bar{M}_n$ was 2.3%. The repeatability was determined from the analysis of six sample preparations of poloxamer 188 injected in the same sequence. Table 3 shows that an $\bar{M}_n$ value of 6600 g/mol with an RSD of 2.3% and an $\bar{M}_m$ value of 7900 g/mol with an RSD of 0% were obtained for poloxamer 188.

Reproducibility of the method was investigated by injecting eight different sample preparations of poloxamer 407 on four different occasions. Table 4 shows that the RSD for $\bar{M}_n$ was 6.9%, while the RSD for $\bar{M}_m$ was 1.6%.

3.1.7. *Influence of sample concentration*

In an SEC method the sample concentration is of minor importance. The sample is separated according to the size of the polymer molecules in the SEC column and the relative concentration of the individual molecules making up the polymer sample is used to calculate the molar mass. However, an overload of the column may give band broadening, which affects the determination. Table 5 shows samples with a variation in concentration between 6.3 and 37.2 mg of poloxamer 407 dissolved in 10 ml of eluent. The RSD for $\bar{M}_n$ was determined to be 4.9% and the RSD for $\bar{M}_m$ to be 3.0%. The variation for $\bar{M}_m$ is larger than previously seen and the values obtained at the higher concentrations are slightly higher compared to those at the lower concentrations. The analysis will give a more accurate molar mass distribution if the concentration of the poloxamer sample is lower than 3.7 mg/ml.

The two different columns showed quite different performances when different types of eluent composition were tested with the poloxamers. The PL-aquagel-OH column showed non-exclusion behaviour as no separation of poloxamer 407 was ever achieved and when it gave a separation for poloxamer 188, the peak shapes were slightly non-Gaussian. For the TSKgel column, a satisfactory separation was achieved with all the eluent compositions tested. Poloxamer 407 contains a higher amount of the lipophilic propylene oxide part, which might explain the retention of that material to the PL-aquagel-OH column. The poloxamer contains many oxygen groups in the

Table 2
RSD for retention time and area for six consecutive injections of PEG 600, PEO 23 000 and poloxamer 188

Sample	RSD retention time (%)	RSD area (%)	$\bar{M}_n$ (g/mol)	$\bar{M}_m$ (g/mol)
PEG 600	0.03	2.9	–	–
PEO 23 000	0.04	1.0	–	–
Poloxamer 188	0	1.0	6700 (RSD 2.3%)	8000 (RSD 0.65%)

In the case of poloxamer 188 the RSDs for $\bar{M}_n$ and $\bar{M}_m$ are also presented.

Table 3
Six injections of different sample preparation of poloxamer 188

Injection	Sample weight (mg)	Retention time	$\bar{M}_n$ (g/mol)	$\bar{M}_m$ (g/mol)
1	20.5	13.68	6400	7900
2	20.6	13.68	6800	7900
3	23.7	13.68	6600	7900
4	20.0	13.68	6500	7900
5	20.6	13.68	6500	7900
6	19.8	13.68	6600	7900
Average		13.68	6600	7900
RSD		0%	2.3%	0%

polymer backbone that can give hydrogen bonding to hydroxyl functionality in the stationary phase and a difference in the stationary phase of the two columns may explain their different behaviour.

3.2. SEC-MALS/RI

The major advantage by adding a multi-angle light scattering detector to the traditional SEC-RI system is that an absolute method for determination of molar mass and size is at hand. By measurement of sample concentration and scattering intensity simultaneously the molar mass can be determined through the well-known, simplified relationship valid in dilute polymer solutions [14],

$$M = \frac{R_\theta}{KcP_\theta},$$

where c is the sample concentration, R_θ is the Rayleigh ratio, θ is the scattering angle and K is an instrumental constant. The form factor P, which is related to the size of the macromolecule, is approaching unity for small polymers having a radius below 10 nm where the angular dependence is weak. Obviously, by this approach, there is no need for molar mass calibration of the SEC-column which is advantageous in the case of poloxamers where no such calibrants exist. The obtained molar mass against the elution volume is illustrated in Fig. 8 for both poloxamers 188 and 407. The chromatographic peaks are similar to those obtained in the SEC-RI system. At a first sight, the poor separation between the peaks

Table 4
Poloxamer 407 analysed on four different occasions

Occasion	Sample weight (mg)	Retention time		$\bar{M}_n$ (g/mol)		$\bar{M}_m$ (g/mol)	
			Mean		Mean		Mean
1	23.9	13.50		5500		8100	
	21.3	13.49	13.50	5600	5600	8200	8100
2	17.8	13.50		5300		7900	
	17.4	13.49	13.50	5200	5300	8000	8000
3	23.7	13.47		5400		8100	
	17.5	13.46	13.47	5600	5500	8200	8200
4	18.7	13.45		6200		8200	
	19.9	13.45	13.45	6200	6200	8300	8300
Average		13.48	13.48	5600	5700	8100	8200
RSD		0.2%	0.2%	6.8%	6.9%	1.6%	1.6%

Table 5
Influence of sample concentration on the molar mass calculation for poloxamer 407

Sample weight (mg)		Retention time		$\bar{M}_n$ (g/mol)		$\bar{M}_m$ (g/mol)	
	Mean		Mean		Mean		Mean
17.8		13.50		5300		7900	
17.4	17.6	13.49	13.50	5200	5300	8000	8000
6.7		13.48		5500		8000	
5.9	6.3	13.48	13.48	4900	5200	7800	7900
38.3		13.47		5600		8300	
36.1	37.2	13.47	13.47	5700	5700	8400	8300
Mean		13.48	13.48	5400	5400	8100	8100
RSD		0.09%	0.1%	5.4%	4.9%	2.9%	3.0%

Samples dissolved in 10 ml of eluent.

indicates a quite small difference in molar mass between the poloxamers 188 and 407 samples. However, the superimposed molar mass data from MALS are generally higher for the poloxamer 407 sample which reflects their different chemical composition. Thus, by using MALS, the existing molar mass difference is clearly identified.

The cumulative molar mass distributions for both poloxamers are depicted in Fig. 9. The distribution for the poloxamer 188 sample is

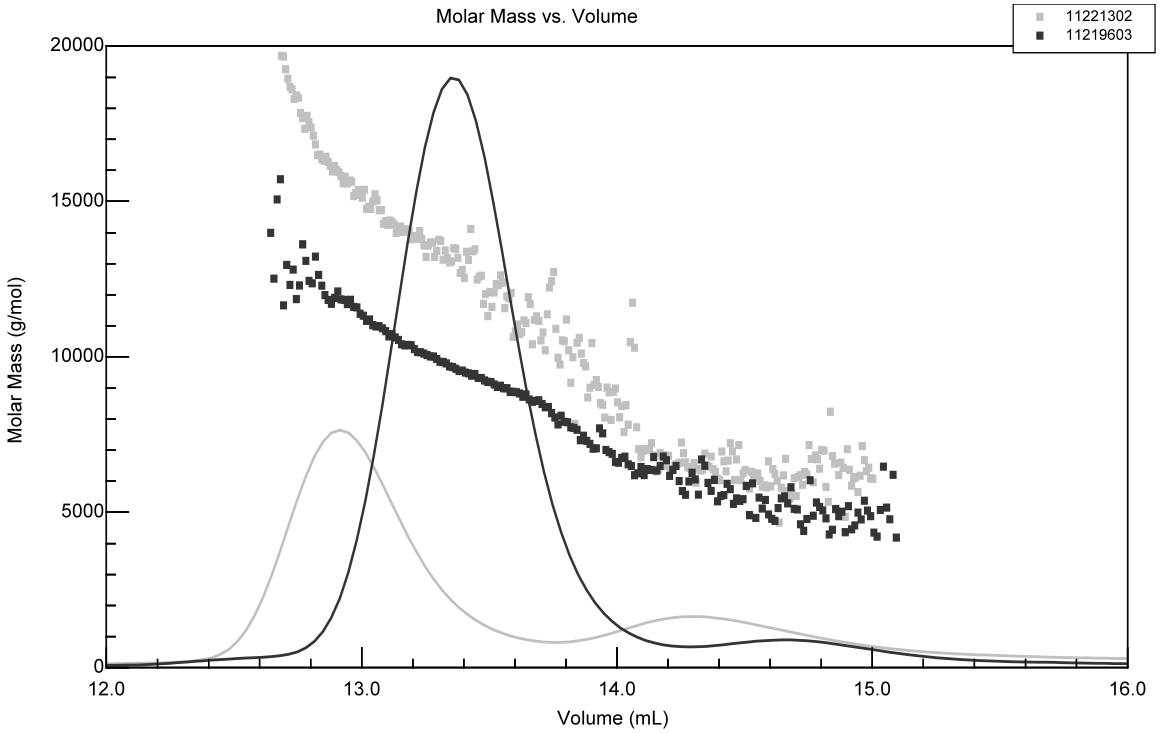

Fig. 8. Molar mass against elution volume for poloxamers 188 (black) and 407 (grey). The refractometer signals are superimposed for both samples.

centred around 9000 g/mol with a $\bar{M}_m$ of 9700 g/mol (Table 6). Presence of smaller sample components is detected, a fraction corresponding to approximately 5% of the total sample has a molar mass about 5000 g/mol. This fraction may consist of PEO–PPO blocks ($M \sim 5100$ g/mol) or even unreacted PEO chains ($M \sim 3500$ g/mol). The broad, clearly bimodal shape of the poloxamer 407 sample (Fig. 8) reveals an even more pronounced presence of low molar mass components. Still, about 70% of the sample has a molar mass of 12 000 g/mol or higher (Fig. 9). This part should represent the tri-block polymer whereas the low molar mass fraction, centred at 6500 g/mol and representing approximately 20% of the sample, probably contains PEO–PPO blocks.

The obtained $\bar{M}_m$ for the poloxamer 407 sample is about 13 500 g/mol which is significantly higher than the corresponding $\bar{M}_m$ determined with the SEC-RI method. The reason for this discrepancy is most likely the calibration with PEO standards having a different conformation than the poloxamers. Thus, obtained molar mass data from this approach would be 'PEO-equivalent' and a deviation from the absolute molar mass is expected.

Table 6
Obtained molar mass data for poloxamers 188 and 407 using the SEC-MALS method

Sample	$\bar{M}_m$ (g/mol)	$\bar{M}_n$ (g/mol)	$\bar{M}_m/\bar{M}_n$
Poloxamer 188	9400	9000	1.04
Poloxamer 407	13 500	11 400	1.18

3.3. MALDI-MS

The use of MALDI-MS as a tool in polymer characterisation is growing steadily [15]. Recently, MALDI-MS has been employed as a tool to study poloxamers. van Rooij et al. have utilized a high resolution instrument for a detailed investigation

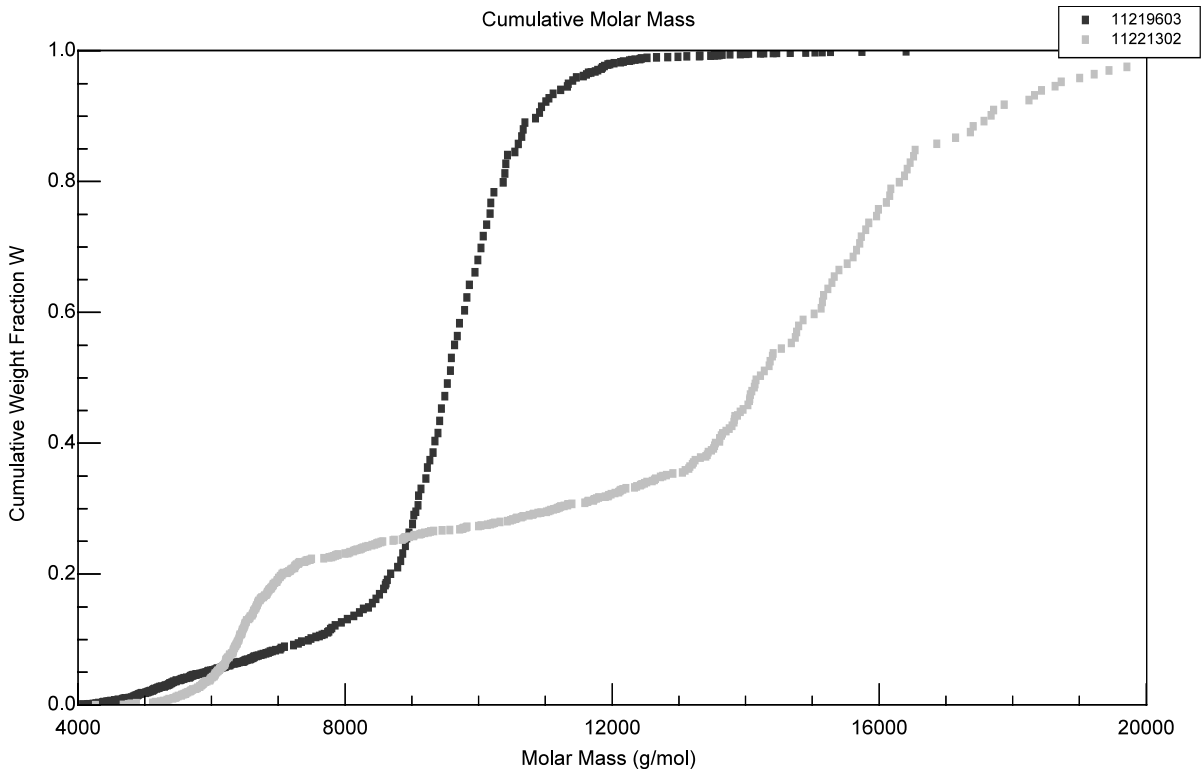

Fig. 9. Cumulative molar mass distribution for poloxamers 188 (black) and 407 (grey).

of poloxamer 101 (Pluronic® L31) [16], Gallet et al. have studied thermal degradation of poloxamer 407 [12], and Takátz et al. have analysed five different poloxamers, including 188 and 407 [17]. MALDI mass spectra of the two poloxamers of the present investigation are shown in Fig. 10. Since isotopic information was out of reach in reflector mode the samples were analysed in linear mode, which gives substantially higher signal intensity. The figure displays the raw spectra, before background subtraction. For both poloxamers there are two major distributions visible (cf. SEC-RI and SEC-MALS/RI sections above). Both these distributions have also been reported earlier [12,17]. Before calculating $\bar{M}_n$ and $\bar{M}_m$ the spectra were background subtracted.

The most intense distribution of poloxamer 188 (Fig. 10a) has $\bar{M}_n \approx 8900$ g/mol, and corresponds to the expected tri-block polymer PEO–PPO–PEO. The smaller distribution has $\bar{M}_n \approx 3900$ g/mol, which falls in between the masses of a mono-block of PEO (3500 g/mol) and a di-block of PEO–PPO (5100 g/mol). The measured mass instead corresponds to what can be expected if the polymerisation of propylene oxide is terminated by chain transfer reactions [13]. Since the isotopes were unresolved, the slight possibility that this distribution comes from doubly charged species could not be ruled out. However, the obtained $\bar{M}_n$ is more than 10% off compared to the expected value of 4450 g/mol, i.e. half the value of the singly charged distribution (cf. discussion of poloxamer 407 below). Peaks are also visible at even lower masses but it is unclear whether those are intact species or fragments. Since these low mass peaks interfere with the smaller distribution the calculated $\bar{M}_n$ is somewhat uncertain. Unfortunately, MALDI-MS is unreliable as a quantitative method. The response often varies with molecular mass and chemical composition. Therefore, no estimate can be made about the relative amounts of the different components.

Also for poloxamer 407 two distributions are visible (Fig. 10b). In this case, however, the low mass distribution is relatively pronounced having roughly the same intensity as the high mass distribution. The high mass distribution has $\bar{M}_n \approx 13\,000$ g/mol and corresponds to the tri-block

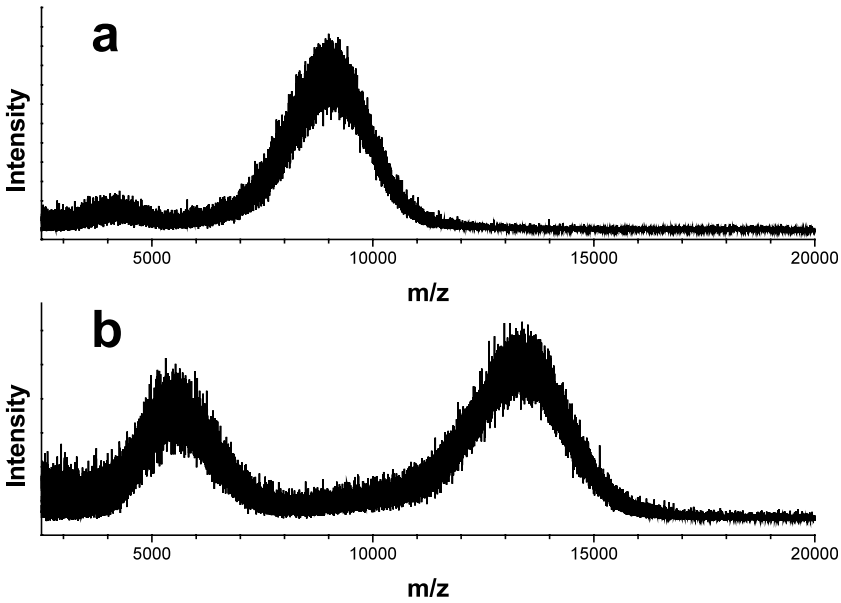

Fig. 10. MALDI mass spectra of poloxamers: (a) 188; and (b) 407. The spectra were calibrated externally.

polymer PEO–PPO–PEO. The low mass distribution, which also in this case is subjected to interference from low mass peaks, has $\bar{M}_n \approx$ 5500 g/mol, a value that also for this poloxamer falls in between those of the mono-block PEO (4400 g/mol) and the di-block PEO–PPO (7700 g/mol). As for poloxamer 188 the obtained mass corresponds with species resulting from chain tranfer reactions during the propylene oxide polymerisation [13]. Also in this case it could be argued that this distribution might originate from doubly charged species, but here the discrepancy is even greater than for the poloxamer 188. The expected $\bar{M}_n$ would be 6500 g/mol so the discrepancy is about 15%. The fact that the distributions are visible with SEC also strongly suggest that these distributions are singly charge species, and thus actual components of the analytes.

MALDI-MS analysis often gives slightly lower number than SEC does [18], which most likely accounts for the discrepancy between the data sets. Table 7 lists the numbers obtained from the MALDI-MS analysis. The table also includes the mass ranges that were used for calculating $\bar{M}_n$ and $\bar{M}_m$. This is of course, together with the background subtraction method, a likely source of moderate error, because of the difficulty to determine the background level for unresolved peaks such as the poloxamer distributions.

4. Conclusions

Two different columns were tested with different eluent compositions to find a suitable SEC-RI method that could be used instead of the pharmacopoeia method for determination of the molar mass for poloxamers 188 and 407. The best separation was obtained using a TSKgel 3000PW column with sodium chloride (0.01 M)–methanol (90:10, v/v) as eluent. The method gave the following molar mass values relative to PEG/PEO standards: poloxamer 188 $\bar{M}_n$ 6900 g/mol and $\bar{M}_m$ 8000 g/mol, and poloxamer 407 $\bar{M}_n$ 6200 g/mol and $\bar{M}_m$ 8600 g/mol. The difference between the two poloxamers is smaller than that obtained using titration. In view of their different molar mass distribution, together with the difference in figures, an SEC method can be used to differentiate between poloxamers 188 and 407.

The proposed method was validated by investigating the linearity, range, precision and the influence of sample concentration. The investigated parameters showed the suitability of the proposed method for determining the molar mass distribution of poloxamers 188 and 407.

The SEC-RI method was found to be a good alternative to the pharmacopoeia method for quality control. It was also shown that if information about the absolute molar mass is needed, e.g.

Table 7
The number average and mass average molecular masses and the polydispersity for poloxamers 188 and 407 measured by MALDI mass spectrometry

Analyte	Low mass distribution[a]			High mass distribution[b]		
	$\bar{M}_n$	$\bar{M}_m$	$\bar{M}_m/\bar{M}_n$	$\bar{M}_n$	$\bar{M}_m$	$\bar{M}_m/\bar{M}_n$
Poloxamer 188	3892	4003	1.028	8913	9009	1.011
	3991	4087	1.024	8888	8991	1.012
Average	3942	4045	1.026	8901	9000	1.011
Poloxamer 407	5399	5540	1.026	13 001	13 123	1.009
	5514	5639	1.022	13 067	13 191	1.010
Average	5457	5589	1.024	13 034	13 157	1.009

Spectra were background subtracted prior to calculation.

[a] The mass range used for calculating $\bar{M}_n$ and $\bar{M}_n$ was 2500–5500 g/mol for poloxamer 188 and 3000–8500 g/mol for poloxamer 407.

[b] The mass range used for calculating $\bar{M}_n$ and $\bar{M}_n$ was 5600–13 000 g/mol for poloxamer 188 and 8500–20 000 g/mol for poloxamer 407.

for a better comprehension of the possible chemical composition of components within the distribution, both SEC-MALS and MALDI-MS offers an opportunity to determine that. Similarly to the SEC-RI method they will give information about the distribution, as opposed to the pharmacopoeia method. They will not have the lack of specificity and the result will not be affected be the presence of unsaturated chain ends.

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