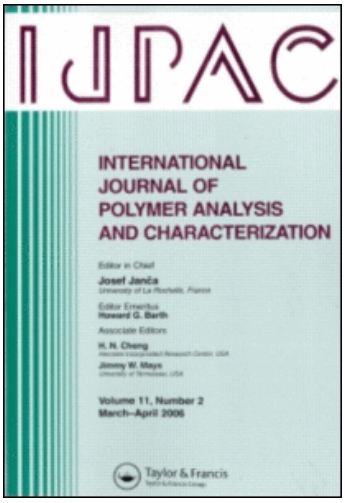


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# Characterization and Micellization of a Poloxamer Block Copolymer

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# Characterization and Micellization of a Poloxamer Block Copolymer

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**Abstract:** Several poloxamers that are symmetrical EPE block copolymers (E and P are ethylene and propylene oxide, respectively) have been characterized by size exclusion chromatography on Superose columns in water. The poloxamers contain between 12 and 26 wt% of smaller-size UV-absorbing impurities. Poloxamer P94 ($E_{28}P_{48}E_{28}$) forms micelles with increasing temperature, and micellization was investigated by eluent gel permeation chromatography (EGPC). EGPC results demonstrate that P94 impurities are not incorporated into the micelles up to 38°C. The importance of poloxamer heterogeneity for thermodynamic and structural studies is discussed.

**Keywords:** Eluent gel permeation chromatography; Micelles; Micellization; Pluronics; Poloxamer; Polydispersity

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## INTRODUCTION

Block copolymers often form micelles in selective solvents. Poloxamers are symmetrical triblock copolymers composed of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide), with the composition $E_xP_yE_x$, where $x$ and $y$ denote the number of monomers per block. Poloxamers exhibit a fascinating range of structures in solution depending on concentration, copolymer composition, co-solutes, and temperature.[1–3] Poloxamers are dissolved in water as individual polymer chains, unimers, at low temperatures, and spherical micelles with a core of poly(propylene oxide), PPO, are formed with increasing temperatures.[4–6] Micellization of poloxamers is often analyzed in terms of a closed association model with a dynamic equilibrium between unimers and micelles:

$$n \text{ unimers (aq)} \rightleftarrows \text{micelle}_n \text{ (aq)} \quad (1)$$

where $n$, the number of unimers per micelle, denotes the cooperativity of the process. Static light scattering studies on Pluronic P94 (also known as poloxamer 284, $E_{28}P_{48}E_{28}$) show that micelles contain 37 unimers per micelle at 40°C.[7] The value of $n$ for poloxamers is determined both by the block length of PPO and by the ratio $y/x$, and $n$ increases with temperature.[1,5,7,8] The characteristic time scale for motions between unimers and micelles of poloxamers is faster than milliseconds,[9,10] which ensures that solutions are in near-equilibrium states.

A number of studies have shown that the association model in Equation (1) is too simple to give an accurate description of the micellization process.[11–16] In a recent study, we have shown that the copolymers included in micelles formed at temperatures where both unimers and micelles are present have higher relative PPO contents and molar masses than the remaining unimers.[14] Poloxamers are prepared by base-catalyzed polymerization,[1] and for these low molar mass polymers, a molar mass distribution should be expected even in an ideal synthesis. Pluronic P85 ($E_{27}P_{39}E_{27}$) contains such a distribution of both PPO and poly(ethylene oxide) (PEO) block lengths, and the width of the micellization transition seen in differential scanning calorimetry (DSC) was partly due to the PPO block length distribution.[16] This study also demonstrated that P85 contains about 12 wt% smaller-size contaminants, which were attributed to a chain transfer reaction during polymerization, resulting in a diblock impurity.

In this study, we have investigated whether poloxamers other than P85 contain similar smaller-size contaminants and whether these contaminants are included in the micelles that are formed by the intact triblock copolymers. We chose to investigate P94 in more detail by eluent gel permeation chromatography (EGPC), since this copolymer has been

characterized in several studies.[7,10,14] EGPC is a special form of size exclusion chromatography (SEC) in which the eluent contains the sample to be investigated. EGPC is especially useful for studies of dynamic equilibria systems, e.g., the unimer micellar system, and was developed by Booth to investigate poloxamers.[17] In a recent investigation on P94 solutions, the shift in unimer micellar equilibrium was studied as a function of temperature.[18] We have improved this type of EGPC analysis by finding a column material with a minimum of interaction effects between the copolymers and the column.

## MATERIALS AND METHODS

### Polymer Samples

Pluronics samples with different PPO and PEO block lengths were from BASF (Parsippany, N.J., USA), except for P94, which was purchased from Serva (Heidelberg, Germany). In the Pluronic nomenclature the first letter denotes the physical state of the copolymer at room temperature (L = liquid; P = paste; F = flakes). Details about the expected block lengths are summarized in Table I. All aqueous solutions were prepared gravimetrically using double-distilled water.



### Liquid Chromatography

A Shimadzu high-performance liquid chromatography (HPLC) system consisting of a LC-10AD pump, a SPD-M10A photo diode-array detector, and a RID 10A differential refractive index (RI) detector was used in

**Table I.** Poloxamer characterization according to producers and measured content of smaller-size impurities determined by SEC

| Sample | Poloxamer | Molar mass (g/mol) | PPO block length | Impurity content (wt%) |
|---|---|---|---|---|
| P85 | 235 | 4630 | 39 | 12 |
| P94 | 284 | 5250 | 48 | 16 |
| L101 | 331 | 3750 | 54 | 16 |
| P103 | 333 | 4900 | 54 | 17 |
| P104 | 334 | 5850 | 54 | 17 |
| P105 | 335 | 6480 | 54 | 22 |
| F108 | 338 | 14400 | 54 | 24 |
| L122 | 402 | 5030 | 67 | 25 |
| P123 | 403 | 5730 | 67 | 26 |

different types of chromatography.[16] The RI signal is proportional to the weight concentration, since PEO and PPO have very similar differential refractive index increments.

Size exclusion chromatography (SEC) was used to characterize poloxamers according to hydrodynamic size. Different column materials were investigated, and Superose columns from Pharmacia[19] were found to be ideal SEC columns for this purpose. Two Superose columns, HR-6 and HR-12, with lengths and diameters of 30 and 1 cm, respectively, were used. The two columns have different molar mass exclusion limits and were used either individually or in series in order to cover a broader range of sizes. The temperature of the columns and the injection unit were controlled to $\pm 0.1$°C by immersing them in a water thermostat from Heto (Birkerød). EGPC experiments were done on the same Superose columns, but the eluent contained 1 mg/mL P94 in water. The samples to be investigated and sucrose were dissolved in this eluent. EGPC chromatograms were determined at temperatures between 15° and 38°C.



### Scanning Calorimetry

Differential scanning calorimetry was performed using a Nanocal scanning calorimeter from CSC (Calorimetry Science Corporation, Provo, Utah, USA) with a cell volume of 0.322 mL, as described elsewhere.[16] Scans were performed with a scan rate of 30°C/h and analyzed using the Origin software. The thermal lag was negligible at this scan rate and lower scan rates.

## RESULTS AND DISCUSSION

The chromatograms in Figure 1 show SEC for various Pluronics samples on a Superose HR-12 column in water at 15°C. Under these conditions, even homopolymers of PPO with a molar mass of 2000 g/mol elute according to hydrodynamic size, as a result of minimal hydrophobic interactions with the column material. Pululans with molar masses of 380 and 853 kDa elute in the exclusion volume at 7.3 mL, and the total volume of the column is close to the elution volume of sucrose, marked with S on the chromatograms. The chromatograms for all eight Pluronics samples show a major eluting peak followed by a smaller later eluting peak, which for some of the samples (especially L101 and L122) extends almost to the sucrose peak. The results in Table I illustrate that the major peaks in Figure 1 elute in good agreement with the expected molar masses of the intact triblocks. The relative areas of the small peaks were calculated by integrating the areas under the two peaks. The table shows that

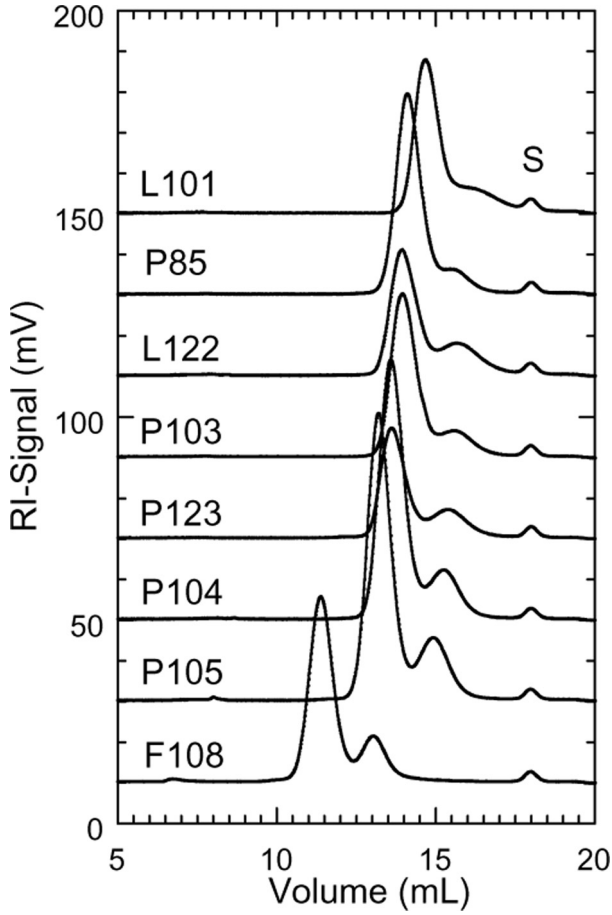


**Figure 1.** Size exclusion chromatography of various poloxamers in water at 15°C on a Superose HR-12 column. Refractive index signal as a function of elution volume is shown. A 0.1 mL amount of approximately 4 mg/mL poloxamer solutions with 0.1 mg/mL sucrose were injected on the column. Elution peaks marked S are due to the internal standard sucrose.

impurities make up between 12 and 26 wt% of the poloxamer samples. It is also seen that the content of impurities is high for poloxamers with long PPO block lengths and it is higher for samples with long PEO chains for the same PPO block copolymers.

Micellization of poloxamers in water can be studied by several techniques.[1–3] DSC has been used to investigate micellization of a 1 mg/mL P94 solution in water at a scan rate of 30°C/h. This solution is the eluent used for the EGPC measurements below. The heat flow,

which is proportional to the heat capacity, is shown in Figure 2, after baseline correction.[16] The thermogram is similar to previous results on P94[14,18] and shows a broad endothermic transition with an onset temperature of about 27°C and a peak temperature at 32°C. The curve marked B shows the normalized integral of the heat capacity curve and illustrates that half of the transition is completed at 34°C and that 92% is completed at 38°C.

Traditional size exclusion chromatography studies in water of a dynamic unimer-micelle system are difficult, since the equilibrium shifts during the passage of the sample through the column. Unimers released from micelles due to dilution, as seen in Equation (1), will move slowly through the column compared with the micelles, and this dilution of the sample will shift the equilibrium towards monomers. EGPC is an elegant way to avoid such problems. If the eluent contains the dissolved copolymer and the column is at a temperature where both unimers and micelles are present there will be both uimers and micelles throughout the column. If an excess of polymer is injected on the column, it will partition to the micelles already present in the eluent. By changing the temperature of the column, the critical micellization temperature can



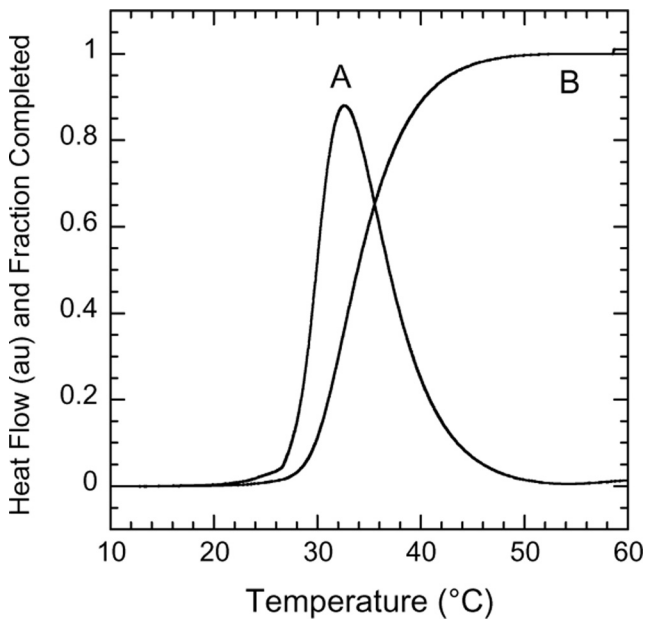


**Figure 2.** Differential scanning calorimetry of 1 mg/mL P94 solution in water at a scan rate of 30°C/h. Heat flow (A) in arbitrary units after baseline correction is plotted together with the fraction of transition completed (B) against temperature.

be determined. Only unimers and impurities were observed at temperatures between 15° and 26°C. The chromatogram at 27°C shows the first appearance of an earlier eluting peak. This temperature is in excellent agreement with the onset temperature of micellization observed in Figure 2. A UV detector at 203 nm was also used together with the RI detector. Figure 3 shows a small UV absorption of the micelles, which is primarily assigned to light scattering from the micelles, and a small UV signal of the unimers. The main UV absorption is due to the impurities even though the concentration of impurities is much lower than the unimer concentration as seen from the RI signals. The impurities are chemically different from the intact triblocks, and the absorbance is assigned to double bonds formed in a chain transfer reaction.[16] Additional support for this assignment was obtained from nuclear magnetic resonance (NMR) data on the impurity fractions (data not shown). UV signals in chromatograms of other poloxamers confirm that impurities contain UV-absorbing components.



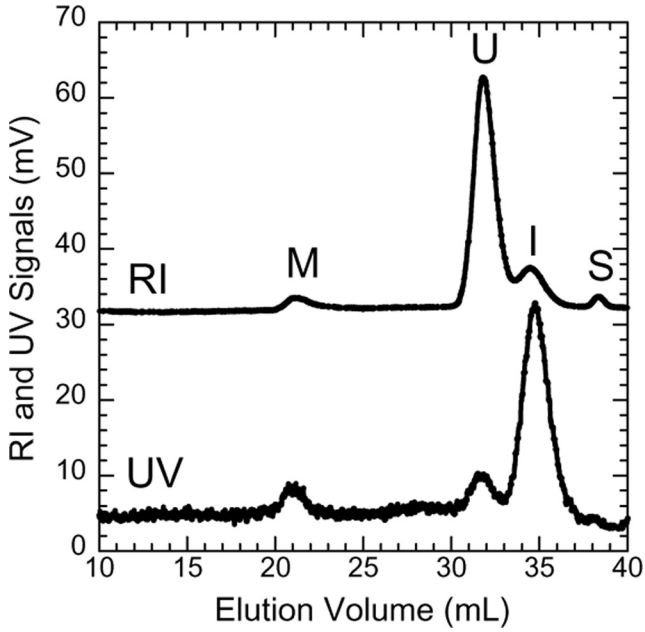


**Figure 3.** Eluent gel permeation chromatography of P94 at 27°C on Superose HR-6 and HR-12 columns in series. Refractive index and UV absorption at 203 nm signals are plotted. Eluent is 1 mg/mL P94, and 0.1 mL of 6 mg/mL P94 solution with sucrose was injected on the column. Refractive index and UV absorbances at 203 nm are shown as a function of elution volume. Peaks are assigned as: M for micelles, U for unimers, I for impurities, and S for sucrose.

EGPC chromatograms at selected temperatures are shown in Figure 4. At 21°C only unimers, impurities, and sucrose elution peaks are seen. When the temperature is increased from 27°C the micellar peak gradually increases in area, whereas the area of the unimer peak decreases. The midpoint of the transition in DSC occurred at 32°C, and at this temperature integration of the peaks indicate that 55% of the triblocks are in micelles and the rest are unimers. Integrations of the micellar and unimer peaks show that 95 wt% of the triblocks are in

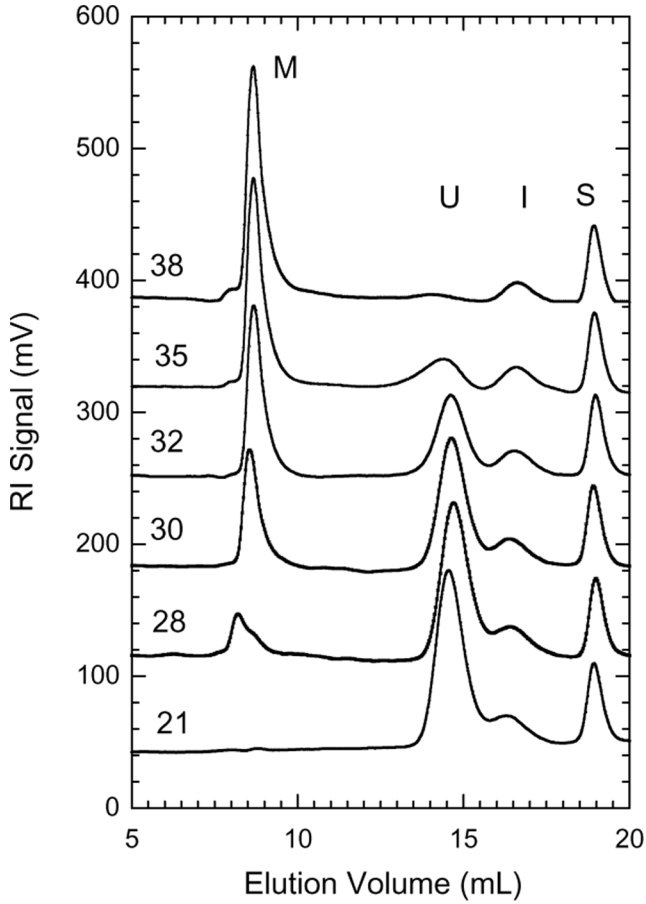


**Figure 4.** Elution gel permeation chromatography of P94 on a Superose HR-12 column at the different temperatures indicated in °C. A 0.1 mL amount of 2 mg/mL P94 is injected into 1 mg/mL P94 eluent. Refractive index signals at selected temperatures are shown as a function of elution volume. Peaks are assigned as: M for micelles, U for unimers, I for impurities, and S for sucrose.

the micellar state at 38°C, compared with 92% of the transition completed in DSC. The area of the impurity peak is virtually independent of temperature from 21° to 38°C and far exceeds the unimer area at 38°C. This shows that the impurities, even at the highest temperature, are not incorporated into micelles.

Our observation that all the poloxamers contain significant amounts of impurities, which in the case of P94 are not included in the micelles, is important for precise determinations of poloxamer micellization properties. If, for example, 20 wt% of the copolymers are not included in the micelles, DSC enthalpies will be 25% underestimated for intact triblocks when expressed in enthalpy per mol or gram of polymer. The same problem will occur in the determinations of several other thermodynamic properties such as heat capacity and volume changes. A consideration of impurities is also important for structural studies in which the calculations of volume fractions occupied by micelles are likely overestimated if a significant fraction of the sample is not able to form micelles. If precise studies of triblocks are required, the copolymer sample should be fractionated or purified or at least characterized carefully.



## CONCLUSIONS

Our results have demonstrated that all the investigated poloxamers contain smaller-size impurities. These contaminants are present in concentrations between 12 and 26 wt%, and they are UV absorbing due to a double bond resulting from a transfer reaction during polymerization. A 1 mg/mL P94 solution forms micelles between 27° and 38°C as seen in DSC and EGPC. The impurities in P94 are not included in the micelles at the highest temperatures. If 10–25% impurities in poloxamers are not included in micelles, the measured calorimetric enthalpies are in error. Similar problems arise in many types of both structural and thermodynamic determinations of micelles.

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