Investigation of Block Copolymer Micellization by High-Performance Size-Exclusion Chromatography

PAVEL ŠPAČEK and MIROSLAV KUBÍN, *Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, 162 06 Prague 6, Czechoslovakia*

Synopsis

The formation and decomposition of micelles of the three-block copolymer polystyrene-block-poly(ethene-stat-butene)-block-polystyrene (Kraton G 1652, Shell) were investigated by high-performance size-exclusion chromatography in a mixed solvent dioxan–heptane. The association equilibrium may be shifted from the pure unimer to pure micelles by changing the composition of the mobile phase, and also (at constant composition) by a change in temperature. The shape of the chromatograms observed under conditions of comparable equilibrium concentrations of both species may be explained by the disturbance and reestablishment of the association equilibrium during separation in the chromatographic column. Experiments with changing flow rate show the dominating effect of degrading separation efficiency with increasing linear velocity of the mobile phase.

INTRODUCTION

In dilute solutions, block copolymers dissolved in a solvent selective for one of the blocks may form associates,[1] which by analogy with low-molecular-weight soaps and surfactants have been called micelles. Association (micellization) is a reversible process which may be controlled by changing the thermodynamic quality of the solvent. The formation and decomposition of micelles were investigated by, e.g., light scattering[2–6] and osmometry.[6,7] These methods provide information about the average molar mass of all particles in solution, and thus indirectly about the equilibrium between the micelles and the molecularly dissolved copolymer which is here usually called the unimer.[8] On the basis of low-angle x-ray scattering data,[9,10] a simple model of two concentric spheres has been suggested for the structure of block copolymer micelles, in which the compact core of the micelle consists of insoluble blocks and the shell contains solvated chains of the other block, soluble in the given medium. Little so far is known about the kinetics of micellization and decomposition of the associates.

Separation of micelles from the unimer by size-exclusion chromatography (SEC) was studied[11] with the system polystyrene-block-polyisoprene in *N,N*-dimethylacetamide (a selective solvent for polystyrene); the transition micelle $\rightleftharpoons$ unimer was controlled by changes in temperature. The authors[11] explained the shape of the chromatograms by the sensitivity of the association equilibrium to dilution during the chromatographic separation, and by positive adsorption of the unimer on the packing at lower temperatures; they did not observe any effect of the flow rate of the mobile phase.

It is the aim of the present study to elucidate the behavior of micellizing

Journal of Applied Polymer Science, Vol. 30, 143–150 (1985)
 CCC 0021-8995/85/010145-08$04.00

block copolymers under conditions of high-performance size-exclusion chromatography. Since in the chromatographic column the equilibrium is continuously disturbed and reestablished, in a favorable case the results could contribute to the elucidation of the kinetics of micellization and decomposition of associates; since, however, the theory of separation of associating macromolecules under real chromatographic conditions is still in a rather unsatisfactory state, only qualitative conclusions may be expected.

The three-block copolymer polystyrene–hydrogenated poly-butadiene–polystyrene in a binary solvent 1,4-dioxan–heptane was investigated; thus, the thermodynamic quality of the mobile phase could be controlled not only by temperature changes, but also by changes in composition (dioxan is a selective solvent for polystyrene).

EXPERIMENTAL

The three-block copolymer polystyrene-block-poly(ethene-stat-butene)-block-polystyrene (Kraton G 1652, Shell product) contained 29 wt % of polystyrene as two outer blocks of the same length on both sides of the central block of hydrogenated butadiene; M_w by light scattering was $\cong$ 60,000. Heptane (analytical grade, Loba Chemie, Fischamend) was used as received. 1,4-Dioxan (analytical grade, Lachema, Brno) was distilled with Cu_2Cl_2 and used fresh.

The apparatus for high-performance size-exclusion chromatography has been described earlier.[12] The column system consisted of one column 500 mm long, i.d. 4 mm, packed with macroporous silica of our own production (particle diameter 8 μm, molecular weight exclusion limit for polystyrene in tetrahydrofuran $M_e = 1 \times 10^5$) in series with two identical columns (250 mm long, i.d. 6 mm) packed with silica having $M_e = 1 \times 10^6$. The concentration in the eluate was monitored with a variable wavelength UV detector (Cecil Instruments, Cambridge, England) at 254 nm in series with a differential refractometer (type R 401, Waters Assoc., Milford, Conn.). Signals obtained from the two detectors were recorded with a two-channel potentiometric recorder (Goerz, Vienna, Austria); the differential refractometer served mainly for checking whether the concentration of the eluate was already stabilized at the new level after a change in the composition of the mobile phase. The whole apparatus was placed in an air thermostat.

A stock solution of the copolymer (26 mg mL^{-1}) in a mixture heptane/dioxan (30/70 by volume) was prepared in a sealed ampoule. The solution was injected directly onto the top of the first column; solutions for calibration of the detector response were prepared from the stock solution.

RESULTS AND DISCUSSION

The temperature dependence of the association equilibrium micelle $\rightleftharpoons$ unimer was investigated for the composition of the mobile phase heptane/dioxan 30/70 by volume and at the flow rate of 0.5 mL/min; 20 μL of the stock solution were injected. The shape of the resulting chromatograms can be seen in Figure 1. Below 28°C a single, very narrow and symmetrical peak of micelles was observed at the retention volume V_R = 9.5 mL. On the contrary, above 41°C the elution curve consisted of a single symmetrical

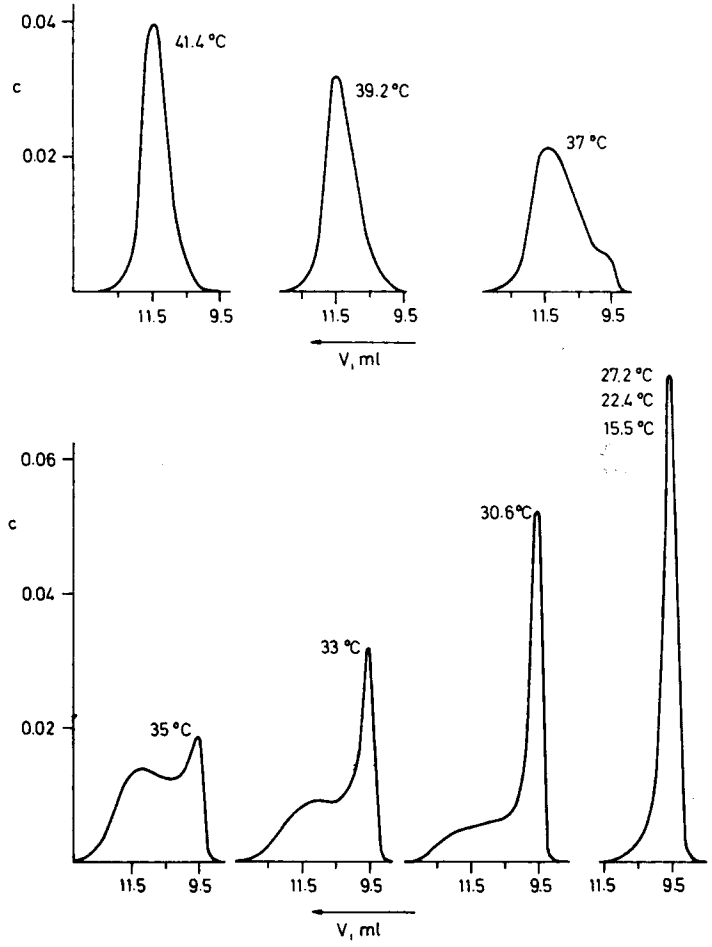

Fig. 1. Effect of temperature on the shape of elution curves of Kraton G 1652 in the mobile phase heptane: 1,4-dioxan 30:70 by volume. Flow rate 0.5 mL/min, 20 μL of the stock solution injected; c is the copolymer concentration in the eluate in wt. %.

peak at V_R = 11.5 mL, which (owing to the disappearance of the typical bluish tint of the stock solution at elevated temperatures) must be attributed to the pure unimer. By superimposing both peaks it can be demonstrated that with the given system of three columns an almost baseline separation of the micelles from the unimer could be achieved. However, the actual chromatograms recorded at temperatures between these extremes, when both species coexisted in the system at comparable concentrations, never represented a mere superposition of peaks of the pure components (Fig. 1), but were deformed in such a manner that the first and second peak showed strong tailing and fronting, respectively. This is in agreement with the assumed continuous establishment of association equilibrium in the column which is superimposed on the chromatographic separation of the two components: As soon as the two peaks are separated as a result of their different rates of migration in the column, the system tends to restore the original concentration equilibrium, so that in the peak of the micelles (unimer) a

certain concentration of the unimer (micelles) is established and these newly formed species then migrate through the column at their own inherent rate. Hence, as the separation continues, the areas under the two peaks of pure components gradually decrease, and this deficit of concentration shifts into the interjacent range of elution volume, in a qualitative agreement with the shape of real chromatograms in Figure 1. This process is further complicated by the sensitivity of the equilibrium between the unimer (U) and micelles (M)

$$n\mathrm{U} \rightleftharpoons \mathrm{M}$$

(where n is the association number) to concentration which in each peak varies continuously from the maximum value to zero.

A change in the composition of the mobile phase at constant temperature of 29°C has an analogous effect (Fig. 2; the flow rate was 0.5 mL/min, the injected volume was 20 μL). At a higher content of dioxan (a selective solvent for polystyrene) than about 75 vol %, only the peak of micelles at the retention volume 9.5 mL can be observed; on the other hand, if the dioxan content in the mobile phase drops below 60 vol %, the system consists virtually of pure unimer. The shape of the elution curves in the composition range between these values is then very similar to that in Figure 1.

For a better understanding of the observed elution profiles, one chromatogram [obtained at 35°C with the mobile phase containing 70 vol % of dioxan, flow rate 0.2 mL/min was decomposed (Fig. 3) into the peak corresponding to pure unimer (V_R = 11.5 mL, area A] and the peak of pure micelles (V_R = 9.5 mL, area B); these peaks were assumed to be strictly symmetrical in the decomposition. The difference (hatched) area represents that part of the copolymer which has passed through the column partly as micelles and partly as the unimer; the position on the V-axis of the respective fractions inside this peak (in the elution volume interval between

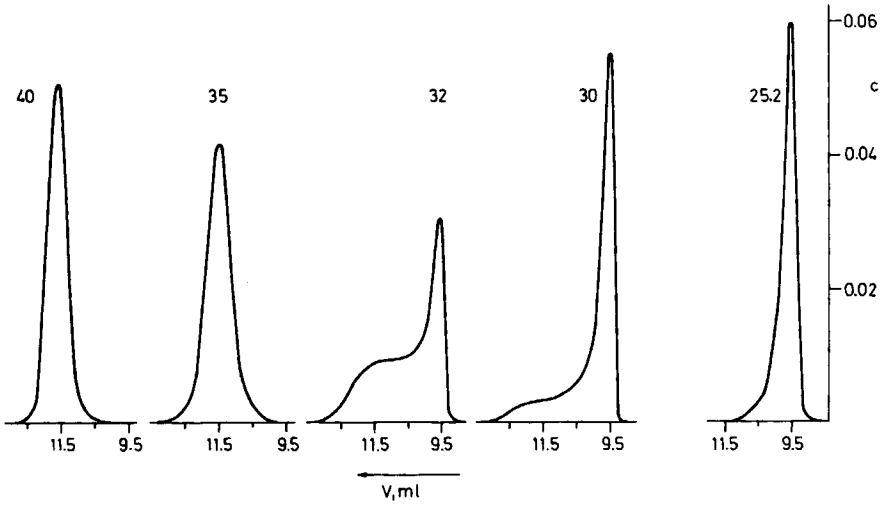

Fig. 2. Elution curves recorded at 29°C with mobile phase of varying composition. Numbers give the heptane content (vol %) in the mixture with 1,4-dioxan.

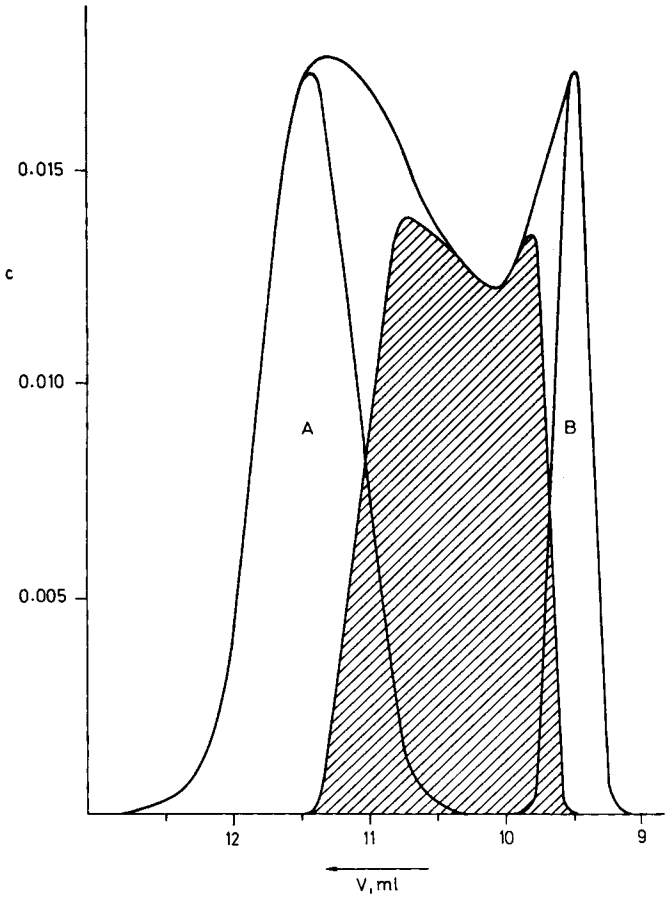

Fig. 3. Decomposition of a typical chromatogram into peaks of pure unimer (A) and pure micelles (B); see text for explanation.

9.5 and 11.5 mL) then indicates the fraction of time spent in the two forms.[13] By comparing the three areas, it can be seen that in this particular case about 14% of the copolymer passed through the column exclusively as micelles, about 36% was present in the column exclusively as the unimer, and roughly 50% participated in the redistribution between these two species.

The peak of the micelles at V_R = 9.5 mL was extremely sharp in all cases, so that the size distribution of the associates was obviously very narrow (cf. Refs. 8 and 10), which corroborates the view that the micellization of block copolymers proceeds via the mechanism of closed association.[5,8] No changes in the retention volume of micelles were observed at different temperatures and with different mobile phases; it does not follow, however, that micelles with the same association number n are formed under widely different conditions: The sensitivity of the method was clearly insufficient to discern differences in the hydrodynamic volume of the compact associates formed.

No comprehensive theory of separation of associating macromolecules

under real chromatographic conditions is available.[14-16] Accordingly, it is difficult to determine quantitative characteristics of the rate of formation or decomposition of micelles in the given system from the shape of observed elution curves. It can be only estimated that the halftime of at least one of the reactions that proceed in the system is not negligibly small in comparison with the duration of the chromatographic experiment. By analogy with the chromatography of *isomerizing* macromolecules (where the transformations in the system formally obey the first-order kinetics) which has been treated theoretically by Belenkii and Vilenchik (Ref. 16, p. 169), it may be expected that only a single peak containing both unresolved components will be observed if both reversible reactions are very fast compared with the time scale of the chromatographic process. On the contrary, if the formation or decomposition of the associates were very slow on the same time scale, the resulting chromatogram would be a superposition of the peaks of both pure components.

With the aim to confirm, at least qualitatively, the validity of this analogy for our system, we varied the time scale of the chromatographic experiment by changing the flow rate of the mobile phase (0.2, 0.4, 0.6, 0.8, and 1.0 mL/min, which corresponds to a shift of the elution time of the unimer from about 60 min to some 11.5 min). The results are shown in Figure 4 for 35°C [when at the given composition of the mobile phase—70 vol.% of dioxan in the mixture—the equilibrium was shifted more or less in favor of the micelles; cf. Fig. 4(a)], and for 37°C [when the equilibrium mixture contained more unimer; cf. Fig. 4(b)]. It follows from the analysis reported in Ref. 16 and valid for an isomerizing system that as the time of the experiment is reduced, the effect of the (constant) rate of isomerization will become less pronounced and the resolution of the two peaks will improve. Contrary to this expectation, however, in our system the resolution became distinctly poorer with increasing flow rate (Fig. 4). Apparently, the decrease in the separation efficiency with increasing flow rate of the mobile phase predom-

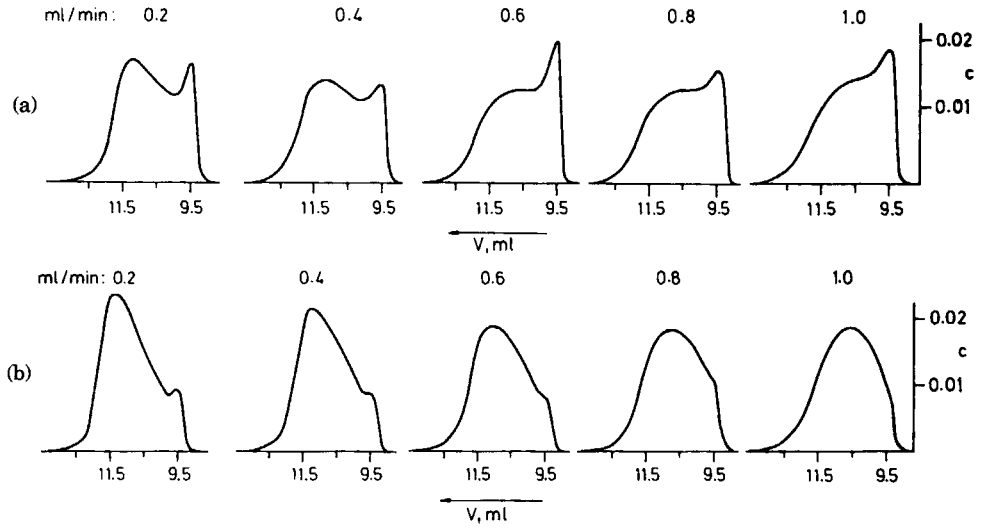

Fig. 4. Effect of flow rate on the chromatographic separation of Kraton G 1652 in the mobile phase heptane: 1,4-dioxan (30:70 by volume) (a) at 35°C, (b) at 37°C.

inates; this effect is generally observed in SEC of macromolecules, and it has been confirmed in a number of papers[17-22] that the separation efficiency is controlled by the finite rate of mass transfer, i.e., the contribution of the stationary phase to the height of the theoretical plate predominates in SEC. (To simplify the calculations, Belenkii and Vilenchik[16] neglected this effect and considered as the only mechanism of spreading the longitudinal diffusion, whose dependence on the flow rate is directly opposite and which has been known for some time to be of minor importance in SEC of macromolecules.)[23,24]

The shift of the association equilibrium in favor of the unimer with decreasing concentration of the block copolymer in a selective solvent was investigated chromatographically by varying the volume of the injected stock solution so that also the mean copolymer concentration in the column was changed; these measurements were performed at the flow rate of 0.5 mL/min. Chromatograms obtained under conditions (35°C, mobile phase 70 vol. % of dioxan in the mixture with heptane) when comparable concentrations of the unimer and micelles coexist in equilibrium (cf. Fig. 1) are shown in Figure 5. With decreasing injected volume the relative fraction of the peak of the unimer at V_R = 11.5 mL gradually increases at the

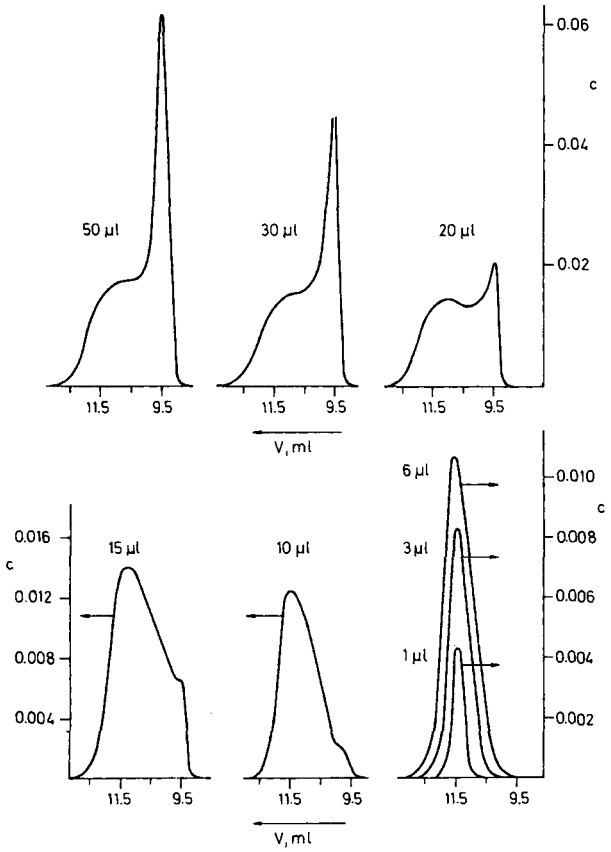

Fig. 5. Chromatograms of the micellizing block copolymer at various injected volumes of the stock solution. Mobile phase heptane: 1,4-dioxan (30:70 by volume), 0.5 mL/min, 35°C.

injected under these conditions, no micelles can be detected in the eluate, and the copolymer passes through the column from the very beginning as the unimer. On the other hand, under conditions when the equilibrium was displaced predominantly in favor of the micelles (the same mobile phase as in Figure 5, 20°C), not a trace of the unimer peak appeared in the range 1–50 μL of the injected stock solution, although at the smallest injected volume the maximum concentration in the peak of micelles leaving the column amounted only to 0.05 mg/mL. On the contrary, under otherwise identical conditions but at 41.8°C (where there exists in the system practically pure unimer—see Figure 1), no micelles could be detected in the eluate even at the highest concentration in the column, i.e., with 50 μL of the stock solution injected. These observations are in agreement with the concentration dependence of the mean molar mass of particles, as observed at various temperatures in solutions of block copolymers in a selective solvent (e.g., Refs. 3 and 4). A quantitative interpretation of our results in terms of the critical micellar concentration is not possible, however, because of the continuously varying concentration across the chromatographic peak and also in view of the decrease in the concentration at the peak maximum during its passage through the column, brought about by axial dispersion.

The authors wish to thank Dr. Z. Tuzar for the sample of Kraton, and also for many valuable discussions.

References

1. Z. Tuzar and P. Kratochvíl, *Adv. Colloid Interface Sci.*, **6**, 201 (1976).
2. S. Krause, *J. Phys. Chem.*, **68**, 1948 (1964).
3. Z. Tuzar and P. Kratochvíl, *Makromol. Chem.*, **160**, 301 (1972).
4. C. Price, J. D. G. McAdam, T. P. Lally, and D. Woods, *Polymer*, **15**, 228 (1974).
5. Z. Tuzar, V. Petrus, and P. Kratochvíl, *Makromol. Chem.*, **175**, 3181 (1974).
6. A. Sikora and Z. Tuzar, *Makromol. Chem.*, **184**, 2049 (1983).
7. J. Periard and G. Riess, *Eur. Polym. J.*, **9**, 687 (1973).
8. H.-G. Elias, *J. Macromol. Sci. Chem.*, **A7**, 601 (1973).
9. J. Pleštil and J. Baldrian, *Makromol. Chem.*, **176**, 1009 (1975).
10. Z. Tuzar, J. Pleštil, Č. Koňák, D. Hlavatá, and A. Sikora, *Makromol. Chem.*, **184**, 2111 (1983).
11. C. Booth, T. D. Naylor, C. Price, N. S. Rajab, and R. B. Stubbersfield, *J. Chem. Soc., Faraday Trans. I*, **7**, 2352 (1978).
12. M. Kubín, *J. Appl. Polym. Sci.*, **27**, 2943 (1982).
13. R. A. Keller and J. C. Giddings, *J. Chromatogr.*, **3**, 205 (1960).
14. G. K. Ackers, *Adv. Protein Chem.*, **24**, 343 (1970).
15. J. K. Zimmermann, *Biochemistry*, **13**, 384 (1974).
16. B. G. Belenkii and L. Z. Vilenchik, *Chromatografia Polimerov*, Chimia, Moscow, 1978, p. 176.
17. L. H. Tung and J. R. Runyon, *J. Appl. Polym. Sci.*, **13**, 2397 (1969).
18. W. W. Yau, C. P. Malone, and H. L. Suchan, *Separ. Sci.*, **5**, 259 (1970).
19. N. Povey and R. A. Holm, *J. Chromatogr.*, **46**, 33 (1970).
20. G. K. Ackers and H. R. Halvorson, *J. Polym. Sci., A-2*, **9**, 245 (1971).
21. A. M. Basedow, K. H. Ebert, H. Ederer, and H. Hunger, *Makromol. Chem.*, **177**, 1501 (1976).
22. K. Unger and R. Kern, *J. Chromatogr.*, **122**, 345 (1976).
23. J. G. Hendrickson, *J. Polym. Sci., A-2*, **6**, 1903 (1968).
24. R. N. Kelley and F. W. Billmeyer, *Separ. Sci.*, **5**, 291, (1970).

Received January 18, 1984
Accepted May 14, 1984