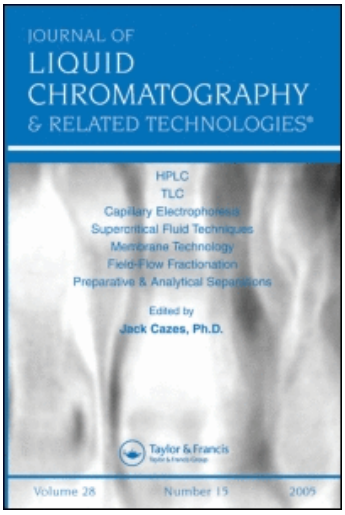

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Behavior of Reversibly Associating Systems in Size Exclusion Chromatography. Interpretation of Experimental Data Based on Theoretical Model

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BEHAVIOR OF REVERSIBLY ASSOCIATING SYSTEMS IN SIZE EXCLUSION CHROMATOGRAPHY. INTERPRETATION OF EXPERIMENTAL DATA BASED ON THEORETICAL MODEL

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ABSTRACT

The shape of the SEC chromatogram of a reversibly associating system in the SEC is a result of several competing processes: (a) SEC mechanism, (b) re-equilibration of the perturbed association equilibrium, and (c) the axial dispersion of individual components in the mobile phase. In some associating (micellizing) block copolymer systems in selective solvents, other secondary processes (e.g. temporary trapping of a polymer in pores of the stationary phase) may influence the chromatogram shape and complicate the analysis of experimental data. In this paper, general aspects of the interpretation of experimental data is discussed on the basis of theoretical models.

INTRODUCTION

In our preceding papers (1,2,3) we dealt with the problems of the chromatographic separation of reversibly associating systems. First, a rather simplified model, describing the competition of the SEC mechanism with the re-establishment of the perturbed association equilibrium and disregarding the axial dispersion, was proposed (1). This model was further modified by taking into account specific chromatographic effects, which may take place in SEC in micellizing block copolymer systems (2). By considering the effect of the axial dispersion, a more realistic model of SEC of reversibly associating systems was created (3). Mathematical solution of the set of derived partial differential equations was rather complicated when compared with the older models. However, numerical solutions for a wide range of variable parameters (i.e. association number, rates of the association/ dissociation processes etc.) correspond well to the experimental chromatograms of associating systems (4-6).

In this work we have studied the effect of the axial dispersion upon the shape of theoretical chromatograms in more detail. We consider some practical aspects of the interpretation of micellizing copolymers. Finally we deal with possible difficulties which may accompany the SEC experiments with associating systems and compare our theoretical results with experimental chromatograms of micellizing block copolymers.

THEORETICAL

Let us consider the passage of a solute through a SEC column. The component U (unimer) associates

reversibly forming an associate A

$$n\,U \overset{K_A}{\rightleftharpoons} A \qquad (1)$$

where n is the association number and K_A is the equilibrium constant. Processes which take place in the unit volume of the homogeneous SEC column of the finite length L, can be described mathematically - under reasonable and not too simplifying assumptions (3) - by a set of partial differential equations

$$\partial c_i^{(m)}/\partial t = D_i(\partial^2 c_i^{(m)}/\partial x^2) - v(\partial c_i^{(m)}/\partial x) + (-1)^i Q - P_i, \qquad (2a,b)$$

$$i = 1,2$$

$$\partial c_i^{(s)}/\partial t = P_i/\gamma_i \qquad (3)$$

where $c_i^{(m)}$ is concentration of the i-th component in the mobile phase, $c_i^{(s)}$ is its concentration in the stationary phase, D_i its axial dispersion coefficient (in the mobile phase), v is the rate of the forced flow of the mobile phase, and γ_i is the ratio of the void volume of pores of the stationary phase, accessible for the i-th component, to the volume of mobile phase. Values of subscript i stand for: a) i=1, for unimer, and b) i=2, for associate. The terms Q and P_i are the source terms describing the conversion of unimer into associate and the phase equilibria of individual forms between the mobile and the stationary phases. For a closed association eq. (1), the simpliest relation which adequately describes the concentration changes in a thin cross-section of the SEC column may be expressed (3) by the equation

$$Q = k_{as}[c_1^{(m)}]^n - k_d c_2^{(m)} \qquad (4)$$

where k_{as} and k_d are the association and dissociation rate constants, respectively.

For the limiting case of SEC (gel filtration) and the instantaneous phase equilibration (IPE model - the instantaneous phase equilibration model) of unimer between the mobile and the stationary phases; thus holds $P_2 = 0$ and $(\partial c_1^{(s)}/\partial t) = K_1 (\partial c_1^{(m)}/\partial t)$, where K_1 is the phase equilibrium constant of unimer between mobile and stationary phases and eq. (3) need not be solved simultaneously with eqs. (2a,b).

In some cases encountered mainly in systems of micellizing block copolymers (7-9), the assumption of the instantaneous equilibration of unimer between mobile and stationary phases is not fulfilled. Micelles that are built from unimer after its diffusion into gel pores, may be trapped in pores and the polymer release from the stationary phase is slowed down (2). We have proposed to use the linear driving force model (10) for the phenomenological description of that effect, (HSR model - the hindered solute release model) regardless of its real molecular mechanism (2).

The linear driving force gives the following expression for the time derivative of the space average concentration in a gel particle, $(\partial c_1^{(s)}/\partial t)$,

$$(\partial c_1^{(s)}/\partial t) = \begin{cases} k_{sp}[c_1^{(m)} - c_1^{(s)}], & \text{for } c_1^{(m)} > c_1^{(s)} \\ k_{ef}[c_1^{(m)} - c_1^{(s)}], & \text{for } c_1^{(m)} < c_1^{(s)} \end{cases} \qquad (5)$$

where k_{sp} and k_{ef}, $k_{sp} > k_{ef}$, are the effective constants describing unimer penetration into and release from, pores of the stationary phase. Different mathematical expressions, e.g. those modelling an adsorption isotherm, might be used, and theoretical concentration profiles

(peaks) for other chromatographic techniques would be obtained. Initial and boundary conditions, describing real SEC experiments were used in our mathematical calculations (1,3).

RESULTS AND DISCUSSION

Effect of the Axial Dispersion

Results obtained in (3) have shown the important role of the axial dispersion. Mainly that of unimer, which is more important due to the higher mobility of its relatively smaller particles. In case of the fast association equilibrium, the axial dispersion of unimer accelerates the decrease in the associate concentration during the passage through the SEC column. In this part, some theoretical concentration profiles $c_1^{(m)}$ and $c_2^{(m)}$ in the column are given, documenting in more detail the influence of the axial dispersion upon the shape of the chromatograms.

Fig. 1a) shows the theoretical concentration profiles of both coexisting components in the mobile phase (S-slow, F-fast components, respectively), (normalized to the equilibrium concentration of the unimer in the inlet pulse, c_1^o), i.e. $S = c_1^{(m)}/c_1^o$ and $F = c_2^{(m)}/c_1^o$, as a function of the reduced position in the column, $X = x/L$ and Fig. 1b) shows the normalized summary chromatograms, i.e. $(S+F)/2$ in the SEC column for the gel filtration and fast association (i.e. under the same condition as in (3)). The following constant reduced parameters simulating realistic experimental conditions have been used: $n = 16$, $w_d = k_d L/v = 10^3$, $w_{as} = k_{as}[\, c_1^o \,]^{n-1} L/v = 10^3$, $D_F = D_2^{(m)}/(vL) = 5.0\times10^{-5}$, and varying values of $D_S = D_2^{(m)}/vL = 5.0\times10^{-5}$, 2.0×10^{-4} and 8.0×10^{-4}.

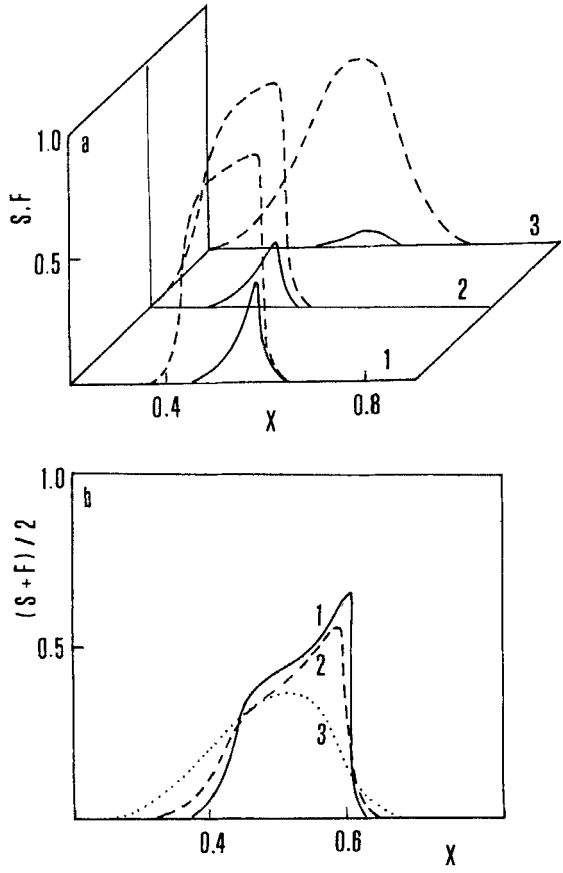

Figure 1. Effect of the axial dispersion on the theoretical chromatograms of fast associating components (association number n = 16, relative rates of association/dissociation processes, $w_{as} = w_d = 10^3$) (a) Reduced concentrations of unimer S, (dashed line) and associate, F, (full line), and (b) the reduced total solute concentration, (S+F)/2 as a function of reduced position in the column, X, for the reduced time of elution $\tau = 0.5$, and the relative coefficient of the axial dispersion, $D_F = 5.0 \times 10^{-5}$ and different values of $D_S = 5.0 \times 10^{-5}$ (curve 1), 2.0×10^{-4} (curve 2) and 8.0×10^{-4} (curve 3). Calculation based on the model of an instantaneous equilibration of unimer between the mobile and stationary phases (IPE model).

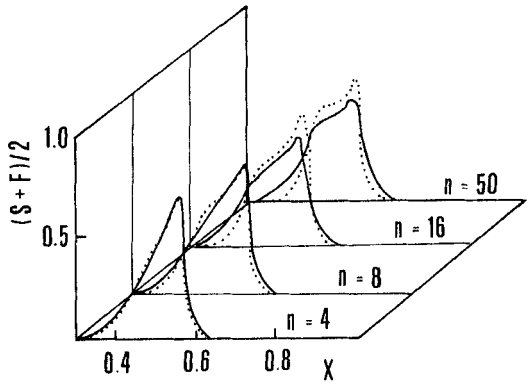

Figure 2. Effect of the axial dispersion on the theoretical chromatograms of fast associating systems for different association numbers, n, ($W_{as} = w_d = 10^{-3}$, $\tau = 0.5$). The meaning of the used symbols is the same as in Fig. 1. Calculations based on the IPE model for the following reduced dispersion coefficients $D_F = 5.0\times10^{-5}$ and $D_S = 5.0\times10^{-5}$ (full line) and 2.0×10^{-4} (dashed line).

It has been shown earlier (1-3) that the typical shape of chromatograms for fast reversibly associating species is that for, $D_S = 5.0\times10^{-5}$, i.e. the shape with two distinct inflection points in the part closer to the column entrance. The increasing effect of the axial dispersion suppresses that shape and complicates the interpretation of experimental chromatograms of such systems.

The main effect of the axial dispersion on the SEC data of fast associating systems with different association numbers, (n = 4-100; only few of them are shown here), may be summarised as follows. The higher is the association number, (a) the faster and more pronounced is the decrease in the associate concentration in the column as compared with a hypothetical system without any axial dispersion,

and (b) the less pronounced is the deviation of chromatogram shape from that typical for fast associating systems without dispersion despite the evident decrease in associate concentration (2).

Evaluation of the Equilibrium Concentrations of the Coexisting Components for fast Association Equilibrium from Experimental SEC Data

For associating systems, the equilibrium concentrations of unimer, $c_1^{(m)}$, and associate, $c_2^{(m)}$, depend on the association constant K_A, on the association number n, and on the total solute concentration by means of the mass action law

$$c_2^{(m)} / [c_1^{(m)}]^n = K_A \qquad (6)$$

Due to the simultaneous action of: (a) the separation SEC mechanism, (b) the re-establishment of the association equilibrium, and (c) the effect of the axial dispersion, the concentration profiles are changing and generally spreading during the passage through the SEC column. The overall solute concentration which leads to a fast decrease provokes the *fast relative decrease* in the associate concentration. In columns with high separation efficiency it may result in the disappearance of the associate from the eluate at the column outlet. The decrease of the mass ratio of associate to unimer, $\xi = \int c_2^{(m)}(x)\,dx / \int c_2^{(m)}(x)\,dx$, as a function of normalized time of elution $\tau = t/t_o$, (where t_o is the time necessary for the passage of the solvent through column i.e. $t_o = L/v$) for different n values, is shown in Fig. 3.

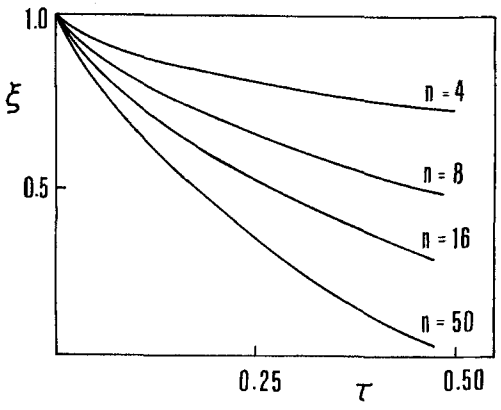

Figure 3. Decrease of the mass ratio of associate to unimer, ξ, in the SEC column as a function of the reduced elution time τ for fast association ($w_{as} = w_d = 10^{-3}$) and for different association numbers, n. Based on the IPE model, $D_S = 2.0 \times 10^{-4}$ and $D_F = 5.0 \times 10^{-5}$.

Fig. 4 gives several calculated concentration profiles (S + F)/2 in the column for different elution times, demonstrating the evolution of measurable chromatograms and gradual decrease of the contribution corresponding to the associate concentration. Results in Fig. 3 and Fig. 4 suggest the following experimental procedure leading to a reasonably accurate estimate of the equilibrium concentration.

By using a set of columns differing in length and extrapolating to L = 0, the equilibrium concentrations may be obtained.

For moderately fast association, the decrease in the associate concentration is less dramatical. Nevertheless, even in cases when two not fully separated peaks are obtained (Fig. 5) the decrease in ξ is still important (Fig. 6). In that case, the extrapolation to L = 0 should be carried out, too.

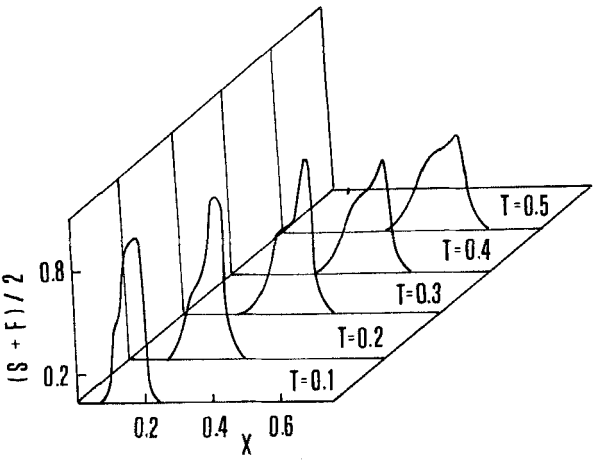

Figure 4. The time development of the theoretical SEC chromatogram for fast associating system ($n = 16$, $w_{as} = w_d = 10^{-3}$). Based on the IPE model, $D_S = 2.0 \times 10^{-4}$ and $D_F = 5.0 \times 10^{-5}$.

The main limiting factor of the accuracy in this case is a rather unexpected shape of the association concentration profile.

As the use of several columns differing in length may be experimentally difficult, different flow rates of eluent could be used and the extrapolation to $1/v = 0$ might be applied. In that case, all flow rates should be low enough not to disturb the SEC mechanism.

When studying fast associating systems by SEC, the re-equilibration of components starts to take place immediately after the peaks have left the column outlet. In case that the combination of a differential refractometer and a light scattering detector are used for detection, the mass average molar mass, M_w, measured by a light scattering detector is given by the mass action law and depends on the overall concen-

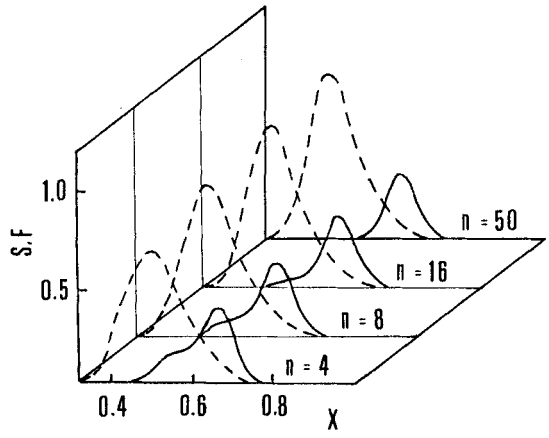

Figure 5. Theoretical concentration profiles, S and F of moderately fast associating systems ($w_{as} = w_d = 20$) for different association numbers n. Based on the IPE model, $D_S = 2.0 \times 10^{-4}$ and $D_F = 5.0 \times 10^{-5}$, $\tau = 0.5$.

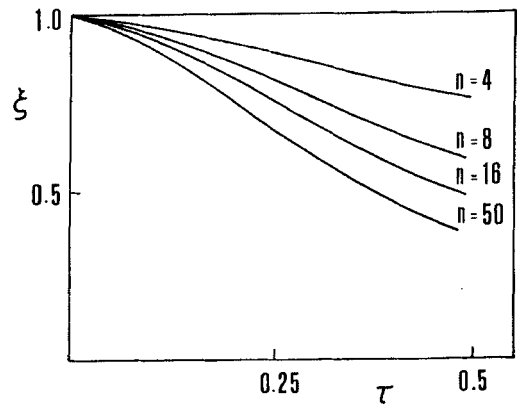

Figure 6. Decrease of the mass ratio of associate-to unimer, ξ, in the SEC column as a function of the reduced elution time τ for a moderately fast association ($w_{as} = w_d = 20$) and different association numbers n. Based on the IPE model, $D_S = 2.0 \times 10^{-4}$ and $D_F = 5.0 \times 10^{-5}$.

tration. Neglecting this fact may lead to serious errors. The light scattering detector should precede the differential refractrometer and the connecting tubes should be as short as possible.

Hindered Release of Solute from the Stationary Phase

It had been observed experimentally that in some micellizing block copolymer systems in selective precipitants (11), the release of the solute from the stationary phase is substantially hindered and its release rate is slow in comparison with the case of polymers (2,4,6-8). This effect was interpreted either as an adsorption (8,9), or as a trapping of associates (micelles) secondarily built in pores of the gel phase (2,7). On the basis of our older experimental studies (2), we have concluded that the second mechanism could be operative in many block copolymer systems in selective precipitants.

The present realistic model, which takes into account the axial dispersion, is capable of describing phenomenologically the hindered release of the solute from the stationary phase, too, regardless of its detailed molecular mechanism by using eq. (7), instead of eq. (6b).

Different finite rates of solute penetration into, and its release from, the gel pores may be monitored by changing the values of $w_{sp} = k_s L/v$ and $w_{ef} = k_{ef} L/v$. The concentration profiles for different w_{sp} and w_{ef} in Fig. 8 show the effect of hindered release of the solute from the gel phase. Only the practically important case of high rates of unimer penetration into gel pores is discussed here ($w_{sp} = 10^3$ and 10^4). For low values of w_{sp} the concentration $c_1^{(s)}$ does not reach the value of $c_1^{(m)}$, and the

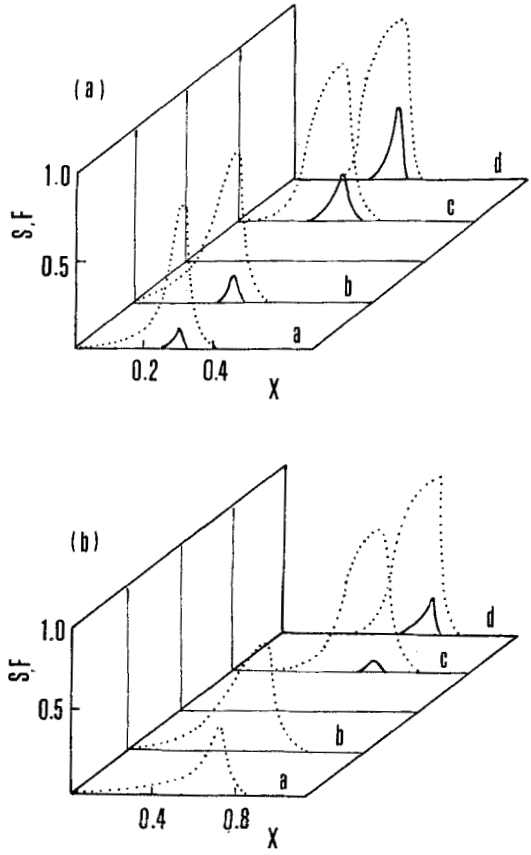

Figure 7. Theoretical concentration profiles, S and F, for fast associating system (n = 16, $w_{as} = w_{d} = 10^{3}$) in case of a hindered release of the solute from the stationary phase for two different reduced elution times τ. Based on the HSR model, $D_S = 2.0 \times 10^{-4}$ and $D_F = 5.0 \times 10^{-5}$, (a) τ = 0.3, (b) τ = 0.5, ((a) $w_{sp} = 10^{3}$, $w_{ef} = 10$; (b) $w_{sp} = 10^{3}$, $w_{ef} = 10^{2}$; (c) $w_{sp} = 10^{3}$, $w_{ef} = 10^{3}$; (d) $w_{sp} = 10^{4}$, $w_{ef} = 10^{4}$).

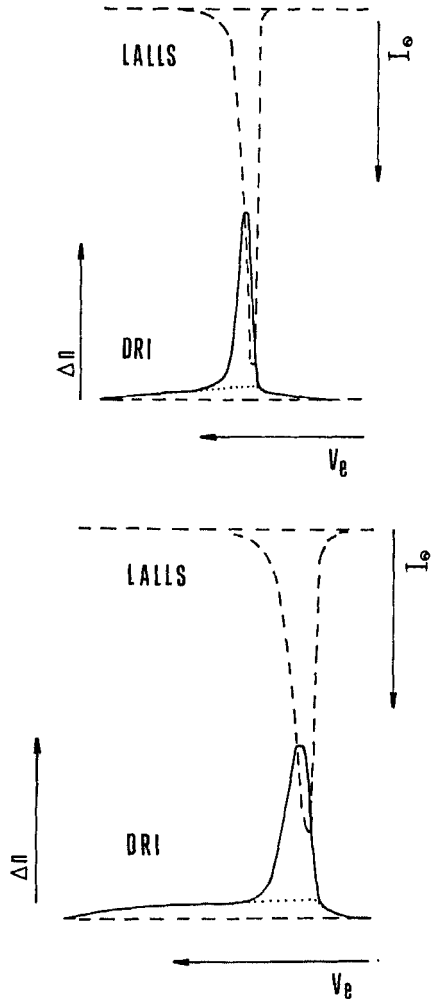

Figure 8. Chromatograms of Kraton G-1650 at 25°C. Mobile phase: THF/24 vol.% ethanol; $c = 0.9 \times 10^{-3}$ g cm^{-3} Flow rate: (a) 1 cm^3 min^{-1}; (b) 0.5 cm^3 min^{-1} dot line indicates theoretical separation of unimer and micelle concentrations.

separation efficiency is slow and unimportant from experimental point of view.

Concentration profiles for parameters modelling a detainment of the solute in the stationary phase (values w_{ef} significantly lower than w_{sp}) demonstrate the pronounced non-symmetrical broadening of unimer peaks. It is evident that the concentration of associates drops to very low values in early stages of the elution ($\tau = 0.3$, Fig. 7a), whereas in the latter stages ($\tau = 0.5$, Fig. 7b), the concentration F reaches zero for $w_{ef} < w_{sp}$. Values $w_{sp} = w_{ef} = 10^3$ do not simulate adequately the case of an instantaneous phase equilibration, whereas values $w_{sp} = w_{ef} = 10^4$ simulate it quite well (cf. Fig. 1 and Fig. 7). From the practical point of view, the presented chromatograms predict correctly the effect of the unimer trapping in gel pores and the numerical calculations are considerably less timeconsuming than those for higher values of w_{sp} and w_{ef}.

The most striking effect of the presented chromatograms is the long tail towards the smaller values of reduced positions in the column, X, and an absolute solute mass deficiency in the mobile phase. In experimental SEC studies with micellizing block copolymers (when elution volume is measured instead of the position in the column), the tail may exceed the low molar mass limit given by the peak of the solvent and an absolute mass deficiency of polymer in the eluent may be observed (2,5). In some cases a zone of a good solvent injected into the selective mobile phase is necessary to wash out the detained polymer. The "fast" peak (i.e. the only peak in this case) starts somewhere in between the elution volume of an "independent" associate (i.e. such, that is not in equilibrium with the unimer) and that of the

unimer. This peak contains usually only unimer molecules. It must be kept in mind that if light scattering detector is used, the presence of an associate may be monitor after leaving the SEC column.

Comparison with Experiment

Experimental SEC data on micellizing block copolymers supporting our numerical calculations will be a subject of another paper (12), nevertheless in this section two experimental results are given to substantionate qualitatively the theoretical conclusions.

The SEC experiments were performed using a Waters ALC/GPC 150 C apparatus (USA) with on-line RI detector and LALLS detector CMX 100 (Chromatix, USA) and four μ-Bondagel E-linear columns. All measurements were carried out at 25°C using injection volume 250 μl and flow rates upto 1 $cm^3 min^{-1}$.

Experiments have been performed with purified three-block copolymer Kraton G 1650 (Shell, USA) polystyrene-block-poly(hydrogenated butadiene)-block-polystyrene, $M_w = 7.4 \times 10^4$ $g.mol^{-1}$, weight fraction of polystyrene units, $w_{sp} = 0.28$, in selective solvents for polystyrene. In the systems studied, the unimer $\rightleftharpoons$ micelle equilibrium was shifted towards micelles with aliphatic cores and protective polystyrene shells.

Fig. 8 shows the decrease in the mass fraction of micelles with increasing elution time (decreasing eluent flow rate). In this figure, two chromatograms for polymer concentration $c = 9.0 \times 10^{-3} g.cm^{-3}$ in selective solvent THF/24 vol.% of ethanol and two different flow rates (0.5 and 1.0 $cm^3.min^{-1}$) are given.

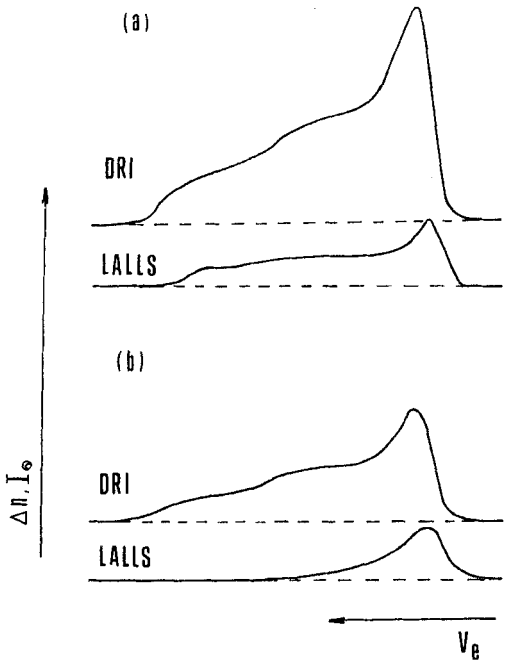

Figure 9. Chromatograms of Kraton G-1650 at 25°C. Mobile phase: 1,4 dioxane/30 vol.% n-heptane; Flow rate 1 $cm^3 min^{-1}$; Injected solution concentration: (a) 2,5x10 $^{-3}$ g cm^{-3}, (b) 1,1x10 $^{-3}$ g cm^{-3}.

Both chromatograms were recorded with the same line recorder rate and it is why half-width of micellar peaks differs appreciably. It is evident that the micelle to unimer mass ratio is considerably higher for flow rate 1.0 $cm^3 min^{-1}$ ($\xi = 6.5$) than for 0.5 $cm^3.min^{-1}$ ($\xi = 2.5$); the equilibrium value $\xi_{eq} = 11.5$ (2). The response of LALLS detector shows no evidence of micelles in the "tail" of chromatogram.

In Fig. 9, the experimental evidence of the unimer $\rightleftharpoons$ micelles re-equilibration outside the SEC column

(before the polymer zone reaches the LALLS detector) is given. Experiments were performed in 1,4-dioxane/30 vol.% of heptane with the flow rate 1.0 $cm^3.min^{-1}$.

In Fig. 9a), the signals from RI detector and from LALLS detector are shown for the input concentration $c = 1.0x10^{-3}$. As can be seen clearly, a presence of high molar mass species (micelles) is detected by LALLS in the "chromatogram tail" corresponding theoretically to the unimer zone. Scale units of refractive index differences, Δn, and scattered intensity, I_o, are chosen arbitrarily. In Fig. 9b), the analogical results for the input concentration $c = 0.5x10^{-3} g.cm^{-3}$ have been recorded with the same scale units as in case (a). Due to lower overall copolymer concentration, the unimer $\rightleftharpoons$ micelle equilibrium (after re-equilibration in the tubing) remains shifted in favour of unimer in the chromatogram part corresponding theoretically to the unimer zone. The LALLS signals drops continually and quickly to zero in this part of the experimental chromatogram.

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