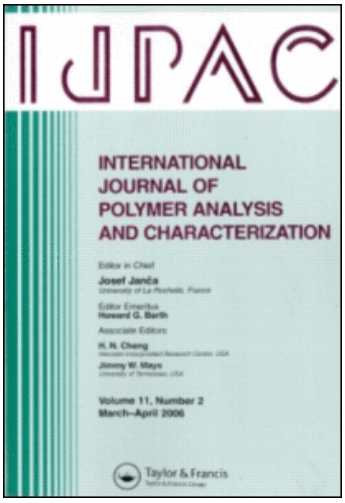

International Journal of Polymer Analysis and Characterization
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713646643

Release of Hydrophobic Substances from Polystyrene-Methacrylic Acid Block Copolymer Micelles into Aqueous Media

Emin Arca[ab]; Minmin Tian[a]; Stephen E. Webber[a]; Petr Munk
[a] Department of Chemistry and Biochemistry and Center for Polymer Research, The University of Texas at Austin, Austin, Texas [b] Chemical Engineering Department, Hacettepe University, Beytepe, Ankara, Turkey

To cite this Article Arca, Emin , Tian, Minmin , Webber, Stephen E. and Munk, Petr(1995) 'Release of Hydrophobic Substances from Polystyrene-Methacrylic Acid Block Copolymer Micelles into Aqueous Media', International Journal of Polymer Analysis and Characterization, 2: 1, 31 — 41
To link to this Article: DOI: 10.1080/10236669508233893
URL: http://dx.doi.org/10.1080/10236669508233893

Int. J. Polymer Analysis & Characterization
1995, Vol. 2, pp. 31–41
Reprints available directly from the publisher
Photocopying permitted by license only

Release of Hydrophobic Substances from Polystyrene-Methacrylic Acid Block Copolymer Micelles into Aqueous Media

EMIN ARCA*, MINMIN TIAN, STEPHEN E. WEBBER and PETR MUNK**

Department of Chemistry and Biochemistry and Center for Polymer Research, The University of Texas at Austin, Austin, Texas 78712

(Received September 26, 1994; in final form November 29, 1994)

Block copolymer micelles are potential carriers for controlled-release drug delivery. In this study, the fundamental aspects of the release of a model compound from the micelles into the surrounding media were explored. A fluorescence-based technique was developed for following the release of the model compound from the cores of block copolymer micelles into aqueous media. The experimental release curves were compatible with theoretical dependences for diffusion controlled release from spherical particles. The diffusion coefficients found were of the order of 10^{18} cm^2/s, which was expected for molecules with sizes of our probes and glassy polystyrene. The partition coefficients are also of the expected order of 10^5 magnitude. The fraction of the probe present outside of the cores at the start of the experiment may serve to characterize the efficiency of various procedures for the loading of various agents into the core.

KEY WORDS Block copolymer micelles, phenanthrene, controlled release, fluorescence.

INTRODUCTION

Copolymers with hydrophobic and hydrophilic blocks may form spherical micelles in aqueous media. These aqueous micellar systems are potentially useful for applications in the fields of pharmacology, ecology, painting and printing industry, and agriculture. They may take up organic materials from water solutions or release them, or may be utilized as delivery vehicles for hydrophobic drugs, pesticides, as scavengers of hydrophobic pollutants, etc. [1–10].

Recently, we have prepared a novel class of micelles that are based on block copolymers of styrene and methacrylic acid [11–14]. In these copolymers the styrene block is hydrophobic whereas the methacrylic acid block is hydrophilic. They are mutually incompatible and interact with solvents differently. Consequently, in aqueous solutions, the copolymers form micelles in which the styrene blocks aggregate into a core, and the methacrylic acid blocks form the shell. We have used fluorescence measurements for studying local mobility of polymer segments within the micelle cores [15–17] and for

*Permanent address: Hacettepe University, Chemical Engineering Department, Beytepe, Ankara 06532, Turkey.
**Correspondence: Professor Petr Munk, Dept. of Chemistry and Biochemist, University of Texas, Austin, Texas 78712

obtaining data on uptake of small molecular by the micelles [18]. In this study we have explored the fundamental aspects of the release of a model compound from the micelles into the surrounding media. The micelles are formed by dissolving the block copolymers of styrene and methacrylic acid in a mixture of a good solvent for both blocks (80 vol% of dioxane in our studies) and a precipitant for the polystyrene block (20% of water). In this mixture the micelles have a narrow distribution of sizes and the polystyrene core is substantially swollen by dioxane [12,14]. As a model compound we have selected phenanthrene because it has a good affinity for polystyrene and is strongly hydrophobic. Moreover, its fluorescent properties are very convenient for following the kinetics of its release from the core.

EXPERIMENTAL SECTION

Materials

Styrene-methacrylic acid diblock copolymers were synthesized via sequential anionic polymerization of styrene and *tert*-butyl methacrylate followed by hydrolysis of the ester groups. The detailed procedures of the preparation and characterization of the copolymers and micelles were described elsewhere [11–14]. Micellar solutions were formed by directly dissolving the copolymers in 80 vol. % dioxane and 20 % water (80D/20W). Two samples of block copolymers, SA-23 and SA-24, were used in this study. Table I lists some properties of the copolymers and micelles in 80D/20W.

Loading of the Micelles

Typically, 100 mg of the copolymer was dissolved in 10mL of the 80D/20W solvent mixture. Then 5 mg of phenanthrene was added to the micellar solution. The mixture was shaken overnight. Presumably, the phenanthrene distributed itself between the core and the outside solvent (including the shell). Evidence suggested that, in the 80D/20W solvent mixture, the polystyrene core was considerably swollen by dioxane and molecular mobility inside the core was substantial. Full equilibrium was likely achieved. The mixture was then quickly frozen in Dry-Ice (–78°C) and freeze-dried at -15.5°C under high vacuum for several hours. The dried copolymers were then redissolved in pH 7 buffers for the release experiments.

TABLE I

Properties of Block Copolymers and Micelles.

Sample	M_w[a]	W_{ps}%[b]	$M_w \times 106$c in 80D/20W	r_{core}(nm)
SA-23	66,300	60.0	9.0	12.5
SA-24	47,700	55.0	3.7	9.0

a. Molecular weight of block copolymer measured by GPC;
b. Weight fraction of polystyrene measured by NMR;
c. Molar mass of micelles in 80D/20W.

Aqueous Solutions of Micelles

In the freeze-dried copolymer, the micellar cores presumably retained their identity. As a result, the freeze-dried material was easily redispersed in any good solvent for the shell. We have redissolved it in the buffer. It should be noted that the original copolymers (ie, materials freeze-dried from their molecular solution in wet dioxane) are not soluble in aqueous buffers. However, using various experimental techniques (*cf*[12,14]), we have found that the particles present in the buffer after redissolving have molar masses ten to thirty times larger than the original micelles in the 80D/20W solution. We believe that these particles are micellar clusters that are held together by the intertwined shell-forming chains. Apparently, the glassy structure of the polystyrene cores does not allow a full separation of individual micelles. Although the nature of the forces holding the micelles together is not apparent, the existence of the multicore micellar clusters was confirmed by our preliminary studies using scanning electron microscopy.

The Release of Fluorophore

A measure of 30 μL of 0.5 mg/mL solution of the micelles loaded with phenanthrene in a phosphate buffer pH 7.0 (ca. 0.1M) was injected into a fluorescence cell containing 3 mL of the same buffer. Presumably this 100× dilution made the chemical potential of phenanthrene outside the micelles much smaller than its chemical potential inside the micellar cores and started the diffusive release of phenanthrene. The quantum yield of phenanthrene fluorescence in buffer solution is different from that in the polystyrene core. In order to obtain the ratio of these quantum yields, we performed the experiment in duplicate: one of the cells also contained 9 mg of thallium nitrate—a contact ionic quencher that cannot penetrate the hydrophobic polystyrene core. No quencher was added to the other cell. In a separate experiment we found that under these conditions the quencher reduced the fluorescence of phenanthrene dissolved in our buffer to 30.0%. Immediately after the injection of micelles, the fluorescence spectra of samples in both cells were measured repeatedly (as a function of elapsed time). The excitation wavelength was 293 nm. The total intensity emitted in the region of 315–450 nm was measured. In order to account for the instrumental drift and possible fluctuation of the lamp intensity, a third reference cell containing a dilute solution of phenanthrene in an organic solvent was measured periodically and data were corrected accordingly. Figure 1, shows the fluorescence spectra of phenanthrene at several times of release.

RESULTS AND DISCUSSIONS

When phenanthrene diffuses out of the polystyrene core, the quantum yield of its fluorescence changes due to (1) the change of environment and (2) the presence of the quencher (in one of the cells). With $x(t)$ being the fraction of fluorophore remaining in the polystyrene core at time t and I_{ps} the unknown intensity corresponding to all fluorophore being in the core, one may write for $I_u(t)$, the fluorescence intensity for sample without quencher as

$$I_u(t) = I_{ps}\,[\,x(t) + (1 - x(t))b\,] \tag{1}$$

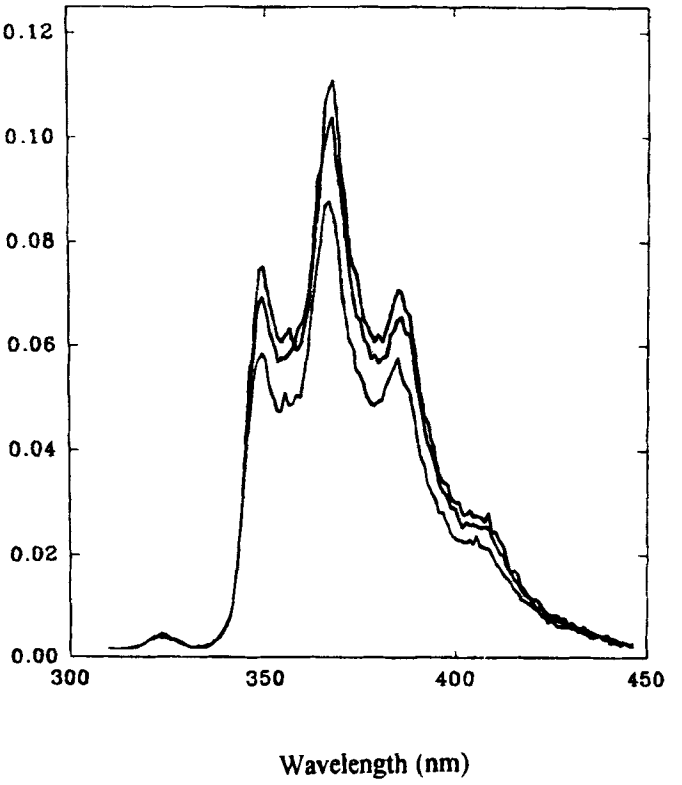

FIGURE 1 Fluorescence spectra of phenanthrene at several times of release. Excitation wavelength 293 nm. From the top to bottom: immediately after the injection (start of release); 7 min after injection; 14 min after injection.

where b is the ratio of fluorescence quantum yields of phenanthrene in the outside aqueous buffer and inside the polystyrene core. The fluorescent intensity of sample with quencher $TINO_3$ in the outside aqueous buffer $I_q(t)$ can be written as

$$I_q(t) = I_{ps}[x(t) + (1 - x(t))bq] \tag{2}$$

Here q is the unquenched fraction of the fluorescence, which was measured by an independent experiment and was found to be 0.300 in this study.

Eliminating $x(t)$ from equations 1 and 2, one obtains:

$$I_u(t) - I_q(t) = I_{ps}\, b(1 - q) - b[I_q(t) - q\, I_u(t)] \tag{3}$$

Figure 2 shows the plot of $[I_u(t) - I_q(t)]$ vs. $[I_q(t) - qI_u(t)]$ for the release of phenanthrene from micelles SA-23 and SA-24. From the slope we obtained b, the ratio of quantum yields of fluorophore in the buffer solution and inside the polystyrene core. From the intercept and knowing the value of b, we obtained the value of I_{ps}.

The fraction of fluorophore remaining inside polystyrene core at the beginning of the experiment and at any time t can thus be evaluated as

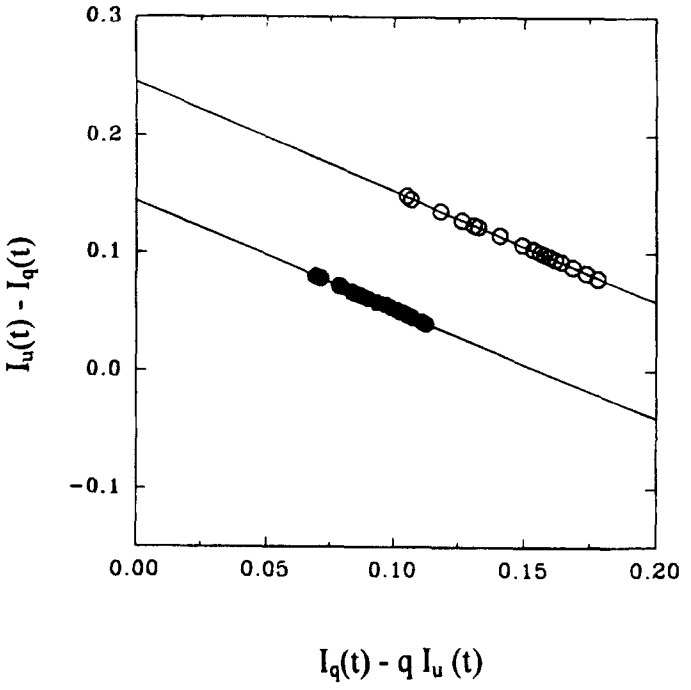

FIGURE 2 Plot of $[I_o(t) - I_q(t)]$ vs. $[I_q(t) - q\ I_u(t)]$ for micelles SA-23 and SA-24 in aqueous buffer (pH 7.0). - —SA-23; ·—SA-24.

$$x(0) = [\,I_q(0) - b\,q\,I_{ps}\,]/[\,I_{ps}(1 - b\,q)\,] \tag{4}$$

and

$$x(t) = [\,I_q(t) - b\,q\,I_{ps}\,]/[\,I_{ps}(1 - b\,q)\,] \tag{5}$$

Figure. 3 shows the fraction of phenanthrene released from polystyrene core of the block copolymer micelle SA-24 as a function of time.

THE KINETICS OF THE RELEASE

Block copolymer micelles are usually modeled as containing a spherical core surrounded by a concentric shell. We expect that the polystyrene cores in aqueous media are not swollen and that any diffusion within the cores will correspond to diffusion in glassy materials and will be very slow. However, diffusion of small molecules within the micellar shells will be only slightly slower than their diffusion in the outside solvent. In any case, it will be orders of magnitude faster than their diffusion in the core. The same will apply to the diffusion of the probe in the intertwined, but otherwise loose shells in the micellar clusters. We have therefore modeled the release from micelles as a release from a sphere that is uniformly loaded with the probe. We further assumed that the diffusion

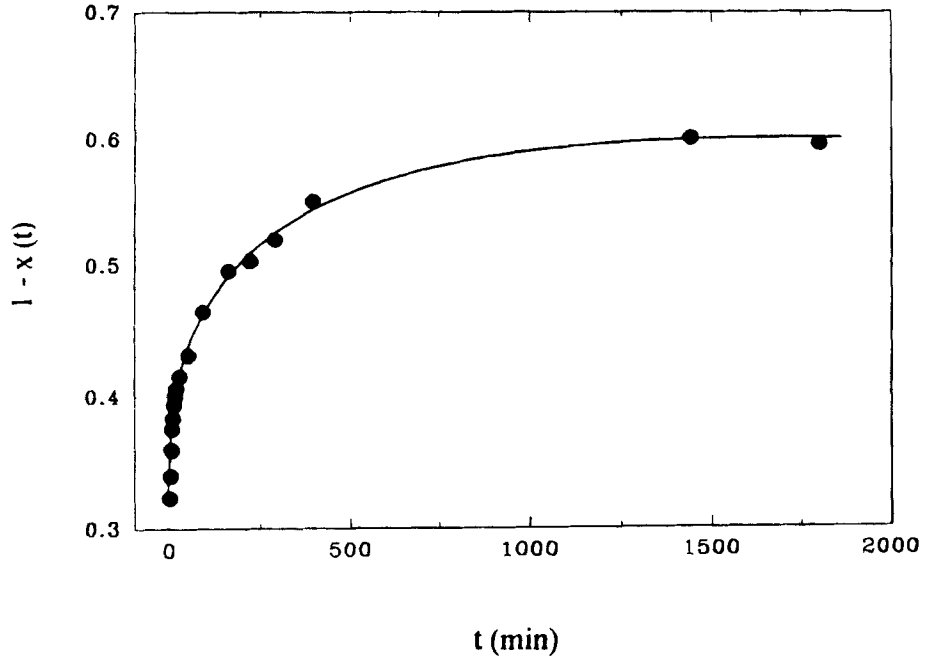

FIGURE 3 Fractional release of phenanthrene from SA-24 in pH 7.0 buffer, as a function of time.

coefficient of the probe within the sphere is constant and independent of its concentration. Once the probe is released from the sphere it is immediately dispersed uniformly throughout the whole volume of the sample. We have found that at the start of the experiment some fraction of the probe was already present outside the sphere. This fraction corresponds to the probe that is either associated with the shells or simply did not enter the micellar region during the loading procedure. After long time the system will reach an equilibrium governed by the distribution coefficient K_d of the probe between the cores and the outside solution. K_d is related to the partition coefficient K_p as

$$K_d = K_p(V_s/V_{\text{out}}) = (c_s^e/c_{\text{out}}^e)(V_s/V_{\text{out}}) \tag{6}$$

where V_s and V_{out} are the volumes of the micellar core and of the outside solution, respectively; c_s and c_{out} are the concentration of phenanthrene inside the micellar core and the outside liquid, respectively. The superscript e refers to the final equilibrium.

We have simulated the diffusion release process using a computer program and the finite difference form of the Fick's law in spherical coordinates [19]. To do so, we divided the spherical core of micelle (radius r_s) into N layers with the same thickness, $\Delta r = r_s N$. We found it convenient to define an effective concentration of the outside liquid, c_{eff} as

$$c_{\text{eff}} = c_{\text{out}} K_p \tag{7}$$

There is no transport across the boundary of the core when c_{eff} is equal to the concentration of the probe in the outermost N-th layer (e. g., at the final equilibrium).

The amount of transport across the layer boundary from the i-th to the $(i + 1)$-th layer m_i, during the time interval Δt is

$$m_i = D\Delta t[(c_i - c_{i+1})4\pi r_i^2]/\Delta r \tag{8}$$

where D is the diffusion coefficient of the probe inside the core; c_i is the concentration in the i-th layer and $r_i = i\,\Delta r$ is its outside boundary. The amount of transport across the outside surface of the N-th layer (the surface of the sphere) is:

$$m_N = D\Delta t[(c_N - c_{\text{eff}})4\pi r_s^2]/(\Delta r/2) \tag{9}$$

The factor of 2 accounts for the fact that the effective distance for the diffusion is only $\Delta r/2$ in this case.
The total amount Δm_i transported to the i-th layer is

$$\Delta m_i = m_{i-1} - m_i \quad \text{for } i > 1; \quad \Delta m_1 = -m_1 \tag{10}$$

The concentration change in the i-th layer is

$$\Delta c_i = \Delta m_i/(4\pi r_i^3/3 - 4\pi r_{i-1}^3/3) \tag{11}$$

The amount of transport into the outside liquid Δm_{out}, the change of the outside concentration Δc_{out} and of the effective concentration Δc_{eff} are given as

$$-\Delta m_N = \Delta m_{\text{out}} = V_{\text{out}}\,\Delta c_{\text{out}} = V_{\text{out}}\,c_{\text{eff}} / K_p = V_s\,\Delta c_{\text{eff}} / K_d \tag{12}$$

The initial conditions of the simulation: The concentration of the probe was the same in all layers; the fraction of the probe within the core was specified; the remaining probe was in the outside liquid. Several simulations were performed for the same initial conditions with different values of the distribution coefficient K_d. We have used $N = 50$; this choice provided sufficiently detailed concentration profiles within the sphere while allowing reasonably fast processing on a personal computer. It turned out that it is convenient to follow the progress of the diffusion in terms of the reduced time τ defined through its increment $\Delta\tau$ as

$$\Delta\tau \equiv D\Delta t/r_s^2 \tag{13}$$

Figure 4 shows the distributions of fluorescence probe at several reduced times for $K_d = 9$ and $x(0) = 1$. It is seen that at the beginning, probes release from the core very fast. This is a behavior of diffusion controlled release that is common to all shapes of the core and was discussed elsewhere [5–8]. After the initial quick release, a slow transport of the probe occurs from inner layers of the micellar core to the outer layers and then to the outside liquid. When $\tau \sim 0.5$, the concentration profiles of the probe inside the core and in the outside liquid become essentially constant throughout the micellar core. By varying values of K_d and $x(0)$, we simulated the diffusion under different initial and boundary conditions. For example, when $x(0) = 0.7$ and $K_d < 3/7$, diffusive uptake of probe from the outside liquid by the micellar core was observed. For the limiting case of $K_d = 0$ and $x(0) = 1$, our simulation result was virtually identical to the analytical time dependence obtained by Crank [19].

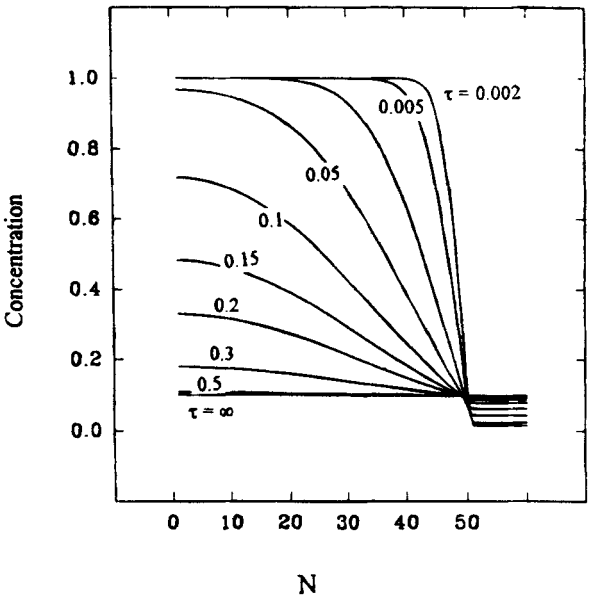

FIGURE 4 Distribution of the probe concentration inside the micellar core at several reduced times. $K_d = 9$, $x(0) = 1$.

In order to compare our computer simulation results to experimental results, we calculated the dependence of fractional release on reduced time for the experimentally found value of $x(0)$ and for a range of values of K_d. We found it convenient to plot the results as f_l ($\equiv 1 - x(t)$), the fraction of the probe residing outside of the cores, against log τ. In these coordinates, the dependences are S-shaped with the original asymptote corresponding to the starting value of the outside fraction and the asymptote at long times reflecting the equilibrium and its distribution coefficient. Dependences of f_l vs. log τ for several values of K_d are presented in Figures 5 and 6. Superimposed on these plots are our experimental curves for the micelles SA-23 and SA-24 plotted in f_l vs. log t coordinates. From the shift along the τ axis it is possible to evaluate the quantity D/r_s^2 for the best fit; the best fit also yields the distribution coefficient K_d. From the known concentration of the micelles and from W_{PS}, the weight fraction of polystyrene in the block copolymers, K_p is then evaluated.

When we freeze-dried copolymer micelles from the 80D/20W solvent mixture and redissolved them in the buffer solutions, we have found that the copolymers form clusters containing several individual micelles. We assumed that the individual micelles preserved the aggregation number they had in the 80D/20W solvent mixture before the freeze-drying procedure. Thus, we have evaluated the radius of the core from M, the molar mass of the original micelles in the 80D/20W solution that was obtained by light scattering, and from the known polystyrene content W_{PS} of the micelles as

$$r_s = [3MW_{PS}/4\pi\rho N_A]^{1/3} \tag{14}$$

where ρ is the density of polystyrene and N_A is the Avogadro number. Knowing r_s we calculated the diffusion coefficient D. The relevant data are collected in Table II. We found

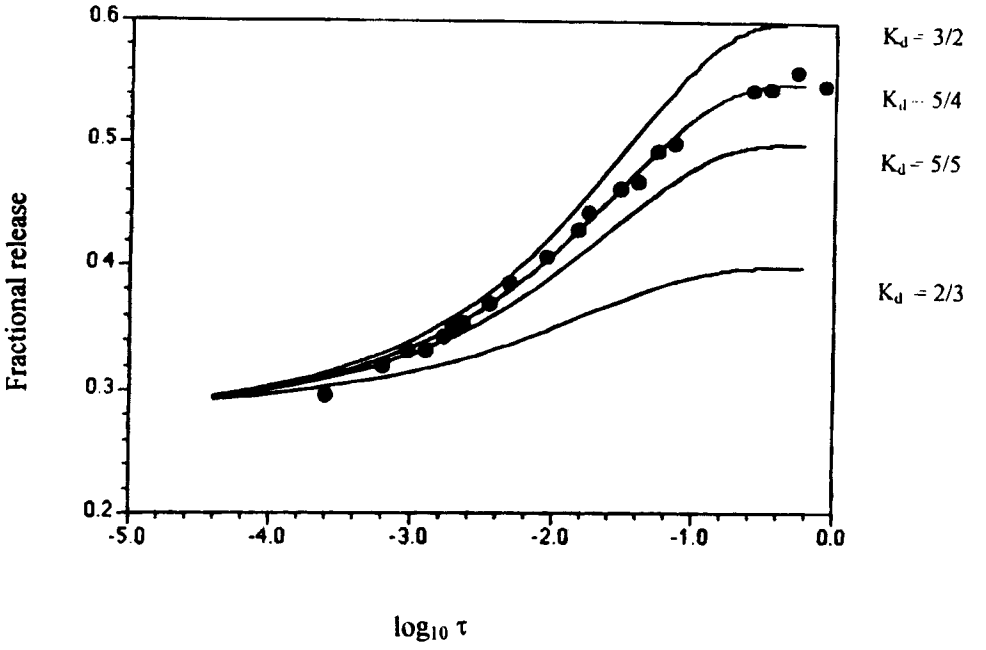

FIGURE 5 Fractional release of phenanthrene from SA-23 micelles in pH 7.0 buffer as a function of $log_{10} \tau$. The curves are the simulation results for f_1 (0) = 0.28 and several values of K_d.

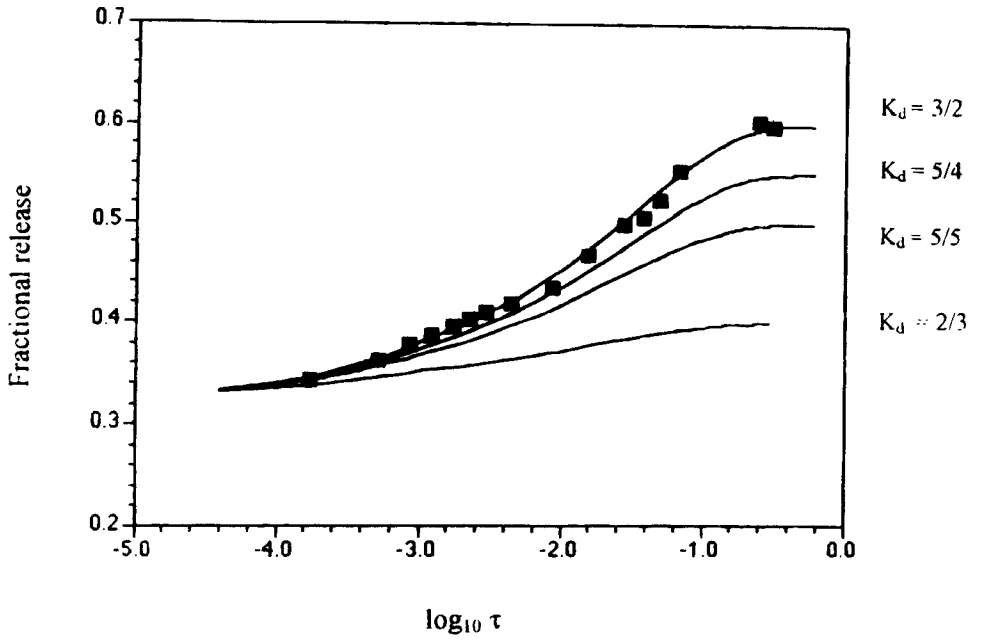

FIGURE 6 Fractional release of phenanthrene from SA-24 micelle in pH 7.0 buffer as a function of $log_{10} \tau$.

TABLE II

Diffusion and Partition Coefficients of Phenanthene in the Micellar Cores.

Micelle	b	$D \times 10^{18}$ (cm²/s)	K_d	K_p
SA-23	0.919	1.33	1.25	4.40×10^5
SA-24	0.934	0.55	1.50	3.45×10^5

that the partition coefficients K_p of phenanthrene are very high in both cases, indicating a strong interaction between phenanthrene and the polystyrene core. The diffusion coefficients are the order of 10^{-18} cm²/s, compared with fluphenazine in bulk poly(methyl methacrylate) 10^{-18} cm²/s and progesterone in silicone rubber 10^{-7} cm²/s [20]. This suggests that the polystyrene core is essentially glassy and is not swollen by aqueous buffers.

CONCLUSIONS

A fluorescence based technique was developed for following the release of hydrophobic substances from the cores of block copolymer micelles in aqueous media. The release curves were compatible with theoretical dependences for diffusion controlled release from spherical particles. The diffusion coefficients found are of the order expected for molecules with sizes of our probes and glassy polystyrene. The partition coefficients are also of the expected order of magnitude. The fraction of the probe present outside of the cores at the start of the experiment may serve to characterize the efficiency of various procedures for the loading of various agents into the core.

Acknowledgment

This research was supported by the U.S. Army Research Office Grant No. DAAL03-90-G-0147 and by the Collaborative Research Grant No. 920166 from the Scientific and Environmental Division of the North Atlantic Treaty Organization.

References

1. Z. Tuzar and P. Kratochvíl in *Surface and Colloid Science*, E. Matijevic Ed., Plenum Press: Vol. 15, New York, 1993.
2. R. Nagarajan, M. Barry, and E. Ruckenstein, *Langmuir* **2**, 210 (1986).
3. M. Chasin and R. Langer "*Biodegradable Polymers as Drug Delivery Systems*", Macel Dekker Inc., New York, 1990.
4. R. Nagarajan and K. Ganesh, *J. Chem. Phys.*, **90**, 5843 (1989).
5. Ahmad Yekta, J. Duhamel, and M. A. Winnik, *J. Chem. Phys.*, **97**, 1554 (1992).
6. P. J. Tarcha, "*Polymers for Controlled Drug Delivery*"; CRC Press, Boca Raton, FL 1991.
7. "*Polymeric Delivery Systems: Properties and Applications*"; M. A. El-Nokaly, D. M. Piatt, and B. A. Charpentier Ed., *ACS Symposium Series* **520**, American Chemical Society, Washington DC 1993.
8. E. W. Merrill, *J. Biomater. Sci. Polym.* Ed. **5**, 1 (1993).
9. Y. Li and T. Kissel, *J. Controlled Release*, **27** 247 (1993).
10. R. Gref, Y. Minamitake, M. T. Peracchia, V. Trubetskoy, V. Torchilin, and R. Langer, Science, **263**, 1600 (1994).
11. C. Ramireddy Z. Tuzar, K. Procházka, S. E. Webber, and P. Munk, *Macromolecules*, **25**, 2541 (1992).

12. P. Munk, A. Qin, M. Tian, C. Ramireddy, S. E. Webber, Z. Tuzar, and K. Prochazka, *J. Appl. Polym. Sci., Appl. Polym. Symp.*, **52**, 45 (1993).
13. M. Tian, A. Qin, C. Ramireddy, S. E. Webber, and P. Munk, *Langmuir*, **9**, 1741 (1993).
14. A. Qin, M. Tian, C. Ramireddy, S. E. Webber, and P. Munk, *Macromolecules*, **27**, 120 (1994).
15. K. Prochazka, D. Kiserow, C. Ramireddy, Z. Tuzar, P. Munk, and S. E. Webber, *Macromolecules*, **25**, 454 (1992).
16. D. Kiserow, K. Prochazka, C. Ramireddy, Z. Tuzar, P. Munk, and S. E. Webber, *Macromolecules*, **25**, 461 (1992).
17. D. Kiserow, J. Chan, C. Ramireddy, Z. Tuzar, P. Munk, and S. E. Webber, *Macromolecules*, **25**, 5338 (1992).
18. T. Cao, P. Munk, C. Ramireddy, Z. Tuzar, P. Munk, and S. E. Webber, *Macromolecules*, **24**, 6300 (1991).
19. J. Crank, *The Mathematics of Diffusion*, 2nd Ed., Clarendon Press, Oxford, 1975.
20. T. J. Roseman in *Controlled Release Technologies: Methods, Theory, and Applications*; A. F. Kydonieus Ed., CRC Press, Boca Raton, FL 1980 pp 21–54.