# Poly(allyl glycidyl ether)-*block*-Poly(ethylene oxide): A Novel Promising Polymeric Intermediate for the Preparation of Micellar Drug Delivery Systems

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**ABSTRACT:** Novel diblock copolymers designed for the preparation of micellar drug delivery systems, consisting of hydrophobic poly(allyl glycidyl ether) (PAGE) and hydrophilic poly(ethylene oxide) (PEO), were prepared, and their self-assembly into micellar structures was studied. Copolymers differing in the length of the polymer blocks were purified and characterized. These amphiphilic copolymers with narrow molecular weight distributions were prepared through the anionic polymerization of allyl glycidyl ether with PEO monomethyl ether sodium salt as the macroinitiator. The PAGE–PEO copolymer readily formed small micelles with narrow size distributions via simple dissolution in water. The addition of pendant double bonds to the hydrophobic part of the chain was intended for further covalent modifications. Catalytic hydrogenation, the radical crosslinking of the micelle core, and the addition of thiol to double bonds of the copolymer were examples of such modifications that were proved to proceed with a quantitative yield for this copolymer. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 95: 201–211, 2005

**Key words:** anionic polymerization; diblock copolymers; drug delivery systems; functionalization of polymers; micelles

## INTRODUCTION

Drug delivery vehicles are usually macromolecules or supramolecular assemblies such as liposomes or micelles. They form complexes with drugs that behave differently than the drugs themselves. These nanosize complexes are delivered to the target site in the organism, and there the drugs are released with the desired kinetics.[1] Because the use of delivery systems is usually more complicated than optimizing the structure of low-molecular-weight substances, the systems are used most frequently when conventional drugs offer only unsatisfactory results, especially in cancer therapy.[1] Nanoparticles,[2] micelles,[3] liposomes,[4] and soluble polymers[5] have been studied as powerful tools for the therapy of solid tumors because they can take advantage of the enhanced permeation and retention (EPR) effect to preconcentrate drugs into tumor tissue. The EPR effect is characteristic of most solid tumors and means that the tumor accumulates large molecules or supramolecular assemblies on the scale of tens of thousands to millions of kilodaltons.[2,5,6] A promising type of drug delivery system uses amphiphilic block copolymers,[7] which form micelles with a hydrophobic core and a hydrophilic shell in an aqueous environment. The systems currently under development most often use hydrophobic interactions to incorporate hydrophobic drugs into the core of the polymer micelle. Polymer micelles studied as drug delivery systems have been prepared by the self-assembly of various diblock and triblock copolymers, such as poly(ethylene oxide)-*block*-polylactide,[8] poly(ethylene oxide)-*block*-polyglycolide,[9] poly(ethylene oxide)-*block*-poly(lactide-*co*-glycolide),[8] poly(ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide) (Pluronic),[10,11] poly(ethylene oxide)-*block*-poly(ε-caprolactone),[12] and poly(ethylene oxide)-*block*-poly(β-benzyl aspartate).[13] Although these systems are relatively easy to prepare, they have some limitations. The most evident disadvantage is that the drug-release kinetics are difficult to control. Micelles based on hydrophobic interactions are in a dynamic equilibrium with a certain amount of the unimer (some molecules of the copolymer are not assembled in micelles), and this causes the instability of micellar systems in an environment in which structures containing hydrophobic domains are present (e.g., serum albumin). These systems with detergent properties may also damage cell membranes (this leads to hemolytic and cytotoxic activity). These problems are especially serious for low-molecular-weight surfactants, which possess a high critical association

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concentration (cac).[9,14] Control over drug release can be achieved by the covalent attachment of the drug to the micelle core by a (bio)degradable bond;[15] the effects associated with a low cac can be overcome by controlled crosslinking of the core.[16] Polymers used for the preparation of drug delivery systems should also have molecular weight distributions as narrow as possible because the biodistribution of such polymers is highly dependent on their molecular weight. Therefore, molecules of low molecular weight tend to be rapidly eliminated; on the other hand, molecules of high molecular weight tend to accumulate in the organism. This is why the development of novel block copolymers with the aforementioned properties is very important to the development of novel micellar drug delivery systems.

In this article, we describe the synthesis of a novel amphiphilic block copolymer, poly(allyl glycidyl ether)-*block*-poly(ethylene oxide) (PAGE–PEO), suitable for the preparation of micellar drug delivery systems through the anionic ring-opening polymerization of allyl glycidyl ether (AGE) on a poly(ethylene oxide) (PEO) monomethyl ether sodium salt macroinitiator. The advantages of using PEO as a hydrophilic block are that this biocompatible and nonimmunogenic polymer protects the micelles from interactions with cells of the reticuloendothelial system, reduces their uptake into liver, and significantly reduces the immunogenicity of the system.[13] It also allows the attachment of a targeting moiety at the end of the hydrophilic chain if the appropriate PEO macroinitiator is used.[17] Poly(allyl glycidyl ether) (PAGE or **III**) as a new hydrophobic block in block copolymers combines multiple properties that are advantageous for micellar drug delivery systems:

- The preparation of copolymers with very narrow polydispersity is easy because of the nature of anionic ring-opening polymerization.
- The purification of block copolymers from the homopolymer is relatively easy and very effective.
- The double bond on the polymer chain is reactive and can be readily used for numerous selective synthetic transformations to modify the physicochemical properties of the polymer chain, covalently crosslink the micelle core, and covalently attach the drug.
- AGE is an inexpensive and commercially available monomer.
- Micelles can be prepared by the simple dissolution of PAGE–PEO block copolymers in water without dialysis.

## EXPERIMENTAL

### Materials

AGE (Aldrich Chemical Co., Inc., Milwaukee, WI) was dried with anhydrous magnesium sulfate and distilled under reduced pressure (bp = 51°C at 2 kPa), benzene (Lachema A.S., Lachner S.R.O. Neratovice, Czech Republic) was dried with sodium metal and distilled, tetrahydrofuran (THF; Lachema) was kept over sodium metal and distilled before use, and cyclohexane (Lachema) was dried with sodium metal and distilled before use. Amberlite XAD-4 (Rohm and Haas Ion Exchange Resins, Philadelphia, PA) was used after the removal of soluble noncrosslinked polystyrene (by extraction in a Soxhlet apparatus with THF and then washing with water and drying in air). PEO monomethyl ether [**I**; weight-average molecular weight ($M_w$) = 2000 or 5000] was obtained from Fluka (Buchs, Switzerland) and was used without additional purification. Other chemicals were obtained from Lachema and were used without purification.

### Methods

#### Characterization of the polymers

$^1$H-NMR spectra were measured in THF-$d_8$ on a Bruker Avance MSL 200-MHz NMR spectrometer (Bruker Daltonik, Rheinstetten, Germany). IR spectra were recorded in KBr pellets on a PerkinElmer Paragon 1000 PC FT-IT spectrometer (PerkinElmer, Inc., Boston, MA). Gel permeation chromatography (GPC) was performed in THF as mobile phase on a PL gel mixed-B LS (10 $\mu$m) column (Polymer Laboratories, Ltd., Church Stretton, UK) with a Delta Chrom SDS 030 chromatograph (Watrex, Inc., San Francisco, CA) equipped with a PL-ELS 1000 evaporative detector (Polymer Laboratories). PEO was used for the molecular weight calibration. Mass spectra were obtained with a Biflex III matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer (Bruker Daltonik) with dithranol as a matrix and sodium trifluoroacetate as a cationizing agent.

#### Preparation of PAGE–PEO (**IIa–IIj**) by anionic ring-opening polymerization

The molecular weights of the PEO macroinitiators and the weights of the other reactants used for the preparation of the samples are given in Table I. **I** was dissolved in dry benzene (30 mL), and benzene was then removed *in vacuo* to azeotropically dry **I**. This procedure was repeated once more. Sodium hydride (after the removal of mineral oil through washing with dry cyclohexane) was then added, and the mixture was heated to 100°C under nitrogen with stirring until the hydride dissolved in the melt of **I** (ca. 30 min). AGE was then added, and the mixture was heated with stirring under nitrogen at 100°C for 6 h. Then, acetic acid (2 mmol/mmol of sodium hydride) was

**TABLE I**
**Preparation and Characteristics of PAGE–PEO (II) Copolymers**

| Polymer | IIa | IIb | IIc | IId | IIe | IIf | IIg | IIh | IIi | IIj |
|---|---|---|---|---|---|---|---|---|---|---|
| Starting PEO $M_w$ | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 | 5000 | 5000 | 5000 | 5000 |
| PEO (g) | 8.00 | 6.00 | 5.00 | 3.00 | 2.50 | 2.00 | 10.00 | 6.00 | 5.00 | 3.00 |
| AGE (g) | 2.00 | 3.00 | 5.00 | 6.00 | 7.50 | 8.00 | 2.00 | 3.00 | 5.00 | 6.00 |
| PEO (mmol) | 4.0 | 3.0 | 2.5 | 1.5 | 1.3 | 1.0 | 5.0 | 3.0 | 2.5 | 1.5 |
| NaH (mmol) | 4.0 | 3.0 | 2.5 | 1.5 | 1.3 | 1.0 | 5.0 | 3.0 | 2.5 | 1.5 |
| NaH (mg) | 96 | 72 | 60 | 36 | 30 | 24 | 120 | 72 | 60 | 36 |
| $M_w$ (PAGE, theoretical) | 500 | 1000 | 2000 | 4000 | 6000 | 8000 | 1000 | 2500 | 5000 | 10000 |
| $n$ (PAGE, theoretical) | 4.4 | 8.8 | 17.5 | 35.1 | 52.6 | 70.1 | 8.8 | 21.9 | 43.8 | 87.6 |
| $n$ (PAGE, $^1$H-NMR) | 3.9 | 6.4 | 9.0 | 22.6 | 24.2 | 25.3 | 7.0 | 14.3 | 19.9 | 19.3 |
| $w$ (PAGE, $^1$H-NMR) | 0.19 | 0.28 | 0.35 | 0.58 | 0.60 | 0.59 | 0.14 | 0.25 | 0.31 | 0.32 |
| $w_h$ ($^1$H-NMR) | 0.01 | 0.07 | 0.23 | 0.21 | 0.38 | 0.51 | 0.03 | 0.11 | 0.28 | 0.51 |
| $M_n$ (PAGE–PEO, GPC) | 2170 | 2730 | 2800 | 4110 | 4110 | 4120 | 5790 | 7030 | 6930 | 7300 |
| $M_w$ (PAGE–PEO, GPC) | 2260 | 2830 | 3010 | 4470 | 4450 | 4350 | 6130 | 7240 | 7390 | 7470 |
| $M_w/M_n$ (PAGE–PEO, GPC) | 1.04 | 1.04 | 1.07 | 1.09 | 1.08 | 1.06 | 1.06 | 1.03 | 1.07 | 1.02 |
| $M_w/M_n$ (PAGE–PEO, MALDI-TOF) | 1.02 | 1.02 | 1.02 | 1.01 | 1.02 | 1.15 | 1.01 | 1.01 | 1.02 | 1.01 |

$w$ = weight fraction of PAGE block in PAGE–PEO copolymer; $w_h$ = weight fraction of PAGE homopolymer in crude PAGE–PEO (gravimetrically, after the evaporation of isopropyl alcohol).

added, and the mixture was cooled to room temperature.

The course of the polymerization was followed with IR spectrometry (the epoxide band at 763 $cm^{-1}$) and $^1$H-NMR [the epoxide C**H**$_2$, chemical shift $\sigma$ = 2.45 (m, 1H) and $\sigma$ = 2.62 (t, 1H, $J$ = 4.8 Hz)]. The polymerization at 100°C was complete in all cases within 6 h.

The crude PAGE–PEO (900 mg) was dissolved in THF (40 mL) and added to silica gel (Kiesergel 60, Fluka; 10 g), and the solvent was then removed *in vacuo*. The polymer-coated silica was then transferred onto a column and washed with isopropyl alcohol (120 mL). The eluate, containing the PAGE homopolymer and other impurities, was discharged, and a mixture of **I** and **II** was then washed from the column with a mixture of isopropyl alcohol and chloroform (2:3 v/v, 200 mL). The solvent was removed from the eluate *in vacuo*, and the residue was then directly used in the next purification step.

The solid residue from the previous step was dissolved in THF (20 mL), and extracted Amberlite XAD-4 (5.5 g) was added. After 1 h, 200 mL of water was added, and the mixture was shaken at room temperature while it foamed (until the surface-active diblock copolymer was present in the solution after ca. 6 h). After that, the resin was filtered off, washed with water, and extracted with THF (100 mL) for 24 h with shaking at room temperature. The THF extract was then filtered and evaporated, and this yielded a residue of pure PAGE–PEO.

The polymerization degree ($n$; i.e., the average number of AGE units per chain) of the PAGE block was estimated via $^1$H-NMR as follows:

$$n = S_1/S_2 \quad (1)$$

where $S_1$ is the sum of the integral signals of the protons on the unsaturated bond of the AGE block [$\sigma$ = 5.07 (d, 1H, trans ═C**H**$_2$), $\sigma$ = 5.22 (d, 1H, cis ═C**H**$_2$), and $\sigma$ = 5.86 (m, 1H, —C**H**═)] and $S_2$ is the integral signal of the methyl end group of the **I** chain [$\sigma$ = 3.27 (s, 3H)].

### Preparation of PAGE by anionic ring-opening polymerization

The weights of the reactants used for the preparation of the particular polymer are given in Table II. Benzyl alcohol was mixed with AGE, and then sodium hydride (after the removal of mineral oil by washing with dry cyclohexane) was added. The reaction mixture was stirred at room temperature under nitrogen while hydrogen evolved (ca. 30 min) and was then heated at 100°C for 6 h. After that, acetic acid (2 mmol/mmol of sodium hydride) was added, and the mixture was cooled to room temperature. Polymer **III** was purified by GPC on a column packed with Sephadex LH-60 with THF as the mobile phase.

**TABLE II**
**Preparation and Characteristics of the PAGE (III) Homopolymers**

| Polymer | IIIa | IIIb |
|---|---|---|
| AGE (g) | 10.0 | 10.0 |
| $M_w$ (PAGE, theoretical) | 2000 | 6000 |
| NaH (mmol) | 5.0 | 1.7 |
| NaH (mg) | 120 | 40 |
| Benzyl alcohol (mmol) | 5.0 | 1.7 |
| Benzyl alcohol (mg) | 541 | 180 |
| $M_n$ (MALDI-TOF) | 1455 | 2532 |
| $M_w$ (MALDI-TOF) | 1705 | 3249 |
| $M_w/M_n$ (MALDI-TOF) | 1.17 | 1.28 |
| $M_n$ (GPC) | 1440 | 2508 |
| $M_w$ (GPC) | 1697 | 3282 |
| $M_w/M_n$ (GPC) | 1.18 | 1.31 |

The course of the polymerization was followed as described previously for the preparation of **II**. The polymerization was complete in all cases within 6 h.

### Hydrogenation of PAGE–PEO

The purified PAGE–PEO (**IId**; 500 mg, 2.52 mmol of C═C bonds) was dissolved in THF (5.0 mL), and then methanol (5.0 mL), a palladium catalyst (5% Pd on charcoal, 80 mg), and cyclohexa-1,4-diene (1.0 mL, 855 mg, 10.7 mmol) were added. The mixture was refluxed with stirring for 12 h (bath temperature = 80°C). The solid was filtered off and washed with THF, and the filtrate was evaporated; this yielded poly(glycidyl propyl ether)-*block*-poly(ethylene oxide) (**IV**).

The course of the hydrogenation was followed by $^1$H-NMR from a relative reduction in the signals of the protons on the unsaturated bonds of the AGE block [$\sigma$ = 5.07 (d, 1H, trans ═C**H**$_2$), $\sigma$ = 5.22 (d, 1H, cis ═C**H**$_2$), and $\sigma$ = 5.86 (m, 1H, —C**H**═)]. None of these signals were observed after 12 h of hydrogenation. The integral signals of the propyl groups [$\sigma$ = 0.90 (t, 3H, $J$ = 7.4 Hz, C**H**$_3$—CH$_2$—CH$_2$—O—), $\sigma$ = 1.54 (m, 2H, CH$_3$—C**H**$_2$—CH$_2$—O—), and $\sigma$ = 3.37 (t, 2H, $J$ = 6.5, CH$_3$—CH$_2$—C**H**$_2$—O—)] proportionally appeared.

### Crosslinked nanoparticles from PAGE–PEO

The purified PAGE–PEO (**IId**; 141 mg, 0.71 mmol of C═C bonds) and PAGE (**IIIb**; 39 mg, 0.34 mmol of C═C bonds) were dissolved in THF (1.5 mL), and 4-methoxystyrene (27 mg, 0.201 mmol) and 2-hydroxy-2-methyl-1-phenylpropan-1-one (15 mg, 0.091 mmol) were added. The solution was diluted with water (30 mL), allowed to stand for 10 min at room temperature, and then irradiated with a UV lamp for 25 min (2-mm layer, 40 mW cm$^{-2}$). After that, the mixture was dialyzed against water containing charcoal (3 wt %) for 24 h and then filtered through a 0.22-mm filter; this yielded **V** in water.

### Addition reaction of methyl sulfanyl acetate on PAGE–PEO

The purified PAGE–PEO (**IId**; 1.49 g, 7.52 mmol of C═C bonds) was dissolved in THF (4.0 mL), methyl sulfanyl acetate (4.63 g, 43.6 mmol, 4.0 mL) and 2,2′-azobisisobutyronitrile (213 mg, 1.3 mmol) were added, and the mixture was heated to refluxing within 1 h and was refluxed under nitrogen for another 3 h. After that, all volatiles were removed *in vacuo* (105°C bath, 13 Pa) to produce a highly viscous liquid (**VI**).

The reaction was followed by NMR. The signals of the double bonds [$\sigma$ = 5.07 (d, 1H, trans ═C**H**$_2$), $\sigma$ = 5.22 (d, 1H,, cis ═C**H**$_2$), and $\sigma$ = 5.86 (m, 1H, —C**H**═)] completely disappeared, and the signals of the product [$\sigma$ = 1.81 (m, 2H, —O—CH$_2$—C**H**$_2$—CH$_2$—S—), $\sigma$ = 2.69 (t, 2H, $J$ = 7.1 Hz, —O—CH$_2$—CH$_2$—C**H**$_2$—S—), $\sigma$ = 3.22 (s, 2H, —S—C**H**$_2$—COOCH$_3$), and $\sigma$ = 3.65 (s, 3H, —S—CH$_2$—COOC**H**$_3$)] appeared. Also, the 1732-cm$^{-1}$ band in the IR spectrum proved the presence of ester.

The S content (10.41%, 3.25 mmol/g) agreed well with the NMR-based content calculation (10.38%, 3.24 mmol/g).

### Preparation of the aqueous micellar solutions

The preparation of the aqueous micellar solutions of PAGE–PEO was simple because the PAGE homopolymer was viscous liquid, and so the copolymer dissolution was relatively fast. Micelles of a very narrow size distribution were formed by the simple dissolution of the block copolymer in water under shaking (within a few hours, depending on the hydrophobic chain length). Solutions of micelles (2.0 mg mL$^{-1}$) were measured and characterized by light scattering methods.

The cac values were determined from the fluorescence intensity of the $I_1$ band ($\lambda_{emission}$ = 367 nm; the first peak on the emission spectra with $\lambda_{excitation}$ = 339 nm) of pyrene fluoroprobes in solutions with different concentrations of the polymer and a constant concentration of pyrene according to a previously reported method[18] (see Fig. 1 for an example of such concentration/fluorescence-intensity dependence).

### Static light scattering (SLS)

SLS measurements were performed with a goniometer with the vertically polarized light of a He–Ne laser at a wavelength of $\lambda$ = 632.8 nm, an angular range of $\theta$ = 30–140°, and a temperature of 25°C. The apparatus was calibrated with toluene as a standard. The processed data are represented (unless otherwise noted) as follows:

$$Kc/R(\theta, c) = [M_w P(\theta)]^{-1} + 2A_2 c \qquad (2)$$

where $K$ is the optical constant, which includes the square of the refractive-index increment ($dn/dc$); $R(\theta)$ is the Rayleigh ratio, proportional to the intensity of light scattered from solutions; $A_2$ is the second virial coefficient; and $c$ is the (co)polymer concentration (g mL$^{-1}$). $P(\theta)$ is the particle scattering function. $M_w$ was evaluated from the zero angle and concentration limit of $Kc/R(\theta,c)$. For the micelles under study, the concentration dependence was neglected, and this seemed to be justified by the low concentrations of the micellar solutions ($2 \times 10^{-3}$ g mL$^{-1}$) and by the low value of $A_2$ for the micelles.[19] The extrapolation of $Kc/R(\theta,c)$ to the zero scattering angle was carried out by linear or quadratic fits of the scattering curves.

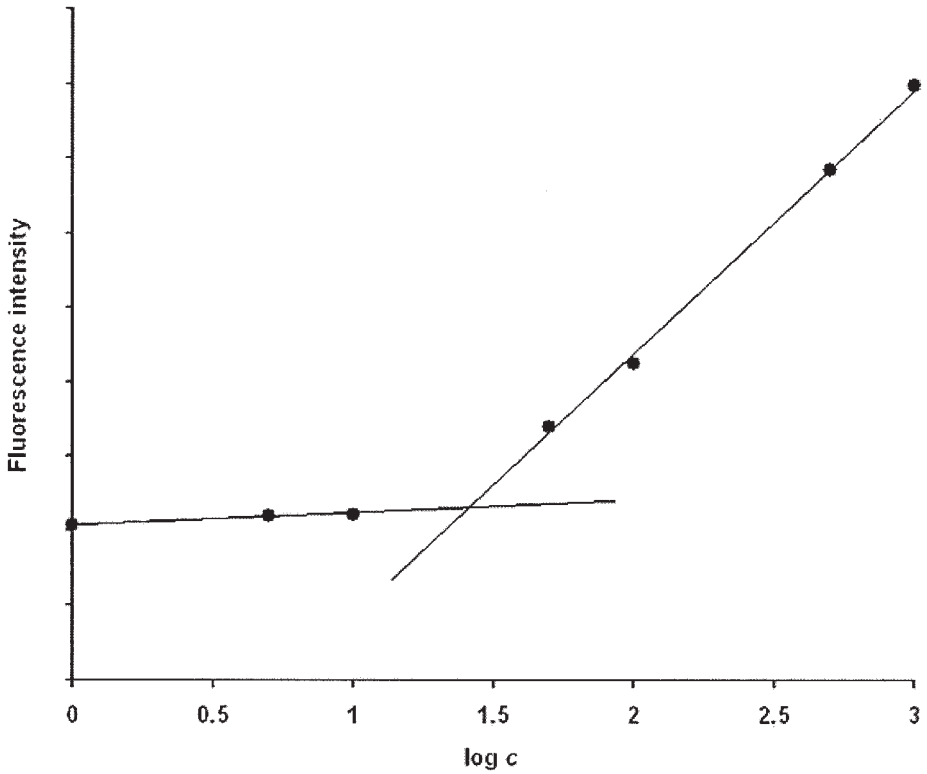



**Figure 1** Log $c$ (mg $L^{-1}$) versus the fluorescence intensity of the $I_1$ band ($\lambda_{emission}$ = 367 nm; the first peak on the emission spectra with $\lambda_{excitation}$ = 339 nm) of pyrene fluoroprobes in solutions with different concentrations of polymer **IIi** and a constant concentration of pyrene.

The refractive-index increment of the PAGE homopolymer was measured with a Brice–Phoenix differential refractometer at $\lambda$ = 630 nm in THF. $dn/dc$ of PAGE in water (0.147) was estimated with respect to the difference between the refractive indices of THF and water. $dn/dc$ of PEO in water (0.139) was taken from the literature.[20] The increments of the PAGE–PEO copolymers were calculated as the weight averages of the increments of corresponding copolymer blocks (see Tables III and IV).

## Dynamic light scattering (DLS)

Polarized DLS measurements were made in the angular range of 30–135° with a light scattering apparatus equipped with a He–Ne laser (632.8 nm) and an ALV 5000 multibit (ALV G.m.b.H., Langen, Germany), multi-$\tau$ autocorrelator covering approximately 10 decades in delay time $t$. Because the sizes of the micelles were low, the hydrodynamic radius ($R_h$) being about 10 nm, the angle dependence of the apparent diffusion coefficient could be neglected. Therefore, most of the DLS measurements were made at the scattering angle of $\theta$ = 90°.

The inverse Laplace transform with the REPES[21] method of constrained regularization (which is similar in many respects to the inversion routine CONTIN[22]) was used for the analysis of the autocorrelation functions. REPES directly minimizes the sum of the squared differences between the experimental and calculated intensity time correlation functions with non-

**TABLE III**
**Characteristics of Micelles of PAGE-PEO (II) Copolymers with Short PEO Blocks**

| PAGE–PEO | $M_w$ | $R_h$ (nm) | $dn/dc$ | cac |
|---|---|---|---|---|
| **IIb** | $3.4 \times 10^5$ | 59 | 0.137 | $1.1 \times 10^3$ |
| **IIc** | $1.3 \times 10^5$ | 6.4 | 0.138 | $5.2 \times 10^2$ |
| **IId** | $6.1 \times 10^5$ | 9.4 | 0.141 | 9.5 |
| **IIe** | $8.5 \times 10^5$ | 10.1 | 0.141 | 6.7 |
| **IIf** | $7.4 \times 10^5$ | 10.6 | 0.141 | 4.6 |

$M_w$ of the PEO block was 2000 mg/L cac in distilled water.

**TABLE IV**
**Characteristics of the Micelles of PAGE–PEO (II) Copolymers with Long PEO Blocks**

| PAGE–PEO | $M_w$ | $R_h$ (nm) | $dn/dc$ | cac |
|---|---|---|---|---|
| **IIg** | $8.4 \times 10^5$ | 62 | 0.135 | $9.0 \times 10^2$ |
| **IIh** | $1.5 \times 10^5$ | 11.0 | 0.136 | $1.0 \times 10^2$ |
| **IIi** | $4.7 \times 10^5$ | 12.3 | 0.137 | 21 |
| **IIj** | $9.4 \times 10^5$ | 13.3 | 0.138 | 24 |

$M_w$ of the PEO block was 5000 mg/L cac in distilled water.

linear programming. This method uses an equidistant logarithmic grid with fixed components (10 components per decade) and determines their amplitudes. As a result, a distribution function [$A(\tau)$] of the decay times is obtained. From the characteristic decay times [$\tau_i$; i.e., the peak positions of $A(\tau)$], the corresponding average diffusion coefficient [$D_i(90°)$] was calculated with a standard procedure. The apparent average $R_h$ value was calculated from $D_i(90°)$ with the Stokes–Einstein equation. The experimental error of the $R_h$ determination for the micelles was typically about 3%.

### Evaluation of $M_w$ of the micelles in the presence of aggregates

In the presence of aggregates in solutions, $M_w$ of the micelles was estimated with a combination of DLS and SLS experiments.[23] If two or more kinds of particles are present in solution, the total scattered intensities [$I_{st}(\theta)$] can be expressed as a sum of individual contributions. For a mixture of micelles and aggregates

$$I_{st}(\theta) = I_{sm}(\theta) + I_{sa}(\theta) \quad (3)$$

where $I_{sm}(\theta)$ and $I_{sa}(\theta)$ are the total scattering intensities generated by micelles and aggregates, respectively. In addition to the hydrodynamic size of the micelles and aggregates, the analysis of DLS data also provides us with the relative scattering amplitude [$fr_a(\theta) = I_{sa}(\theta)/I_{st}(\theta)$]. Therefore, eq. (3) can be rewritten as follows:

$$I_{sm}(\theta) = I_{st}(\theta) - I_{sa}(\theta) = I_{st}(\theta)[1 - fr_a(\theta)] \quad (4)$$

Thus, $I_{sm}(\theta)$ generated only by micelles can be extracted from $I_{st}(\theta)$. The procedure can be successfully used when $I_{sm}(\theta)$ is comparable to $I_{sa}(\theta)$ (a small number of aggregates) and the distribution of aggregate sizes is sufficiently different from the average micelle size. Because both assumptions are fulfilled in the systems under investigation (see the Results and Discussion section), $M_w$ of the micelles can be estimated from eq. (2) with $I_{sm}(\theta)$ values and the concentration of the micelles ($c_m$), instead of $c$. $c_m$ is generally unknown, but for these investigated copolymer solutions, the mass fraction of aggregates is small. If we assume that the same hard-sphere model is applicable to both types of particles ($M_w$ of the particles is proportional to $R_h^3$), then the mass fraction of aggregates for $I_{sm}(\theta) \sim I_{sa}(\theta)$ is about 0.01.

## RESULTS AND DISCUSSION

### Preparation and purification of the block copolymers

PAGE–PEO block copolymers with blocks of different chain lengths were prepared with two different **I** samples [number-average molecular weight ($M_n$) = 2000 or 5000] as macroinitiators and different monomer/**I** ratios (see Table I for the results).

The temperature was chosen according to a low-molecular-weight analogy (ring-opening addition of sodium alcoholate onto epoxide)[24] and the preparation of a random PAGE–PEO copolymer.[25] Acetic acid was used to quench the polymerization active center (alcoholate).

In our first experiments, the lengths of the PAGE blocks obtained with different methods of determination (GPC, MALDI-TOF, and $^1$H-NMR) did not match well. This was due to the presence of contaminants (PEO and PAGE homopolymers) in crude **II**, and so an efficient method of purifying **II** was developed.

### Separation of the PAGE homopolymer

The PAGE homopolymer had a retention factor ($R_f$) of 1.00 (it was s not adsorbed on silica at all because of its low polarity), and both the PEO homopolymer and PAGE–PEO block copolymer had $R_f$ values of 0.00 (they were not eluted at all) in isopropyl alcohol as a mobile phase in thin-layer chromatography (TLC) on polar silica gel. PEO and the PAGE–PEO copolymer were eluted from silica by a mixture of isopropyl alcohol and chloroform (2:3 v/v). This could be used for the separation of the PAGE homopolymer from the mixture. A mixture of PEO, PAGE–PEO, and PAGE was adsorbed onto silica and subsequently washed with isopropyl alcohol until PAGE was completely eluted (followed by TLC). Then, PEO and the PAGE–PEO diblock copolymer were eluted with a mixture of isopropyl alcohol and chloroform (2:3 v/v).

### Separation of the PEO macroinitiators

A reverse approach was used to remove possible traces of PEO in the diblock copolymer. A hydrophobic adsorbent had to be used to selectively adsorb the partly hydrophobic PAGE–PEO diblock copolymer but not fully hydrophilic PEO homopolymer. Therefore, the prepurified copolymer was dissolved in THF with a hydrophobic adsorbent [Amberlite XAD-4; i.e., macroporous poly(styrene-*co*-divinylbenzene) beads], diluted with water, and shaken while it foamed. The foaming was due to the detergent behavior of the diblock copolymer; when the mixture did not foam, the adsorption was complete. After adsorption, the beads were made hydrophilic and readily wettable with water. Then supernatant was filtered off, the residue was washed with water, and the block copolymer was extracted with THF and dried.

The removal of the PAGE homopolymer was essential for further use of the copolymer. It was completed after one purification procedure (no PAGE homopolymer was found with MALDI-TOF; see Fig. 2 for a

typical MALDI-TOF spectrum of purified PAGE–PEO). The removal of the PEO homopolymer was not absolutely necessary (there were only traces in the polymerization mixture because of the mechanism of polymerization). Although this was proved (no **I** was found with MALDI-TOF, and the $^1$H-NMR molecular weights matched those from GPC and MALDI-TOF), all samples were purified in the way previously described to obtain polymer as pure as possible. The purified PAGE–PEO had to be used for further modifications as soon as possible because the purified copolymer was relatively stable only in solutions and, especially when dried, rapidly (within days) formed an insoluble crosslinked matrix through a radical polymerization of double bonds. Lowering the storage temperature did not significantly reduce this crosslinking. Similar behavior was observed for polyesters containing allyl ether moieties.[26]

The molecular characteristics of the purified copolymers are given in Table I. The copolymers had very low polydispersity because of the living nature of the anionic polymerization ($M_w/M_n$ was, on average, 1.06 according to GPC and 1.03 according to MALDI-TOF), which was essential for their possible use in medicine. Moreover, the crosslinking via double bonds was negligible in the freshly prepared copolymers.

### Effect of the copolymer purity on the properties of aqueous solutions of the copolymers

Most polymeric micellar drug delivery vehicles cannot be prepared by a simple dissolution of an amphiphilic diblock copolymer combined with a drug in water because the hydrophobic copolymer core is solid and hardly soluble in water. In such cases, the block copolymer and drug are dissolved in a thermodynamically good solvent for both blocks of the copolymer and the drug (usually *N,N*-dimethylacetamide), and the solution is stepwise dialyzed against water.[27,28] For PAGE–PEO, the preparation of the micelles was easier because the hydrophobic PAGE block was a viscous liquid (as proved by the preparation of PAGE with sodium benzyl alcoholate as an initiator). Thus,

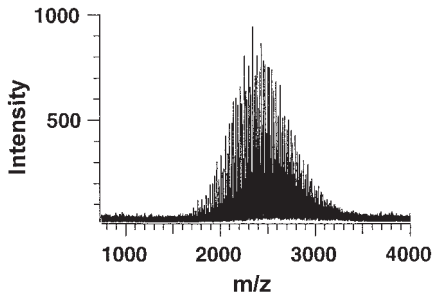



**Figure 2** Typical MALDI-TOF spectrum of a PAGE–PEO block copolymer (**IIb**).

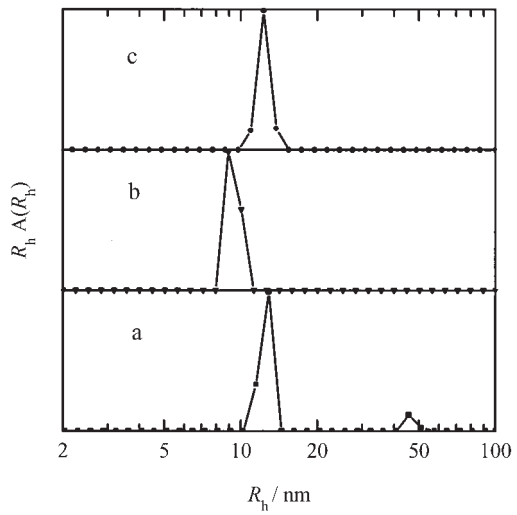



**Figure 3** $R_h$ distribution of particles observed (a,b) in aqueous solutions of crude **IId** and purified **IId**, respectively, and (c) in a solution of crude **IId** filtered through a filter with a porosity of 50 nm. The distribution was calculated from time correlation functions measured at $\theta = 90°$ and $c = 0.2$ wt %.

the aggregation–deaggregation kinetics were relatively fast, and simple dissolution in water could be used for the preparation of micellar solutions.

When we followed the size distribution of PAGE–PEO micelles before and after copolymer purification, we observed the effect of the presence of the PAGE homoblock in the copolymer on the self-assembly of the copolymers in aqueous solutions. The PAGE–PEO copolymers with a PAGE homopolymer admixture (crude copolymers) with sufficiently long hydrophobic PAGE blocks formed a mixture of copolymer micelles and aggregates in aqueous solutions. As a result, a bimodal distribution of $R_h$ was obtained. The purified diblock copolymers formed predominantly micelles. Such behavior is demonstrated for the crude **IId** copolymer in Figure 3(a) and for the purified copolymer in Figure 3(b), in which the distributions of $R_h$ are shown. The distribution obtained from a solution of the crude copolymer filtered through a filter with a porosity of 50 nm is shown in Figure 3(c) for comparison. Large particles were removed by this filtration. Although $R_h$ of the micelles observed in the filtered solution of the copolymer was practically identical to that of the micelles in solutions of the crude copolymer, $R_h$ of the micelles of the purified copolymer was slightly smaller. This difference in $R_h$ was probably due to homopolymer PAGE molecules entrapped in the micellar core of the copolymer micelles. The values of $R(\theta)/Kc$ from the crude copolymer solution were higher than those from the solution of the purified copolymer because of the presence of PAGE homopolymer molecules incorporated into the aggregates and micellar cores (see Fig. 4).

Large particles could be related to aggregates of insoluble homopolymer impurities (PAGE), which

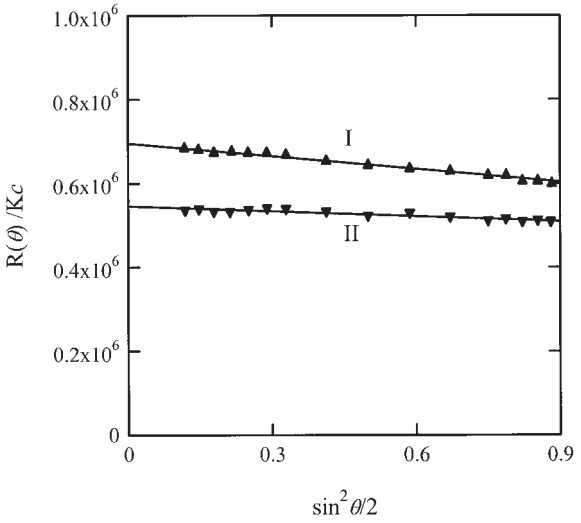



**Figure 4** $\theta$ dependence of $R(\theta)/Kc$ for (I) crude and (II) purified **IId** copolymer solutions ($c$ = 0.2 wt %).

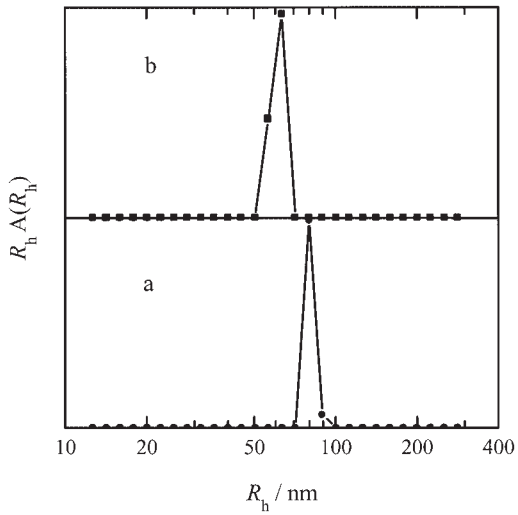



**Figure 5** $R_h$ distribution of particles observed in aqueous solutions of (a) crude and (b) purified **IIg**. The distribution was calculated from time correlation functions measured at $\theta$ = 90° and $c$ = 0.2 wt %.

came from the copolymer preparation, stabilized by diblock copolymers against flocculation (emulsion-like particles). The existence of such large particles in solutions of a homopolymer (A) and a diblock copolymer (AB) in a selective nonsolvent for the A homopolymer has been observed previously.[29,30] Thus, hydrophobic homopolymer impurities could form emulsion-like aggregates or be dissolved in the cores of micelles.

The crude copolymers with short hydrophobic PAGE blocks, which could not form copolymer micelles, could form emulsion-like aggregates of the PAGE homopolymer in aqueous solutions. Such a situation is demonstrated in Figure 5, in which $R_h$ distributions [Fig. 5(a,b)] and $R/Kc$ versus $\sin^2 \theta/2$ (Fig. 6) are shown for solutions of the **IIg** copolymer. Rather large monodisperse particles were found in the solutions of the crude and purified copolymers. Although $R_h$ of the aggregates decreased only slightly after purification, the apparent molecular weight $R(0)/Kc$ decreased by an order of magnitude. Thus, the relationship of the observed particles and the PAGE impurities was clearly demonstrated in the SLS experiment. The copolymers with short PAGE blocks were not sufficiently hydrophobic (polar) for micelle formation but were able to stabilize homopolymer aggregates against flocculation.

### Effect of the chemical composition on the parameters of the micelles

The effect of the PAGE chain length of the PAGE–PEO copolymers on the formation of micelles, for PEO with $M_w$ = 2000, showed the following trends (see Fig. 7 and Table III). First, copolymers with low-molecular-weight PAGE blocks ($M_w < 2000$) formed well-defined large aggregates (emulsion-like particles) with a narrow $R_h$ distribution. Second, the copolymers with longer PAGE blocks ($M_w \geq 2000$) formed micelles with $R_h \approx 10$ nm. A bimodal $R_h$ distribution (micelles and aggregates) was only found for the copolymer with the longest hydrophobic PAGE block. $R_h$ and $M_w$ of the micelles slightly increased with an increasing length of the PAGE blocks (see Table III). Similar behavior was found for block copolymers with longer PEO blocks ($M_w$ = 5000; see Fig. 8 and Table IV). The transition from emulsion-like aggregates to micelles was shifted to the PAGE copolymer of $M_w$ = 2500. Micelles were also larger with $R_h > 10$ nm. The particle characteristics of the micelles are given in Table IV.

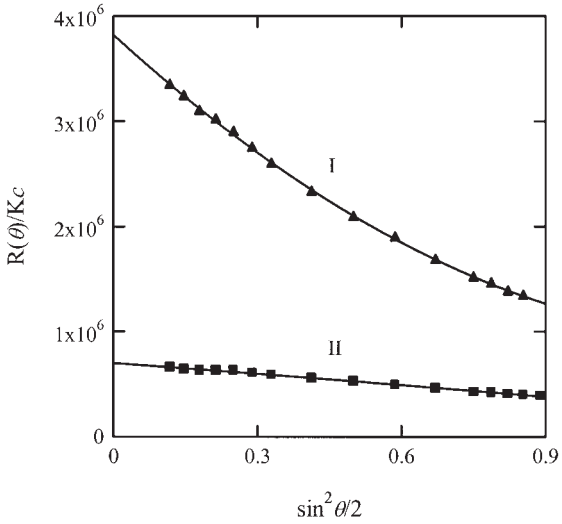



**Figure 6** $\theta$ dependence of $R(\theta)/Kc$ for (I) crude and (II) purified **IIg** ($c$ = 0.2 wt %).

Following the effect of the polymer block length on the micellar behavior of PAGE–PEO, we found that the general trends were similar for similar PAGE blocks and that these trends only slightly depended on the PEO block length. The main differences were that the micelles of the polymers of similar PAGE block lengths but higher PEO chain lengths were larger and that block copolymers prepared from PEO of $M_w$ = 5000 had a greater tendency to form aggregates at higher PAGE lengths. The most plausible explanation for the origin of the aggregates was that even after careful purification, some homopolymer PAGE chains remained, which aggregated to form emulsion-like particles. The weight fraction of the aggregates was anyways negligible.

The cac decreased as the length of the hydrophobic chain increased, whereas the hydrophilic chain had a constant length (see Table III for cac values of polymers prepared from PEO chains with $M_w$ = 2000 and Table IV for polymers prepared from PEO chains with $M_w$ = 5000). The cac also increased as the length of the hydrophilic chain increased, whereas the hydrophobic chains were similar in length [e.g., **IId** (Table III) vs **IIi** (Table IV)]. There was also observed a shift of the pyrene (0,0) band in the excitation spectra ($\lambda_{emission}$ = 390 nm) for $c >$ cac from $\lambda_{excitation}$ = 333 nm to $\lambda_{excitation}$ = 338 nm, proving the incorporation of pyrene into a less polar micelle core.

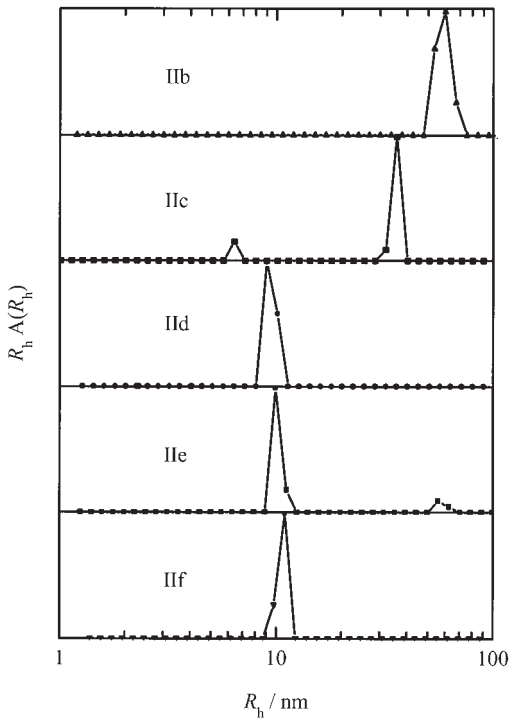



**Figure 7** $R_h$ distributions of particles observed in aqueous solutions of purified PAGE–PEO copolymers with short PEO blocks ($M_n$ = 2000) and with a variety PAGE blocks ($c$ = 0.2 wt % and $\theta$ = 90°).

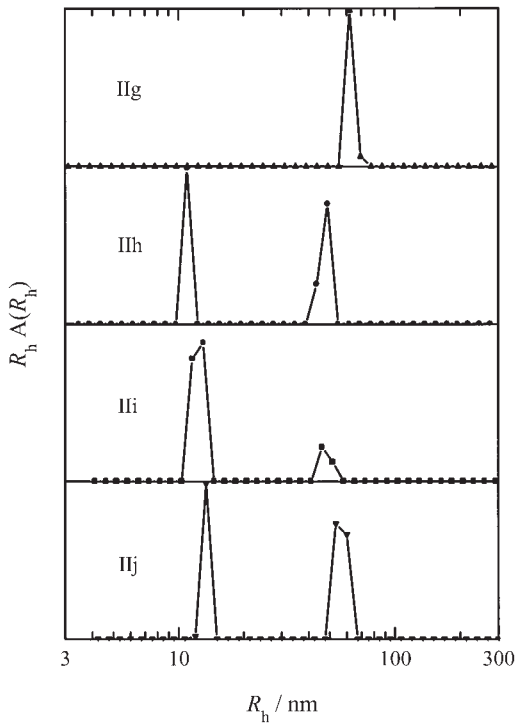


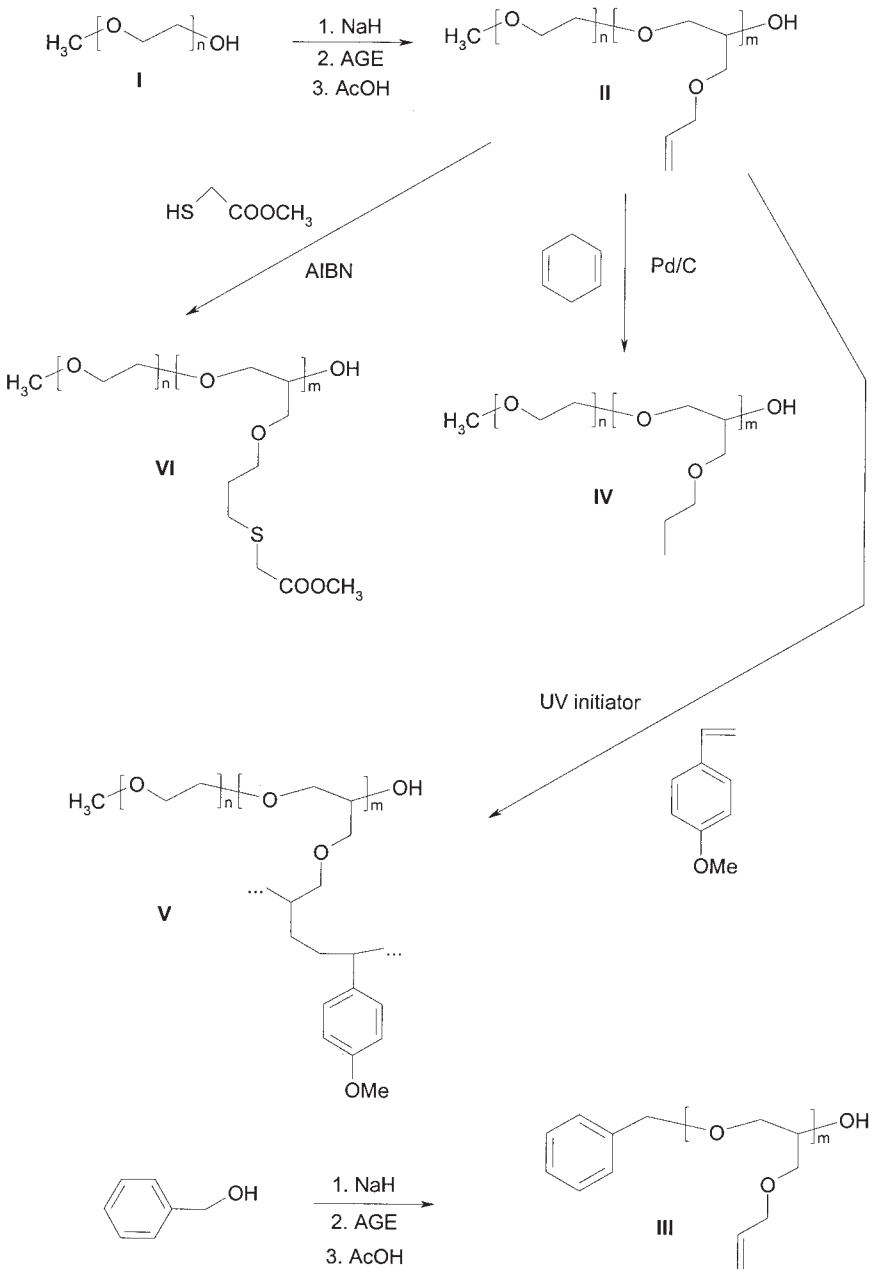

**Figure 8** $R_h$ distributions of particles observed in aqueous solutions of purified PAGE–PEO copolymers with long PEO blocks ($M_n$ = 5000) and with a variety PAGE blocks ($c$ = 0.2 wt % and $\theta$ = 90°).

### Examples of the covalent modifications of the PAGE block

The possibilities of covalent modification of the double bond of the PAGE block copolymers were demonstrated by three examples.

First, PAGE–PEO was hydrogenated into **IV** (see Scheme 1) by transfer hydrogenation with cyclohexa-1,4-diene as the hydrogen donor (see ref. 31 for low-molecular-weight analogies) to estimate the effect of double bonds on the biological properties of the copolymer (**IId** and **IV** differed only in the presence of double bonds; see Scheme 1). The hydrogenation was complete after 12 h (as determined by $^1$H-NMR). Crosslinking was negligible (GPC: **IId**, $M_w/M_n$ = 1.09, and **IV**, $M_w/M_n$ = 1.10; MALDI-TOF: **IId**: $M_w/M_n$ = 1.01, and **IV**, $M_w/M_n$ = 1.02).

Second, the micelle core containing bonds was crosslinked by the radical copolymerization of the copolymer double bonds with 4-methoxystyrene with 2-hydroxy-2-methyl-1-phenylpropan-1-one as a photoinitiator. The $R_h$ distribution of the resulting core-crosslinked nanoparticles (**V**; see Scheme 1) is shown in Figure 9 ($M_w$ = 1.1 × $10^5$ and $R_h$ = 16 nm). The particles were more polydisperse than the starting micelles. The $R_h$ distribution showed a tail toward higher values of $R_h$ resulting from intermicellar crosslinking of the copolymer micelles. Nevertheless,




**Scheme 1**

the particle size seemed to be suitable for possible medical applications.

The methoxybenzene ring was reactive in electrophilic reactions such as iodination, so such nanoparticles could be iodinated (e.g., with $^{125}$I) to investigate their biodistribution.[32,33] Solutions of the nanoparticles could not be dried or lyophilized, or irreversible denaturation took place. Crosslinking seemed to be very efficient because under the same conditions (except that THF was evaporated after the mixing of the components and water was not added), a completely insoluble matrix was obtained.

Third, methyl sulfanyl acetate was added to double bonds of **II** to add an ester moiety to the polymer chain (**VI**; see Scheme 1), which could be used after the deprotection of the ester moiety to bind a drug with a biologically cleavable bond. The UV-initiated radical photochemical addition, realized in analogy to low-molecular-weight compounds,[34] and the addition of cysteamine to double bonds of a random oxirane/AGE copolymer[25] (by mere dissolution in methanol) offered low yields only. The radical addition to double bonds of **II** initiated by the thermal decomposition of 2,2′-azobisisobutyronitrile in analogy to low-molecular-weight compounds[35] offered quantitative yields. The reaction was followed by $^1$H-NMR and IR (see the Experimental section for details). Crosslinking was almost negligible (GPC: **IId**, $M_w/M_n$ = 1.09, and **Vd**,

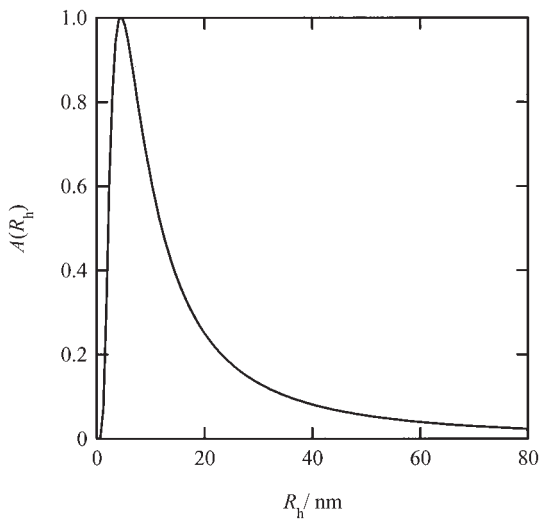



**Figure 9** $R_h$ distribution of core-crosslinked nanoparticles (**V**) found in aqueous solutions at $\theta = 90°$ and $c = 0.2$ wt %.

$M_w/M_n = 1.13$; MALDI-TOF: **IId**, $M_w/M_n = 1.01$, and **Vd**, $M_w/M_n = 1.03$), probably because the thiol group of methyl sulfanyl acetate served as an inhibitor of crosslinking. This was a difference from a low-molecular-weight analogy to a double bond of acrylates, which are easy to polymerize, and thiol groups serve as chain-transfer agents.[36]

## CONCLUSIONS

Novel block copolymers designed for use in drug delivery systems (PAGE–PEO) were prepared with different block sizes and characterized. These amphiphilic copolymers of narrow molecular weight distributions were easily prepared by the anionic polymerization of AGE with **I** sodium salt as a macroinitiator. The initiator was prepared from the corresponding alcohol and sodium hydride. A novel method of separation for free PAGE and PEO homopolymers was developed and used for the purification of the copolymers. The copolymers readily formed micelles of narrow polydispersity by simple dissolution in water. The pendant double bonds on the hydrophobic part of the chain could be used for further covalent modifications. Catalytic hydrogenation, the radical crosslinking of the micelle core, and the addition of thiol to double bonds were examples of such modifications that were proved to proceed with quantitative yields for these copolymers.

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