Facile method for labelling polystyrene with various fluorescent dyes

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A low concentration of chloromethyl groups (up to ≈0.1% monomer unit) has been incorporated into monodisperse polystyrene (PS) at the pendant phenyl ring by reaction with chloromethyl ethyl ether and boron trifluoride–etherate in methylene chloride. This method is both easier and safer to carry out than the conventional chloromethylation procedure. If the reaction is done at a suitable concentration, the molecular weight and polydispersity of the polymer are unchanged after the reaction. The chloromethylated PS reacts readily with the caesium salts of carboxylic acid derivatives of naphthalene, anthracene, pyrene, 7-nitrobenzo-2-oxa-1,3-diazole (NBD) and the potassium salt of carbazole to yield labelled PS. At high polymer concentration the reaction gives insoluble gel product, and in very dilute polymer solution and high concentration of the ether or the catalyst, intramolecularly crosslinked 'microgel' is obtained. Preliminary measurements on the mutual and tracer diffusion coefficients of the NBD-labelled PS particles stabilized by a block copolymer in 20% toluene/80% methanol mixture reveal that the two diffusion coefficients are identical under our experimental conditions.

(Keywords: polystyrene; fluorescent labels; chloromethylation; diffusion coefficient)

INTRODUCTION

In the past decade there has been increasing interest in the use of fluorescence spectroscopy to study various aspects of polymer chemistry and physics. Examples of studies include polymer blend compatibility[1], cyclization dynamics[2], polymer complexation[3], colloid morphology[4] and diffusion measurements[5]. In many cases the polymer samples are labelled covalently with a fluorescent dye[2–5], the most common being anthracene and pyrene. A polymer of particular interest is polystyrene (PS), which is possibly the most widely used material in polymer physics research. In this paper we will introduce an easy method to label it with different fluorescent dyes.

The simplest way to perform such labelling is first to introduce a reactive chloromethyl group to the phenyl ring. The classical method for doing so involves the use of chloromethyl methyl ether and stannic chloride[6]. However, chloromethyl methyl either is very dangerous to use because of its high carcinogenicity[7], thus preventing the widespread use of this approach. In earlier work, Sparrow very briefly described the chloromethylation of crosslinked PS beads with chloromethyl ethyl ether (CMEE) and boron trifluoride etherate ($BF_3{\cdot}Et_2O$)[8]. This method is much safer to employ because CMEE has not yet been established to be carcinogenic. Even if it were, the ethyl ether has a boiling point some 25°C higher than the methyl ether counterpart and therefore is easier to handle and dispose of safely. However, the published procedure used a high concentration of both the CMEE and $BF_3{\cdot}Et_2O$ and we found that it invariably led to crosslinking reactions. Crosslinking is highly undesirable when the labelled polymer is intended to be used for studying single chain behaviour. We report here the reaction conditions that can minimize interchain or intrachain crosslinking.

After the PS chain is chloromethylated, it can be labelled with different fluorescent dyes by reacting it with the caesium salts of the respective dye molecule in dimethylformamide via a simple displacement reaction. The caesium salt has been shown to be the most effective member of the alkaline metal family for complete reaction with the chloromethyl group[9]. The dyes selected for this study were the commercially available carboxylic acid derivatives of four widely used dyes: 1-naphthylactic acid, 9-anthracenecarboxylic acid, 1-pyrenebutyric acid and NBD-aminohexanoic acid. In addition, carbazole was reacted with chloromethylated PS via a similar, simple reaction[10].

All dyes except NBD were chosen because they can form excimers or donor–acceptor pairs, e.g. naphthalene/anthracene and carbazole/anthracene[11]. NBD was selected because it is a popular photobleachable dye and the NBD-labelled PS can be used in diffusion studies using the FRAPP (Fluorescence Redistribution After Pattern Photobleaching) technique[5,12]. This technique gives the tracer diffusion coefficient and has been used to study the molecular weight dependence of the diffusion coefficient of polymer melts[13].

Very recently the fluorescence recovery technique has been applied to measure the tracer diffusion coefficient of a surfactant micelle with solubilized photobleachable dye in aqueous solution[14]. Depending on the interaction between the micelles, the tracer diffusion coefficient may not be the same as the mutual diffusion coefficient, which can be obtained by dynamic light scattering. Measurements of both these diffusion coefficients for a Brownian particle could reveal information about

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Table 1 Conditions that lead to intact PS molecular weight and polydispersity

Reaction	PS (k)	C^* (g cm^{-3})	Amount (g)	CH_2Cl_2 (ml)	CMEE (ml)	$BF_3{\cdot}Et_2O$ (ml)	Tagging (%)
A	50	0.034	1	300	3	0.27	0.135
B	68	0.027	1	380	3	0.27	0.081
C	80	0.023	1	430	3	0.27	0.053
D	110	0.018	1	550	3	0.30	0.026
E	233	0.099	1	1000	3	0.50	0.016

interparticle long-range interaction[15]. We will report here a preliminary experiment involving the use of the NBD-labelled PS in comparing the tracer diffusion coefficient of a PS Brownian particle stabilized by a block copolymer with the mutual diffusion coefficient of the same particles.

Wang *et al.*[16] have labelled monodisperse PS with NBD by a different method, but we think that the approach described in the present study is easier. Moreover, the first step of their method, the metalation of PS by *n*-BuLi, could cause chain scissions, thus inadvertantly affecting the molecular weight of the product.

EXPERIMENTAL

Materials

Monodisperse PS was purchased from Polysciences Inc. and dried in a vacuum oven prior to use. Baker reagent grade methylene chloride was stored over sodium hydroxide pellets overnight to remove HCl and was freshly distilled over calcium hydride powder immediately before use. Dimethylformamide was purchased from EM Science Co. (spectral grade) and was used as received. CMEE, $BF_3{\cdot}Et_2O$, 1-naphthylacetic acid, 9-anthracenecarboxylic acid, 1-pyrenebutyric acid, carbazole, potassium hydroxide, and caesium hydroxide monohydrate were all purchased from Aldrich. $BF_3{\cdot}Et_2O$ was kept under N_2 in an Aldrich Sureseal bottle. NBD-aminohexanoic acid (NBDA) was purchased from Molecular Probes Inc.

Chloromethylation of polystyrene

To minimize the Friedel–Crafts crosslinking reaction, we used the overlap concentration, C^*, as a guideline for polymer concentration. The molecular weight dependence of C^* for monodisperse PS is given by ref. 17

$$C^* = (220 \pm 75) M^{-(0.81 \pm 0.03)} g\, ml^{-1}$$

The polymer concentration used was 1/10 of the calculated C^* value (see *Table 1*). The polymer was first dissolved in dry CH_2Cl_2 followed by the addition of CMEE. $BF_3{\cdot}Et_2O$ was removed from the Sureseal bottle under N_2 via a microsyringe and injected into the reaction mixture. If the solvent was not sufficiently dry, we observed that the solution immediately became cloudy after the addition of the catalyst. The solution was then refluxed for 3 h. A drying tube was attached to the opening of the reflux apparatus to maintain dry reaction conditions. At the end of the reaction the solution was cooled by a dry ice/isopropanol bath, and 5 ml of acidified methanol were added to the reaction mixture to destroy the catalyst $BF_3{\cdot}Et_2O$ and CMEE. The solution was then rotor-evaporated to about 100 ml. The chloromethylated polymer was initially isolated by precipitation in acidified methanol followed by reprecipitation in pure methanol.

Dye labelling reaction

For naphthalene, anthracene, pyrene and NBD, the acid form was first converted into the caesium salt by reacting with caesium hydroxide and flash evaporating to dryness. A large excess of the caesium salt of the dye was mixed with 20 ml of the chloromethylated PS in 20 ml of DMF. The solution was then heated to about 60°C for 4 h. At the end of the reaction, the dye-labelled PS was isolated by precipitation in MeOH. Since the product at this stage still contains some free dye, it was redissolved in THF and precipitated again in methanol. This process was repeated once more. We found that three precipitations in methanol were enough to remove all the free dye molecules detectable by u.v.-visible spectroscopy. However, if the use of this labelled polymer required the removal of all trace amounts of free dye, the labelled PS was further purified by extraction with methanol overnight in a Soxhlet extractor.

For carbazole labelling, we closely followed the procedure described by Gibson and Bailey[10]. Carbazole (0.1 g) was reacted with an equimolar quantity of potassium hydroxide aqueous solution in 15 ml xylene in a Dean–Stark trap overnight. Then xylene and water were removed and replaced by DMF. Chloromethylated PS (0.34 g) was added and stirred at 35°C for 2 h. The isolation and purification of the product were the same as stated above.

Calculation of percentage tagging

The polymer samples listed in *Table 1* were all labelled with NBD and the percentage tagging was obtained by measuring the absorbance of a known amount of the labelled PS in THF at 459 nm. The molar extinction coefficient of NBDA was measured to be $1.5 \times 10^4 M^{-1} cm^{-1}$. Calculation of percentage tagging based on pyrene was found to give identical results.

Characterization

All the samples were characterized by gel permeation chromatography (g.p.c.) using a Waters 150C system equipped with five Ultragel columns from ASI Co. The columns were calibrated with 17 PS standards purchased from Polymer Laboratories having molecular weights ranging from 480 to 8×10^6.

DIFFUSION MEASUREMENTS

Sample preparation

A 0.1% solution of NBD-labelled PS 50k (1 ml) in toluene was mixed with 1 ml of a 0.5% triblock poly(2-vinylpyridine)-polystyrene-poly(2-vinylpyridine)

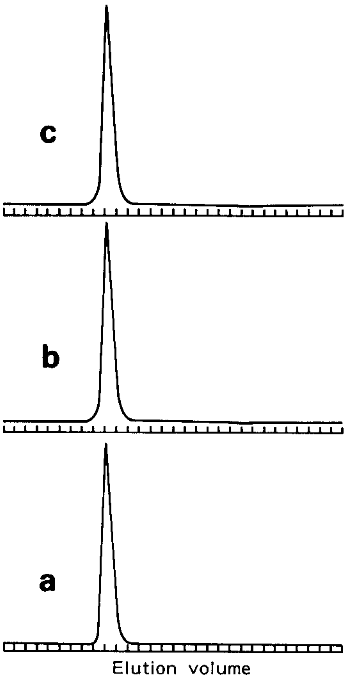

Figure 1 Gel permeation chromatograms of (a) monodisperse polystyrene 50k starting material, (b) chloromethylated polystyrene 50k and (c) NBD-labelled polystyrene 50k

(5k-17k-5k) solution in toluene. Then 80 ml of methanol were added. The block copolymer forms micelles in this mixture[18] but when loaded with PS the size of the micelles increases greatly. For example, the hydrodynamic radius obtained from dynamic light scattering increases from 21 to 65 nm. Thus it is, in essence, a suspension of swollen PS particles. The synthesis of the block copolymer and a detailed study of the block copolymer micelles in toluene/methanol will be published separately[18]. For the FRAPP measurement, the solution was sandwiched between two glass slides. For the dynamic light scattering measurement, the solution was filtered with a 0.45 μm Teflon membrane filter into a cylindrical sample cell.

Instrumentation

The mutual diffusion coefficient of the Brownian particles was obtained by dynamic light scattering. The instrument was a computerized Bookhaven Instrument model BI-200SM goniometer with a 136 channel BI2030 digital correlator. The light source was a Spectra Physics model 124B He–Ne laser operated at 632.8 nm. The tracer diffusion coefficient was obtained by using a FRAPP apparatus, which has been described previously[12]. Briefly, a periodic pattern was generated in the solution containing labelled Browian particles by a strong laser beam and a Ronchi ruling (50 lines per inch). The the fluorescence recovery in the dark region of the pattern as a result of Brownian motion of the particles is observed with a weakened beam. The tracer diffusion of the particles is analysed by fitting the recovery curve with a single exponential function.

RESULTS AND DISCUSSION

Chloromethylation and dye labelling reactions

The results on the chloromethylation of five monodisperse PS samples are presented in *Table 1*. The g.p.c. chromatograms of the 50k starting material as well as the chloromethylated and the dye-labelled products are shown in *Figure 1*. It can be seen that the molecular weight and polydispersity of the sample are virtually unchanged. The very small shoulder of the chromatograms of the chloromethylated and the dye-labelled product on the left amounts to less than 2% of the total area of the peak. This indicates that a very small amount of chain crosslinking did occur for this product. For the other four products, the product chromatogram is identical to that of the starting material. We have shown only the chromatograms for the B reaction in *Figure 2*. From these g.p.c. results and the data on percentage tagging from *Table 1*, we can conclude that the microstructure of the polymer is truly intact after the chloromethylation and the dye-labelling reactions as long as the amount of chloromethyl groups is lower than about 0.1%.

From *Table 1* it can be seen that the concentration of the reactants greatly affects the amount of chloromethyl group attached to the polymer chain. Higher concentration of reactants evidently leads to higher chloromethyl content. This is already well-known in conventional chloromethylation reactions[6]. Based on the g.p.c. results, it can be stated that up to 0.14% (monomer units) of tagging can be introduced in reactions B–E without significant intermolecular crosslinking. This can be achieved by increasing the concentration of CMEE or the catalyst or by increasing the duration of reflux during the reaction.

In *Table 2*, reactions F–H show that polymer concentrations above C^* lead to intermolecular crosslinking. The degree of crosslinking can be controlled by simply varying the amount of catalyst added. *Figure 3* shows the different chromatograms obtained by changing the amount of $BF_3 \cdot Et_2O$ from 0.1 to 0.2 ml. A very high concentration of the reactants causes the polymer to form

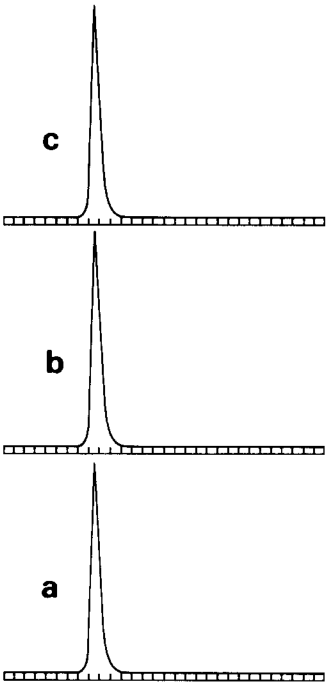

Figure 2 Gel permeation chromatograms of (a) monodisperse polystyrene 68k starting material, (b) chloromethylated polystyrene 68k and (c) NBD-labelled polystyrene 68k

Table 2 Conditions that lead to intermolecular crosslinking

Reaction	PS (k)	*C** (g ml^{-1})	Amount (g)	CH_2Cl_2 (ml)	CMEE (ml)	$BF_3{\cdot}Et_2O$ (ml)	Comment
F	100	0.02	0.5	10	1.5	0.10	Crosslinked, soluble
G	100	0.02	0.5	10	1.5	0.15	Crosslinked, soluble
H	100	0.02	0.5	10	1.5	0.20	Crosslinked, soluble
I	600	0.0046	0.5	20	3.0	0.40	Highly crosslinked, insoluble

Table 3 Conditions that lead to intramolecular crosslinking

Reaction	PS (k)	*C** (g ml^{-1})	Amount (g)	CH_2Cl_2 (ml)	CMEE (ml)	$BF_3{\cdot}Et_2O$ (ml)	Comment
J	110	0.018	0.4	170	1.5	0.04	Crosslinked microgel, soluble

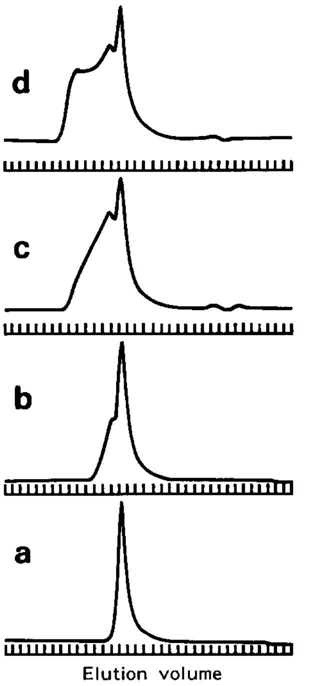

Figure 3 Gel permeation chromatograms of (a) monodisperse polystyrene 100k starting material, (b) product from reaction F, (c) product from reaction G, (d) product from reaction H

an insoluble gel (reaction I). Reaction J in *Table 3* is particularly interesting. When the concentration of the polymer is low but the concentration of the catalyst is high, the chloromethylated product is intramolecularly crosslinked, forming so-called 'microgel' particles (*Figure 4*). Antiotti *et al.* have studied the diffusion of these polystyrene microgel particles prepared using *p*-dichloroxylyene as the crosslinking agent[19]. Here we present an alternative method. Our microgel particles also have chloromethyl groups and can be easily labelled with fluorescent dyes. We have prepared samples that are highly intramolecularly crosslinked and are labelled with the five dye molecules described above.

Except for anthracene, both the absorption and fluorescence spectra of the dye molecules attached to the PS chain are identical to those of the free dyes. In the case of anthracene, the absorption spectrum is unchanged after attaching to the polymer but the shape of the fluorescence spectrum is slightly different from the starting material, 9-anthracenecarboxylic acid, as shown in *Figure 5*.

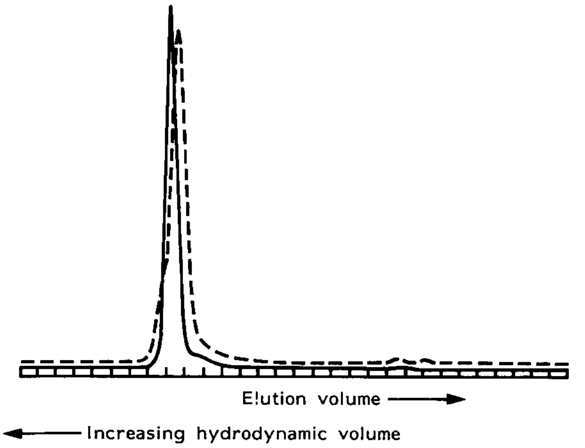

Figure 4 Gel permeation chromatograms of monodisperse polystyrene 110k starting material (———) and the intramolecularly crosslinked product from reaction J (– – –)

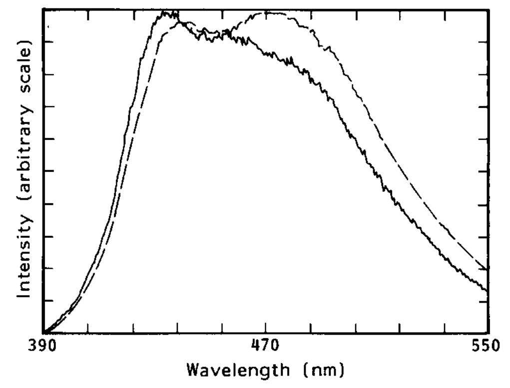

Figure 5 Fluorescence spectra of anthracene-9-carboxylic acid (———) and anthracene-labelled polystyrene 50k (– – –)

Diffusion measurements

The mutual diffusion coefficient of the PS Brownian particles was $5.89 \pm 0.02 \times 10^{-8}$ cm^2 s^{-1} and the tracer diffusion coefficient was $5.73 \pm 0.23 \times 10^{-8}$ cm^2 s^{-1}. Thus the two seem to be identical within experimental error. We note that identical mutual and tracer diffusion coefficients have been observed on haemoglobin and serum albumin at uniform ionic strengths near 0.1 M[20]. Theoretical work has revealed that the two diffusion coefficients should be the same if only hard-sphere particle interaction is important[15]. More systematic work will be reported in the future.

CONCLUSIONS

Chloromethylation and subsequent dye-labelling reactions can be easily carried out on monodisperse PS by using a modification of Sparrow's method. This simple approach should be very useful to those who want to study PS by using photophysical techniques. In an example of such an application, the tracer and mutual diffusion coefficients of PS Brownian particles stabilized by a block copolymer are measured and found to be identical within experimental error.

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