Syntheses of Polyethylene Glycol (PEG) with Different Functional Groups at Each End. I. Preparation of Monotrityl PEG Using Polyvinyl Alcohol (PVA) Matrix*

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SYNOPSIS

The syntheses and separation of monotrityl PEGs with molecular weights in the range of 400–6000 were successful using PVA as matrix. The yield of monotrityl PEG is strongly dependent on the initial mole ratio of PEG–trityl chloride. It is observed that satisfactory results can only be obtained in the range of 1 : 1.7 to 1 : 2.4 (mole number of PEG/mole number of trityl chloride). The effect of reaction conditions on monosubstitution is discussed. © 1992 John Wiley & Sons, Inc.

INTRODUCTION

In the past decade, there has been increasing interest in the synthesis of polyethylene glycol (PEG) with functional ends because these materials are very useful in the synthesis of pro-drugs as pro-moieties,[1] polymeric reagents,[2] and phase transfer catalysts.[3] But up to now the reports were restricted to PEG with the same functional group at both ends or having one end blocked by alkyloxy group.[4] If it is possible to synthesize PEG with different functional end groups, it would be more promising to utilize it in practice and for theoretical research.

But a problem arises: How to separate the PEG with monosubstituted end group from the reaction mixture after the first step of functionalized reaction. In fact, the PEG carrying one functional end group is not differentiated enough from the raw PEG and disubstituted PEG. Moreover, the higher the molecular weight of the starting PEG, the smaller the difference between monosubstituted PEG, disubstituted PEG, and raw PEGs in chemical and physical properties. Many researchers thought[5] it would be very difficult or even impossible to isolate the monosubstituted PEG from the mixture. This study provides a generally useful method to separate the monosubstituted PEG from the reaction mixture. In this work polyvinyl alcohol (PVA) was used as the matrix and trityl chloride, which has been widely used for protection of hydroxyl group, as functionalizing agent.

EXPERIMENTAL

Materials

Diol-type polyethylene glycol (PEG) of $\bar{M}_n$ = 400 (380–420) and tolylene-2,4-diisocyanate (TDI) were obtained from Fluka AG, ERLING & MORTEN LIND, Norway. TDI must be dried and distilled before use. PEG with $\bar{M}_n$ = 1000 (950–1050) and 1500 (1400–1600) were supplied by Hoechst, Germany, and PEG with $\bar{M}_n$ = 3400 (3000–3800) and 6000 (5000–7000) were purchased from KEBO Lab AB Norway. All PEGs were dried by azeotropic distillation[4] before use. PVA (Mowil 4-98, $\bar{M}_n$ = 23320) was obtained from Hoechst, Germany. Trityl chloride was recrystallized twice with benzene before use. Recrystallized products were needlelike crystals, slightly brown red; mp, 112–113°C. Butyl amine was obtained from KEBO Lab AB, Norway, and used without further purification. All solvents were dried by standard methods before use.

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Reaction of PEG with Trityl Chloride

Twelve grams of PEG 6000 (0.004 mol of OH) were dissolved in anhydrous chloroform (400 mL) to which 1.5 mL of dried pyridine (or 2.525 g of dried triethyl amine) and 1 g of trityl chloride (0.0034 mol) were added. After refluxing for 6 h, the reaction mixture was evaporated to about half its volume, and then cooled to room temperature and slowly poured in ether under stirring. After filtration and drying, the precipitate was dissolved in chloroform and again precipitated in ether. The product was dried to constant weight in a vacuum. The synthesis of monotrityl PEG with $\bar{M}_n$ = 400 was conducted as reported in Ref. 6.

Preparation of Isocyanate-Capped PEG

A 2.4-mL (0.02 mol) solution of TDI was injected into 250 mL of chloroform containing 10 g of the above-mentioned product. The system was stirred magnetically for 14 h while refluxing under N_2. The product was precipitated by ether and washed with *n*-hexane and stored in a desiccator.

Grafting of Isocyanate-Capped PEG on PVA

Two methods were used to prepare the grafts:

1. A 4.84-g amount of PVA (Mowil 4-98, $\bar{M}_n$ = 23,320, 0.11 mol of OH) was dried under vacuum at 50°C for 24 h, then suspended in 100 mL of anhydrous pyridine with stirring. Then 100 mL of pyridine containing 12 g of the above-mentioned product was added to the system. The reaction was carried out for 12 h at 70°C in dry N_2. The product was precipitated by dichloromethane with vigorous stirring. After vacuum drying at 50°C overnight, the product (No. 1) was redissolved in DMSO, reprecipitated by chloroform, and finally dried to constant weight in vacuum; yield was 5.45 g (grafting efficiency 12.6%; reacted mole fraction of hydroxyl group in PVA 0.0001).

The percentage grafting was calculated by

$$\text{Percentage grafting (\%)} = \frac{\text{polymer grafted (g)} - \text{PVA used (g)}}{\text{PVA used (g)}} \times 100 \quad (1)$$

The remaining dichloromethane filtrate was distilled to about half its initial volume, then slowly poured into ether. The precipitate (No. 2) was collected and dried.

2. A solution of 100 mL DMSO containing 12 g of isocyanate-capped PEG and 0.05 mL of dibutyltin dilaurate was added dropwise to 400 mL DMSO containing 2.42 g of dried PVA. The reaction was conducted for 12 h at 70°C in dry N_2. The grafted product was precipitated by dichloromethane with vigorous stirring. It could be purified by redissolution in DMSO and reprecipitated in methanol; yield was 8.35 g (grafting efficiency 242.1%; reacted mole fraction of hydroxyl group on PVA 0.0009).

Hydrolysis of Grafted PVA

Nine grams of grafted PVA was hydrolyzed in 200 mL of distilled water contained 0.2 g potassium hydroxide under reflux for 3 h, then precipitated by methanol. After filtration the precipitate was dried, then dissolved in DMSO and reprecipitated in dichloromethane. After filtration the product (No. 3) was washed with dichloromethane and dried. The remaining KOH–methanol–water mixture was neutralized to pH 7 by 0.5*M* HCL and evaporated to dryness. The residue was dissolved in the above-mentioned filtrate of DMSO and dichloromethane, and the solution was filtered again. The filtrate was then distilled to about two-thirds of its volume, poured into ether, and the precipitate (No. 5) was dried under vacuum. The ether mixture solution was distilled to dryness, a brown powder (No. 4) was obtained.

The syntheses and separation of monosubstituted PEG with other molecular weights were carried out according to the same procedure.

MEASUREMENT

^{1}H-NMR characterization of samples was carried out using a Varian XL-300 NMR spectrometer, using TMS as internal standard, $CDCl_3$ as solvent. IR spectra of samples were recorded on a Perkin-Elmer 1310 IR spectrometer (KBr tablet). MS spectra of samples were scanned by a V. G. Micromass 7070 F. The molecular weights of functionalized PEGs were measured by the butyl amine method; number-average molecular weights of functionalized PEGs were calculated by following formula:

$$\bar{M}_n = \frac{X\,W}{(V_1 - V_2)N} \times 1000 \quad (2)$$

where X = mole number of functional groups, W = the weight of sample, V_1 = sulfuric acid volume consumed on blank sample, V_2 = sulfuric acid volume consumed by sample, and N = concentration of sulfuric acid. Elemental analyses were done by

Ilse Beetz Microanalytisches Laboratorium, Germany. Identification data of intermediate compounds used in the syntheses of monotrityl PEG are summarized in Table I.

RESULTS AND DISCUSSION

Concept

In order to separate monosubstituted PEG from the reaction mixture, the essential key is how to increase the difference between disubstituted PEG, starting PEG, and monosubstituted PEG in physical and chemical properties. Our basic idea can be expressed as follow: First, by means of adjustment of mole ratio of starting PEG to trityl chloride and selection of reaction conditions, it is possible to make all of the PEG macromolecules react with trityl chloride, that is, the reaction products were only composed of mono- and disubstituted PEGs. Then TDI would be used to react with the mixture. As is well known, in this case monosubstituted PEG can only be reacted with TDI, the PEG macromolecules with isocyanate group at one end would be grafted to the polymer support with suitable functional groups, such as PVA, polymethacrylic acid (PMA), polyacrylic acid (PAA), polyacryl amine, etc. The PEG grafted on the polymer matrix would show a big difference from disubstituted PEG in its physical and chemical properties, so it is very easy to separate the grafts from the mixture. The monosubstituted PEG could be obtained by hydrolysis of the grafts in dilute potassium hydroxide–water solution under reflux. The whole process can be summarised as follows:

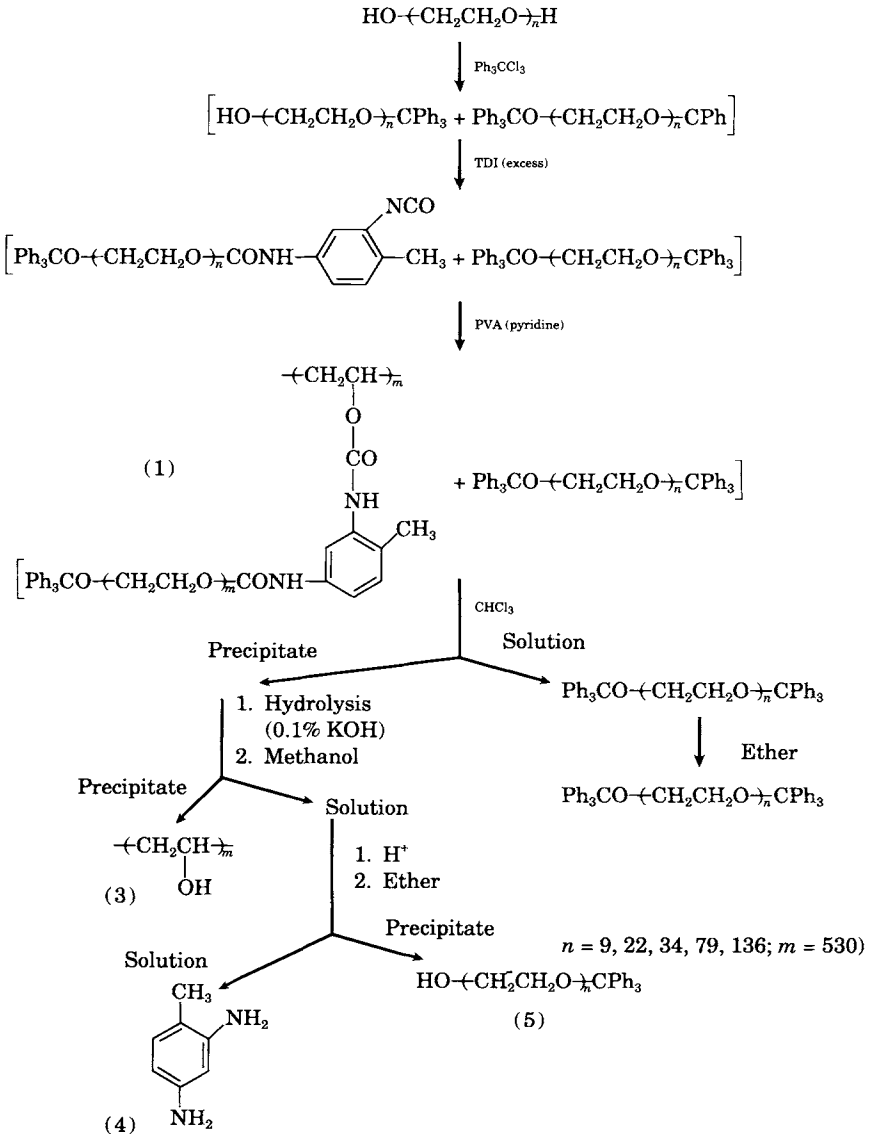
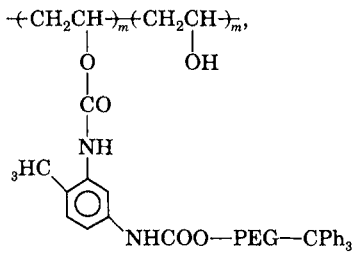
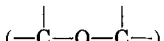
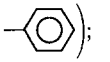
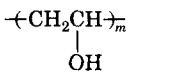
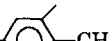
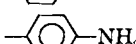
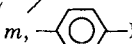
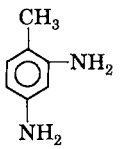
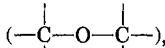


Table I Characterization of Various Compounds Formed in the Process of Syntheses of Monotrityl PEG (6000)

Number of Unknown Compounds	IR (cm^{-1})	^{1}H-NMR (ppm)	MS (*m/e*)	Elemental Analyses		Molecular Structure of Compounds
				Found	Calc.	
(1)	3402 (—OH), 2938, 2865 ($—CH_2—$), 1712 (—CO—), 1119 (—C—O—C—), 765, 821 ()	1.18–1.51 (*m*, $—CH_2—$, $—CH_2\underset{\vert}{C}H—$), 3.68–3.72 (*m*, —OH—, —NH) 6.21–6.83 (*m*, phenyl)				
(2)	2921, 2878 ($—CH_2—$), 1110 (—C—O—C—), 748, 689 ()	3.64 (*m*, $—CH_2CH_2O$), 7.3 (*m*, );	260 (Ph_3COH), 243 (Ph_3C^+), 65 $C^+H_2CH_2OH$)	C 57.03 H 8.92	57.34 8.85	$Ph_3CO\text{-}(CH_2CH_2O)_n\text{-}CPh_3$
(3)	3400, (—OH), 2940, 2853 ($—CH_2—$)	1.43 (*m*, $—CH_2CH—$), 3.8 (*m*, —OH)	64 (C^+H_2=CH—OH) 63 (CH_2=CH—O^+)	C 54.01 H 8.72	54.55 8.85	
(4)	3416, 3368 ($—NH_2$), 2959 ($—CH_3—$)	2.05 (*s*,  , 3H), 3.7 (*s*,  , 2H), 6.08 (*m*,  , 2H), 6.81 (*m*,  , 3H),	122 (M^+, 100), 121 (M^+, —H, 14), 94 (M^+, —H—HCN, 22)	C 68.96 H 8.14 N 22.94	68.85 9.09 22.82	
(5)	2920, 2874 ($—CH_2—$), 1112 (—C—O—C—), 750, 790 ()	3.64 –3.66 (*m*, $—CH_2CH_2O$), 7.26 (*m*, )	243 (Ph_3C^+), 183 (Ph_2C^+OH), 77 (Ph^+) 65 ($C^+H_2CH_2OH$)	C 55.27 H 8.71	55.94 8.97	$HO\text{-}(CH_2CH_2O)_n\text{-}CPh_3$

Determination of Initial Mole Ratio of PEG to Chlorotriphenyl Methane

In order to obtain pure monotrityl PEG, Pozzo et al.[7] used excess PEG to react with Ph_3CCl, but this method is restricted to PEG with low molecular weights (< 1000). When the molecular weight of the PEG is higher than 1000, this method is unsuccessful because there are no sharp differences between reacted PEG and raw PEG in chemical and physical properties. It is impossible to isolate the monosubstituted PEG from the mixture by common chemical methods. Table II shows that when the molecular weight of PEG is lower than 1000, the results of elemental analyses of monotrityl PEG made by the Pozzo method were consistent with the calculated value. When the molecular weight of PEG is above 1000 (for example, 1500), the values of elemental analysis of monotrityl PEG obviously deviated from the calculated values. When the molecular weight of PEG was further increased, the difference between found and calculated values of elemental analyses reduced again due to dropping of the trityl methyl group content in PEG. But the measurements of molecular weights indicated that in this case, the molecular weight of products by reaction with TDI was much higher than the calculated value. The reasons are very clear, that is, in this case excess unreacted starting PEG left in the product-monotrityl PEG. The excess TDI easily reacts with starting PEG, and condensation products with higher molecular weight may be formed between them. Therefore average molecular weight increased. However, it was found that when the mole ratio of raw PEG to Ph_3CCl is in the range of 1 : 1.7 to 1 : 2.4, little of the original PEG is left in the reacted mixture. In other words, the reacted mixture is mainly composed of mono- and disubstituted PEG. Table II indicates that the molecular weight of the product formed by reaction of monotrityl PEG, made from polymer matrix with TDI, is very close to the calculated value. This strongly supports the conclusion that there is no raw PEG left in the reacted mixture.

When the mole ratio of raw PEG to Ph_3CCl is more than 1 : 1.7, it was found that the molecular weight of the products is lower than the initial values. This may be explained as follow: While the end group titration was used to measure the molecular weight of monotrityl PEG with isocyanate group at the other end (NCO–PEG–CPh_3), it was assumed that there is no starting PEG left in the reaction mixture, that is, X, the mole number of functional groups, was denoted as 1 in the formula (2). This supposition is correct when the mole ratio of PEG to Ph_3CCl is less than 1 : 1.7 as shown in Table III.

Table II Characterization of PEG with Monotrityl Group Formed by Pozzo Method and by Polymer Matrix Method

Molecular Weight of PEG (M_n)[a]	Elemental Analyses of PEG–CPh_3				Molecular Weight of Products Formed by Reaction of PEG–CPh_3 with TDI			
		Found[b]			Found[b]			
		P	PM	Calc.	P	PM	Calc.	
400	C	68.12	68.27	68.04	863	879	817	
	H	7.62	7.88	7.91				
1000	C	61.23	61.39	61.45	1947	1456	1417	
	H	6.99	7.32	7.18				
1500	C	56.92	59.95	59.52	2605	1937	1917	
	H	9.12	8.24	8.66				
3400	C	55.28	57.03	56.94	4576	3793	3817	
	H	9.09	9.11	8.89				
6000	C	54.04	55.27	55.94	6634	6189	6157	
	H	9.10	8.71	8.97				

[a] These data were given by the manufacturer.

[b] By P (Pozzo) methods and PM (polymer matrix) method (mole ratio of PEG to Ph_3CCl : 1 : 1.7); both are average values, measured three times.

Table III Effect of Mole Ratio of PEG to Ph_3CCl on the Molecular Weight of Products Formed by Reaction of PEG–CPh_3 with TDI

Molecular Weight of PEG[a]	Mole Ratio of PEG to Ph_3CCl	Molecular Weight of Products Formed by Reaction of PEG–CPh_3 with TDI	
		Calc.	Found
400	1 : 1.0	800	579 (584, 575, 578)
	1 : 1.2		703 (698, 703, 708)
	1 : 1.4		814 (821, 810, 811)
	1 : 1.7		879 (872, 884, 881)
	1 : 2.0		852 (861, 846, 843)
	1 : 2.4		893 (896, 901, 882)
	1 : 2.8		855 (884, 891, 880)
1500	1 : 1.0	1900	1387 (1291, 1396, 1374)
	1 : 1.2		1519 (1524, 1537, 1496)
	1 : 1.4		1812 (1820, 1804, 1812)
	1 : 1.7		1937 (1943, 1934, 1934)
	1 : 2.0		1986 (1971, 1989, 1998)
	1 : 2.4		1920 (1925, 1921, 1914)
	1 : 2.8		1974 (1968, 1972, 1982)
3400	1 : 1.0	3800	3219 (3228, 3234, 3195)
	1 : 1.2		3408 (3426, 3385, 3413)
	1 : 1.4		3746 (3735, 3759, 3744)
	1 : 1.7		3817 (3836, 3782, 3834)
	1 : 2.0		3805 (3780, 3812, 3787)
	1 : 2.4		3846 (3839, 3941, 3858)
	1 : 2.8		3798 (3762, 3822, 3786)
6000	1 : 1.0	6400	4118 (4156, 4122, 3976)
	1 : 1.2		5234 (5230, 5246, 5226)
	1 : 1.4		5906 (5947, 5891, 5880)
	1 : 1.7		6189 (6188, 6231, 6148)
	1 : 2.0		6200 (6236, 6198, 6166)
	1 : 2.4		6193 (6147, 6209, 6223)
	1 : 2.8		6214 (6258, 6226, 6158)

[a] These data were supplied by the manufacturers.
[b] Data were calculated by formula (2), X was denoted as 1. Relative errors are less than 1% (concentration of sulfuric aicd: 0.05N).

But if there is still some starting PEG left in the reaction products as we mentioned before (the mole ratio of starting PEG to Ph_3CCl is above 1 : 1.7), discrepancies may appear. The remaining raw PEG can react with excess TDI; PEG with two isocyanate end groups, that is, OCN–PEG–NCO may be formed. In this case, the average mole number of functional group (X) should be more than 1. If the X is still denoted as 1, the molecular weight calculated by formula (2) should be less than the expected value. This is the reason for the decrease of molecular weight of NCO–PEG–CPh_3 when the mole ratio of PEG to Ph_3CCl was higher than 1 : 1.7. When the ratio is lower than 1 : 2.4, Figure 1 reveals that the yield of monosubstituted PEG (HO–PEG–CPh_3) declined because the content of disubstituted PEG, that is Ph_3C–PEG–CPh_3, increased.

Effect of Reaction Condition on the Formation of Monosubstituted PEG

Solvent

In the preparation of grafts, two solvents were used. One is pyridine the other is DMSO, which is a good solvent for PVA and capped PEG. The grafting efficiency and reacted mole fraction of hydroxyl group in PVA increased from 12.6% and 0.0001 in pyridine to 242.1% and 0.001 in DMSO, respectively. The reasons are very clear. In the good solvent, the chains of PVA and PEG are extended, providing the op-

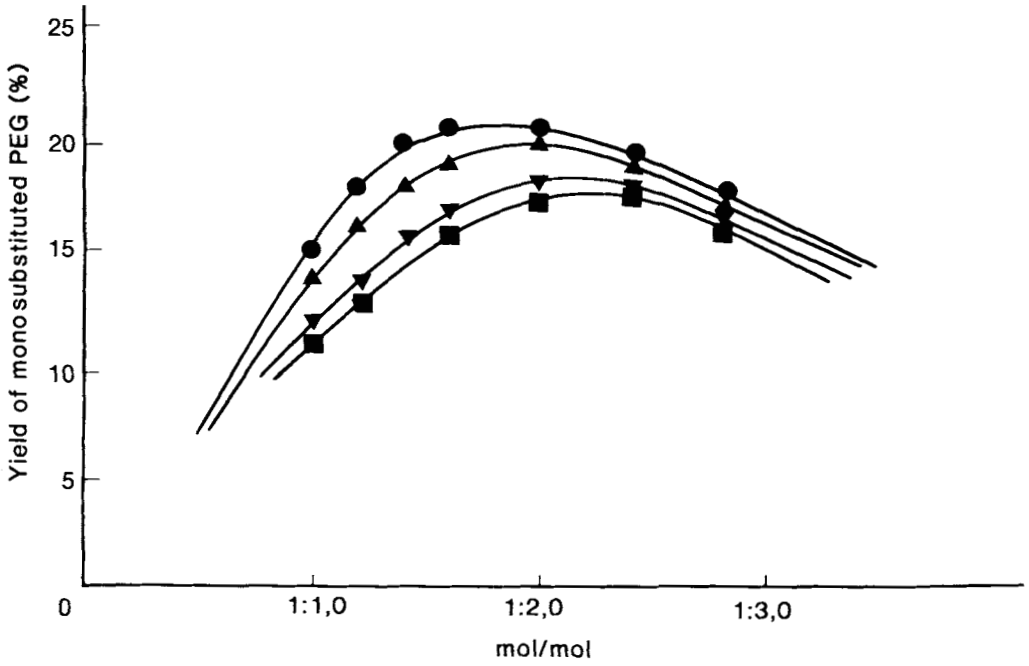


Figure 1 The effect of mole ratio of PEG to Ph_3CCl on the reacted mole fraction of hydroxyl group on PVA (molecular weight of PEG, ● 400, ▲ 1500, ▼ 3400, ■ 6000).

portunity, time, and space for the hydroxyl group in PVA and isocyanate in capped PEG to react with each other. On the other hand, in poor solvent the reactive groups are covered by curling and entanglement of chains of PVA and PEG, so it is not easy for them to move and react each other. Hence the grafting efficiency and reacted mole fraction of hydroxyl group on PVA decreased.

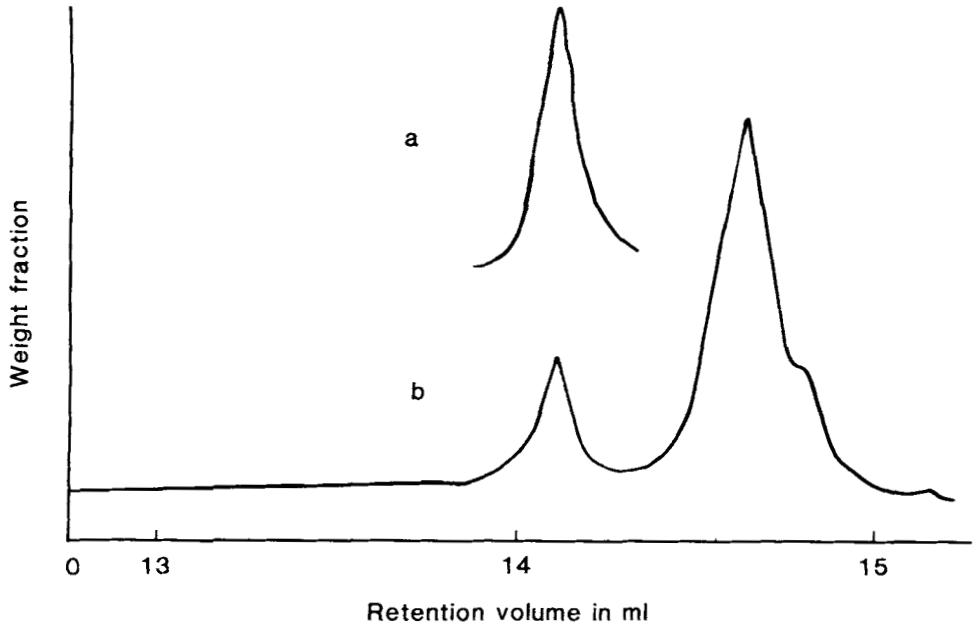


Figure 2 The effect of acid scavenger on stability of functionalized PEG: (a) triethyl amine, (b) pyridine.

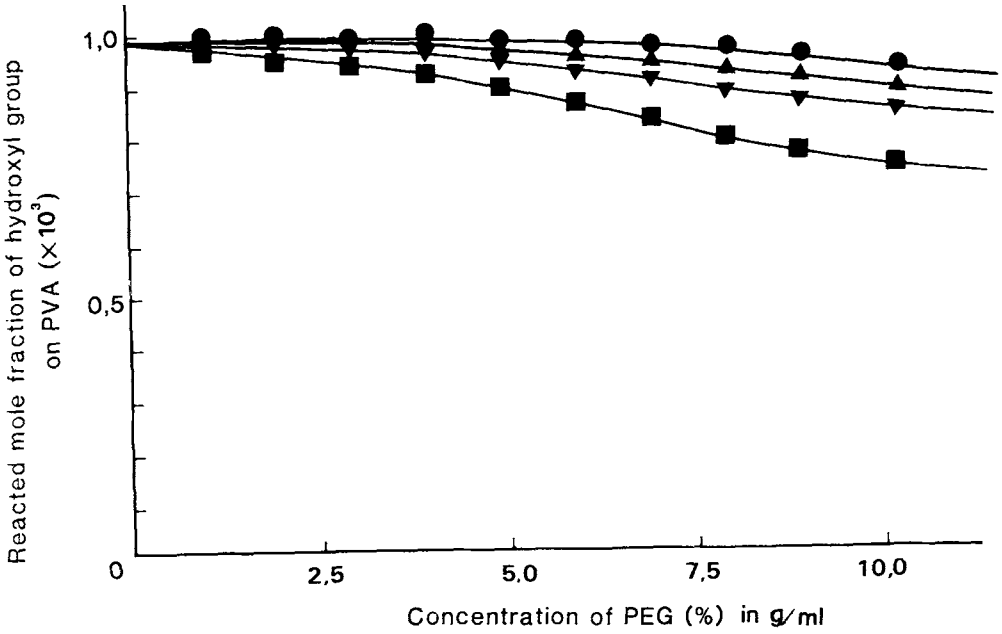


Figure 3 The effect of concentration of PEG on the reacted mole fraction of hydroxyl group on PVA [concentration of PVA ($\bar{M}_n$ = 23,300): 0.6%; molecular weight of PEG: ● 600, ▲ 1500, ▼ 3400, ■ 6000].

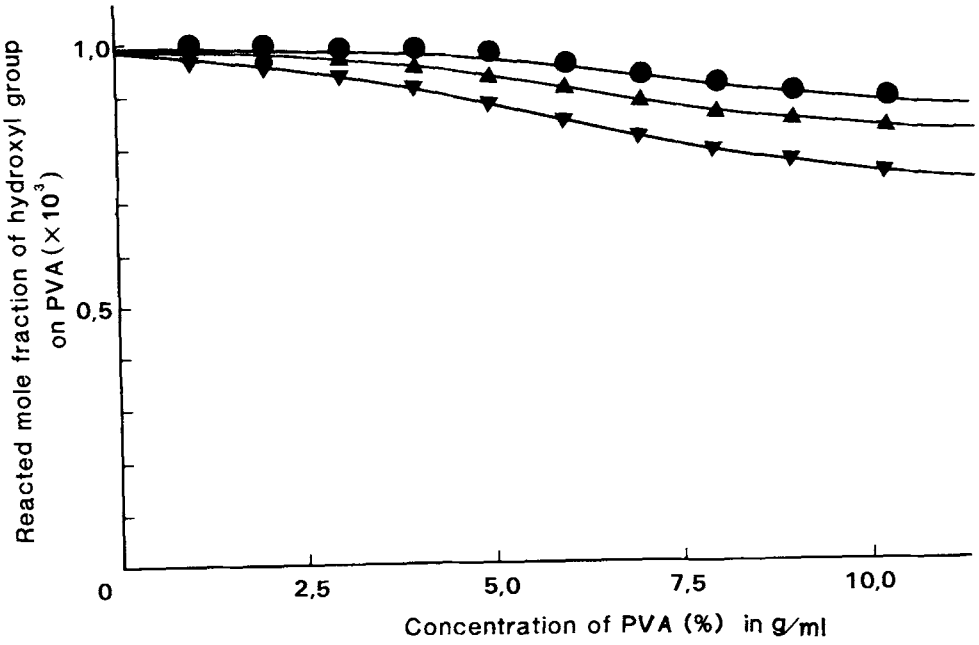


Figure 4 The effect of concentration of PVA on the reacted mole fraction of hydroxyl group [concentration of PEG ($\bar{M}_n$ = 6000): 2%; molecular weight of PVA: ● 10,000, ▲ 16,000].

Catalyst

Pyridine or triethyl amine was used as catalyst for the reaction of PEG with trityl chloride. However, degradation of functionalized PEG occurred when pyridine was used as acid scavenger. The average molecular weight of functionalized PEG decreased one-fourth to one-third of its original value in 24 h, as Figure 2 shows. The degradation mechanism is still unclear. When triethyl amine was used as acid scavenger instead of pyridine, no degradation was detected.

Molecular Weight and Concentration of PVA and PEG

In the dilute solution (concentration of TDI capped PEG < 3%, concentration of PVA 0.6%), the reacted mole fraction of hydroxyl group on PVA is nearly independent of the molecular weight of PEG, as Figure 3 indicates. In dilute solution the macromolecules of PEG with high molecular weight can diffuse to the activated point of PVA without difficulty as the PEG with lower molecular weight does. When the concentration of PEG is more than 3%, Figure 3 shows the reacted mole fraction of hydroxyl group on PVA dropped with the molecular weight of PEG.

In this case the macromolecules of PVA with long branched PEG chain may entangle, and the number of efficient collisions between macromolecules of PVA, PVA with branched PEG chain, and PEG decreased.

Similarly, when the concentration of PVA is more than 1%, the reacted mole fraction of hydroxyl group on PVA decreased with the molecular weight of PVA, as Figure 4 shows. Therefore there are different critical concentrations for PVA and PEG with different molecular weight. For PEG 6000, the critical concentration is 2%; for PEG 3400 and 1500, the values are 3.1 and 5.5% respectively, when the concentration of PVA ($\bar{M}_n$ = 23,300) is less than 1%. For PVA ($\bar{M}_n$ = 23,300), the critical concentration is 1%, and for PVA ($\bar{M}_n$ = 16,000 and 10,000), the values are 2.4 and 3.7%, respectively, when the concentration of PEG ($\bar{M}_n$ = 6000) is less than 1%.

These results support our basic idea that using polymer as a matrix to synthesize and separate monosubstituted PEG is practicable.

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