

# Synthesis of Monomethoxy Poly(ethylene glycol) without Diol Poly(ethylene glycol)

Jing Zhang,<sup>1,2</sup> Yong-Jiang Zhao,<sup>1,2</sup> Zhi-Guo Su,<sup>1</sup> Guang-Hui Ma<sup>1</sup>

<sup>1</sup>National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100080, People's Republic of China

<sup>2</sup>Graduate University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

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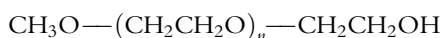
**ABSTRACT:** Since monomethoxy poly(ethylene glycol) (mPEG) inevitably contains diol PEG and is difficult to get high molecular weight through traditional synthesis at high temperature under high pressure, a novel synthetic technique via anionic solution polymerization was reported in this study. With a new initiating system, potassium naphthalene and methanol, was introduced, the polymerization proceeded at ambient temperature and side reactions were well restrained. Furthermore, a slight excess of potassium naphthalene can effectively remove the trace of water and oxygen in the reaction system. Under this condition, mPEG

was nearly quantitatively obtained without containing diol PEG. Its  $M_n$  ranged from 1 to 30 kDa and the polydispersity was kept lower than 1.07. Characterization of the mPEG obtained was carried out using GPC to determine the content of diol PEG and <sup>1</sup>H-NMR and MALDI-ToF MS spectroscopic analysis to confirm the exact structure. © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 105: 3780–3786, 2007

**Key words:** anionic polymerization; ring-opening polymerization; polyethers; high molecular weight; high purity

## INTRODUCTION

In the last three decades, PEGylation, defining the modification of a protein, peptide, or nonpeptide molecule by the linking of one or more poly(ethylene glycol) (PEG), has attracted much attention in the fields of pharmaceuticals and biomedical engineering,<sup>1,2</sup> because exogenous proteins can appreciably reduce their antigenicity and immunogenicity through PEGylation, and blood lifetime of PEGylated biomolecules can be significantly extended.<sup>3,4</sup> Most used one for the modification of proteins, peptides, and nonpeptide molecules is monomethoxy poly(ethylene glycol) (mPEG) having the general structure:



mPEG is usually synthesized by anionic polymerization of ethylene oxide initiated by nucleophilic attack of methoxide ion on the epoxide ring.<sup>5</sup> Generally, the polymerization of ethylene oxide involves monomer addition reaction that accompanies with few chain

transfer and termination steps, and the molecular weight of resulting polymer has a Poisson distribution.<sup>6</sup> The polydispersity of mPEG ranges from 1.01 for low molecular weight (3–5 kDa) to 1.2 for high molecular weight (20 k).<sup>7</sup>

However, since living anionic polymerization can be killed by protonation or reactions with unexpected terminating species such as trace of moisture, oxygen, or other impurities,<sup>8</sup> the mPEG obtained inevitably contains a considerable amount of diol PEG because of the presence of trace amount of water during polymerization. The molecular weight of diol PEG is often twice as that of the targeted mPEG for the propagation at both ends of the polymer chain.<sup>9</sup> The amount of diol PEG range between 1 and 10%,<sup>7</sup> sometimes even exceed 15%,<sup>9</sup> in the mPEG product, which depends on the molecular weight of mPEG (lower for low mass mPEG). Moreover, it has been reported that decomposition reaction of mPEG might occur as well to produce a low molecular vinyl compound depending on the reaction temperature and the amount of catalyst.<sup>10</sup> Such vinyl ether is easily hydrolyzed under acidic condition to produce a hydroxyl group. Thus, if a mineral acid or the like is used to remove the alkaline catalyst, the vinyl ether will become a PEG derivative having a different molecular weight from that of the main component, and it will broaden the distribution of molecular weight of target polymer. On the other hand, if no mineral acid is used to remove the catalyst, the vinyl ether remains to be a vinyl group, which then is left unreacted at the step

Correspondence to: G. H. Ma (ghma@home.ipe.ac.cn).

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of introducing a functional group into the terminal of PEG derivatives. Thus, the vinyl ether remains as an impurity.

mPEG, containing above impurities, will lead to new impurities when mPEG derivative, used for peptide and protein PEGylation, was produced.<sup>11</sup> Since these impurities have physical properties similar to that of the desired compound, they are difficult to be removed effectively. Further, if the mPEG derivative is bonded to drug while containing these impurities, the resulting drug is heterogeneous, making it difficult to invariably provide drug with constant quality. Especially diol PEG, the main impurity in mPEG, will easily lead to unexpected crosslinking of biomedical molecules<sup>7</sup> for the terminal hydroxyl group is the main reacting group in mPEG derivation.<sup>12,13</sup>

Normally, low molecular weight mPEG ( $M_n = 2\text{--}5$  kDa) without diol PEG, whose purity was 96.8%–99.4%, could be prepared in a sealed glass ampoule by the anionic polymerization of ethylene oxide at 70°C, in which alcoholate of methyl ether of triethylene glycol acted as the initiating center.<sup>14</sup> On the other way, the purity of mPEG ( $M_n = 2\text{--}5$  kDa) could rise from 75% to around 99% through the silica gel column chromatographic purification of commercial mPEG.<sup>15</sup>

However, for the physical properties of diol PEG, such as polarity and solubility, are almost the same as those of mPEG when they are both in high molecular weight, the desired high purity mPEG with a high molecular weight cannot be obtained efficiently by usual purification methods such as recrystallization, reprecipitation, ultrafiltration, or silica gel column chromatography. Some methods to solve the problem of diol contamination have been developed. For example, converting PEGs to carboxylic acid, so that mPEG–COOH and HOOC–PEG–COOH can be separated by ion-exchange chromatography.<sup>16</sup> Another route is to strictly control the amount of impurities in all reagents and the polymerization system. For example, after rigorously excluded traces of water, oxygen and other impurities from the polymerization system and reagents for more than 10 h, mPEG with high molecular weight was obtained through anionic polymerization at high temperature around 100°C under a pressure of 3 kg/cm<sup>2</sup> with methanol solution of sodium methoxide as an initiating system.<sup>11</sup> However, it is a pity that the content of diol PEG measured by gel penetration chromatography was still sometimes as high as 8%.

So far several proposals<sup>16,17</sup> have been made to obtain mPEG with high molecular weight and high purity. However, they are complicated and costly because containing several derivation reaction steps and additional separation steps. The target mPEG free of high or low molecular impurities and having a narrower polydispersity degree scarcely has been obtained.

Recently, one of the most common used initiator systems in anionic polymerization, potassium naphthalene, was used in the synthesis of some heterotelechelic poly(ethylene glycol) derivatives by anionic solution polymerization.<sup>18,19</sup> With its high initiating efficiency, it can easily lead to the polymerization of ethylene oxide under mild condition such as ambient temperature and normal pressure, which restrained several side reactions of mPEG and those impurities. In initiating reaction, a slight excess of potassium naphthalene slightly more than stoichiometric amount could convert trace of impurities such as water and oxygen to substances inert to the ring opening of ethylene oxide under mild conditions. Another advantage is that the process of initiating reaction can be monitored by the change of color of the reaction system.

On the basis of high efficiency of potassium naphthalene, it was proposed to be an initiator system with methanol to produce mPEG in this study. With the new initiating system, anionic solution homopolymerization of EO at ambient temperature under normal pressure led to good yields of mPEG almost without diol PEG. Furthermore, the mPEG obtained showed ultra low polydispersity ( $P_d \leq 1.07$ , GPC) in the molecular weight ranging from 1 to 30 kDa.

## EXPERIMENTAL

### Materials

Tetrahydrofuran (THF) was purified by successive distillation over potassium and diphenylketone under dry nitrogen. Methanol was purified by distillation over magnesium and iodine. EO was condensed onto CaH<sub>2</sub> and stayed for more than 24 h, then distilled and kept under inert atmosphere. Potassium, naphthalene, and hydrochloric acid were used as received.

### Synthesis of potassium naphthalene

The mixture of 1.28 g naphthalene (10 mmol) and 0.43 g potassium (11 mmol) was dissolved in 20 mL dry THF, then stirred under an inert atmosphere for several hours at normal temperature until the reaction mixture became deep green. The concentration was determined by titration.

### Polymerization procedure

A round-bottomed glass vessel equipped with a three-way stopcock was degassed and argon gas was introduced. This cycle was repeated three times. After 50 mL THF and 32  $\mu$ L methanol (25.6 mg, 0.8 mmol) were added successively via syringes, THF solution of potassium naphthalene (1.0 mmol, 0.5 mol L<sup>-1</sup>) was added drop by drop. The addition of potassium

naphthalene was stopped when the color turned from colorless to light green and did not fade out in 1 min. After a few minutes agitation, 12.5 mL of EO (11 g, 250 mmol) was added via a cooled syringe. The mixture was allowed to react for 40 h at ambient temperature, then 1.0 mL of hydrochloric acid (4 mol L<sup>-1</sup>, 4 mmol) was added, and the solution was stirred for a further 2 h. The obtained mixture was precipitated in an excess amount of cooled diethyl ether, separated by filtration, and then dried under vacuum. Yield was 10.6 g (96.4%), the obtained product can be used directly in subsequent reactions.

### Polymer characterization

#### FTIR spectroscopy

The polymer sample was ground into fine particles and pressed into the form of KBr disc. FTIR transmission spectra were recorded on a JASCO FT-IR 660 spectrophotometer using 4 cm<sup>-1</sup> resolution and 128 scans.

#### NMR spectroscopy

<sup>1</sup>H-NMR spectra were recorded on an AVANCE Bruker 600 MHz spectrometer using DMSO-d<sub>6</sub> and chloroform-d<sub>1</sub> as solvents separately in 5 mm NMR tubes. In all the spectra TMS was used as the internal reference.

#### Matrix-assisted laser desorption ionization time-of-flight mass spectrometry

MALDI-ToF MS was performed on a Bruker BIFLEX III.  $\alpha$ -Cyano-4-hydroxycinnamic acid (CCA) was used as the matrix for PEG.

#### Thin layer chromatography

The thin layer plate used in TLC was a laminate. The developing solvent was the mixture of chloroform and methanol (85 : 15, v/v). The amount of the specimen solution was from 50 to 100  $\mu$ g. The color development was iodine. About 50 mg of sample was dissolved in 1 mL chloroform to prepare a sample

solution, and 0.2–1  $\mu$ L solution was spotted on the thin layer plate.

#### Gel permeation chromatography

The instrument package was supplied by Waters and comprised following equipment: a Waters 515 HPLC pump, a Waters styragel Columns (HT2+HT3+HT4), and a Waters 2410 Differential Refractometer. THF was used as eluent at a flow rate of 1.0 mL min<sup>-1</sup> at 35°C. The samples were prepared for injection by dissolving 5 mg polymer in 1 mL HPLC grade THF. About 10  $\mu$ L of this mixture was injected and data were collected for 34 min.

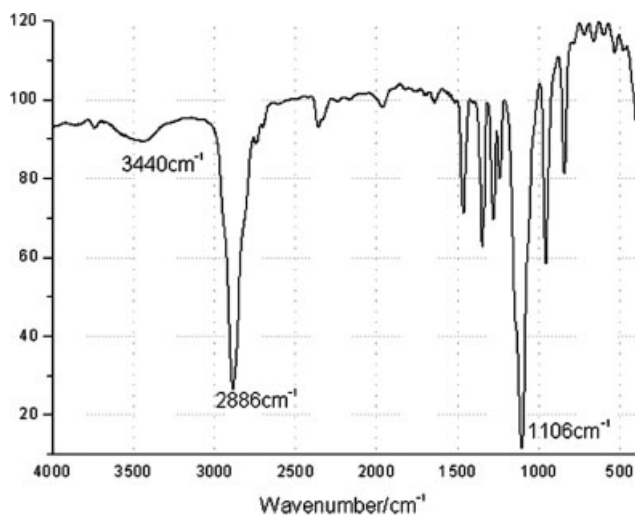
## RESULTS AND DISCUSSION

Details of the linear mPEG synthesized are shown in Table I. The anionic polymerization of EO was initiated by potassium naphthalene and methanol in THF solution at ambient temperature under argon atmosphere. The reaction time of the polymerization varied with the target molecular weight of the polymer. It was almost proportionately prolonged with the increase of the Mn of the obtained mPEG (Table I). At the end of the reaction, an excess amount of hydrochloric acid was added to stop the reaction. The polymerization proceeded smoothly and gave the polymer almost quantitative yield. The molecular weight of the polymers determined from GPC and MALDI-ToF MS agreed well with expected values, which were calculated from the monomer/initiator molar ratio. In addition, the molecular weight distribution of the polymer formed was fairly narrow ( $Pd \leq 1.07$  for all of the samples). All samples were obtained in good yields (> 94.8%), bearing in mind the usual losses during isolation, and the corresponding conversions were close to quantitative.

The essential polyether backbone structure of mPEG can be seen from IR spectra (Fig. 1). The peak of 1106 cm<sup>-1</sup> was the asymmetrical stretching vibration of ether links. The band of 2886 cm<sup>-1</sup> was the stretching vibration of methylene. The presence of hydroxyl group can be seen in the form of the band around 3440 cm<sup>-1</sup>.

**TABLE I**  
Synthesis and Characterization of Monomethoxy Poly(ethylene glycol)

No.	Feed ratio (mol) MeOH/ Naph-K/EO	Reaction time (h)	Yield (%)	Polymer characterization					
				$M_n$			Polydispersity		
				<sup>1</sup> H-NMR	MALDI-ToF MS	Calcd.	MALDI-ToF MS	GPC	
1	1/1.2/40	6	96.0	1,890	1,740	1,792	1.03	1.06	
2	1/1.2/70	9	94.8	3,042	–	3,112	–	1.07	
3	1/1.2/312	20	96.4	12,328	13,270	13,760	1.02	1.06	
4	1/1.1/600	41	95.8	25,685	–	26,432	–	1.07	



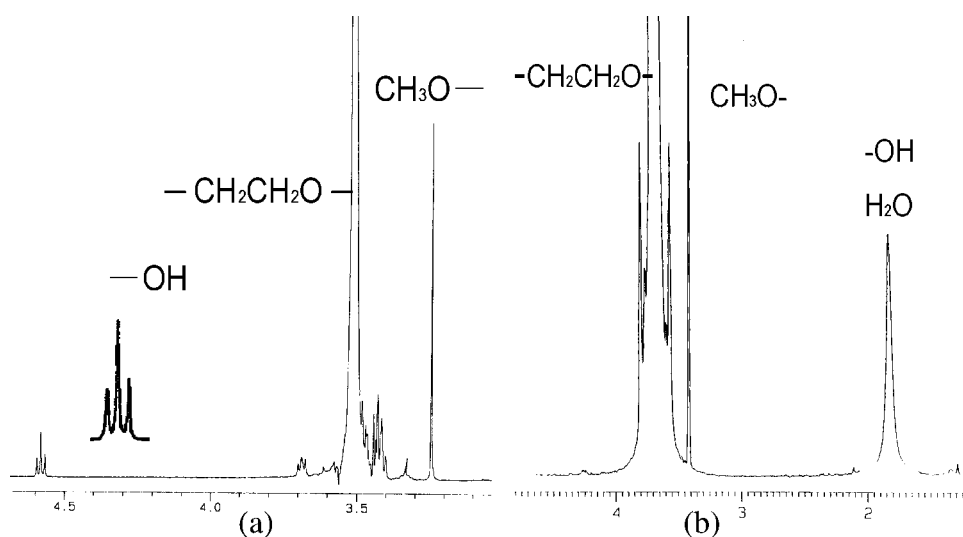
**Figure 1** IR spectrum of the monomethoxy poly(ethylene glycol).

To quantitatively analyze terminal groups of the mPEG obtained,  $^1\text{H-NMR}$  spectra was measured. Using TMS as internal reference in  $\text{DMSO-d}_6$  solution, the singlet appeared at 3.24 ppm shown in the Figure 2(a) was assigned to monomethoxy protons. Hydroxyl proton appeared clearly at 4.58 ppm shown in Figure 2(a) when  $\text{DMSO-d}_6$  was used as solvent, which appeared around 2.0 ppm as a little broader peak in Figure 2(b) when chloroform- $\text{d}_1$  was solvent. Since the comparison of the peak area of hydroxyl proton to that of monomethoxy protons can determine the end groups of mPEG quantitatively,<sup>20</sup> the corresponding ratio of 1 : 3 in Figure 2(a) indicated that the polymer obtained has one hydroxy and one methoxy terminal groups stoichiometrically.

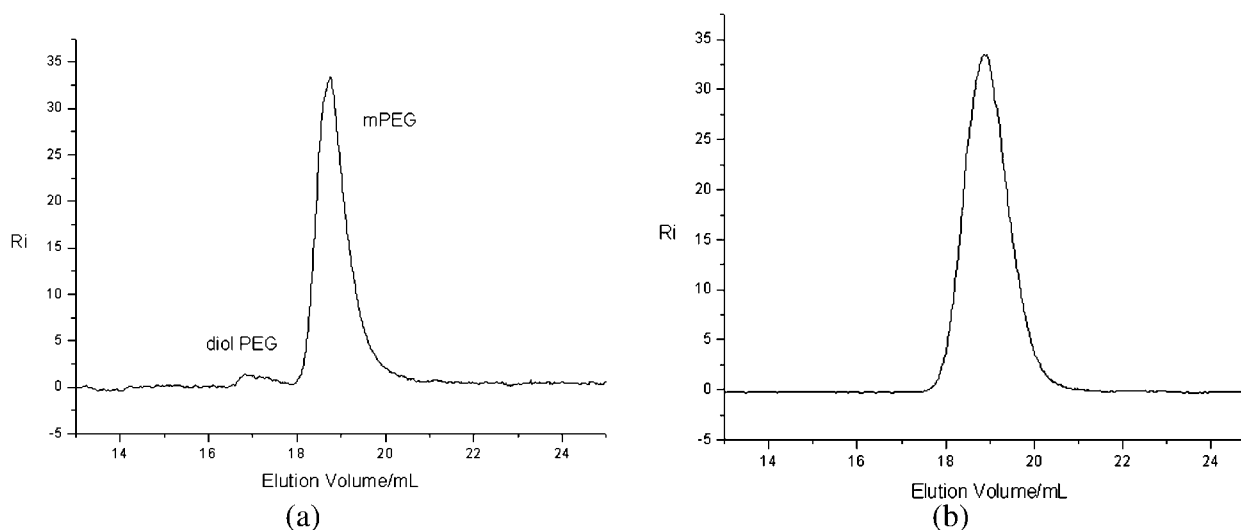
The purity of the obtained mPEG can primarily be determined from TLC. Silica gel TLC separation of mPEG (sample 2 in Table I) using chloroform/methanol (85/15 v/v) as developing solvent showed clearly one spot ( $R_f = 0.3\text{--}0.5$ ), which indicated that the purity of mPEG obtained was high.

However, TLC can hardly distinguish the main by-product, diol PEG, from mPEG, because they have almost the same polarity. It was reported that<sup>5,21</sup> the number of ethylene oxide added to the diol PEG was theoretically twice as the corresponding number added to the mPEG. As a result, the major impurity, diol PEG, showed a double molecular weight compared with that of the obtained mPEG. Therefore, GPC is an effective method to determine the content of diol PEG and to evaluate the homogeneity of mPEG.<sup>5</sup> Figure 3(a) is a GPC chart of commercially available mPEG. In Figure 3(a), diol PEG whose molecular weight is double of that of the target mPEG, showed a small peak a few minutes earlier than the maximum peak of mPEG. In our study, the single elution peak in the GPC of Figure 3(b), without a peak or shoulder in front of it, demonstrated that there was almost no diol PEG formed in the polymerization. From the chromatogram of Figure 3(b), it could be deduced that potassium naphthalene and methanol was an effective initiating system for the polymerization of ethylene oxide to produce mPEG without diol PEG.

In previous reports,<sup>22,23</sup> the MALDI-ToF-MS spectra analysis can distinguish two PEG polymers terminated by different end groups by analyzing the accurate molecular weight of individual polymer molecule. From MALDI-ToF-MS analysis, only parent ions of each polymer molecule are generally observed, meaning no fragments signal can be detected. In con-

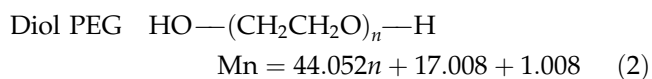
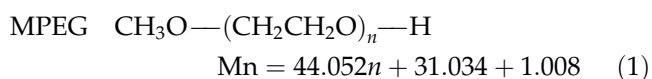


**Figure 2**  $^1\text{H-NMR}$  spectra of the monomethoxy poly(ethylene glycol) (a)  $\text{DMSO-d}_6$  (b)  $\text{CDCl}_3\text{-d}$ .



**Figure 3** GPC of the monomethoxy poly(ethylene glycol) (a) commercially available mPEG (b) prepared mPEG.

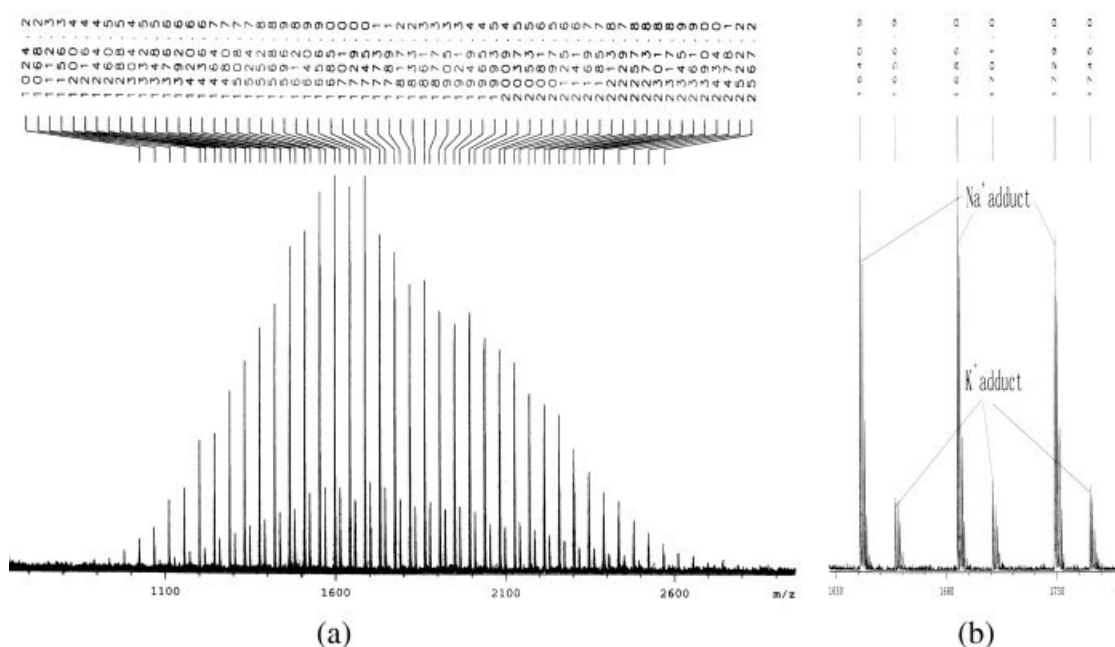
sideration of the  $M_n$  of EO monomer and both end groups, the  $M_n$  of each mPEG and diol PEG should be expressed by following equations separately:



where  $n$  is the degree of polymerization, 44.052, 31.034, 17.008, and 1.008 represent the molecular

weight of ethylene oxide, methoxy, hydroxyl, and hydrogen terminal, respectively.

Figure 4 shows the MALDI-ToF-MS spectrum of mPEG (sample 1 in Table I) appearing around 1740, which is close to the initial monomer/initiator ratio. The polydispersity calculated from MALDI-TOF spectra, 1.03, was narrower than that of GPC. This result is in accordance with literature results<sup>23</sup> and indicates effective initiation ability of potassium methoxide obtained from potassium naphthalene and methanol. The difference in the mass of each peak was roughly estimated as 44, indicating the signals were assignable to the molecular mass of the EO unit. The detected



**Figure 4** MALDI-ToF MS spectrum of the monomethoxyl poly(ethylene glycol).

signals refer to the homologs were 23 mass units larger than those calculated from eq. (1), which were to the sodium adduct of polymer bearing from the matrix eq. (3). For example, peaks detected at 1640.9, 1685.0, and 1729.0, which occurred near the maximum, correspond to the chain lengths of 36, 37, and 38. Small signals in lower intense appearing at 16 mass units larger than each higher signal are generally known as potassium adduct ions of the products, which originated from the initiator for the polymerization eq. (4).

Na<sup>+</sup> adducts of mPEG

$$m/z = 44.052n + 31.034 + 1.008 + 22.99 \quad (3)$$

K<sup>+</sup> adducts of mPEG

$$m/z = 44.052n + 31.034 + 1.008 + 39.098 \quad (4)$$

On the basis of the MALDI-ToF-MS spectrum, it was confirmed that mPEG with a methoxyl group at one end and hydroxyl group at the other end was successfully synthesized in narrow polydispersity. Moreover, there is almost no diol PEG exists in the obtained mPEG which was directly measured without any other purification.

From the earlier qualitative and quantitative analyses, the high purity and high molecular weight of the mPEG obtained was well confirmed. The result was primarily attributed to the high efficiency of the new initiating system, potassium naphthalene and methanol.

At first, new initiating system made it possible to initiate the polymerization of ethylene oxide under mild conditions such as room temperature and normal pressure. Under the circumstances, side reactions of the product such as the decomposition of mPEG, which proceeded more severely at high temperature, were well restrained. Moreover, in the initiation step of the reaction, the excess amount of potassium naphthalene, compared with the mole quantity of methanol, reacted with the trace of impurities in reaction system quantitatively and turned them to substances that are inert to the anionic polymerization under mild conditions. Since the change of color of the reaction system was an apparent symbol of the accomplishment of this quick reaction, potassium naphthalene was added drop by drop, and the addition stopped immediately when the color of reaction system turned from colorless to light green and did not fade out in 1 min. Therefore, when the initiation step was finished, there was no excess potassium naphthalene and impurities existed in reaction system except wanted active center derived from methanol. Consequently, at the end of the polymerization, only mPEG chains, trace of potassium salt and naphthalene

existed in the solvents, and the later two can be removed by filtration and precipitation, which implied the diol PEG formation was well avoided.

## CONCLUSIONS

The main objective of this study was to propose an easy access to monomethoxy poly(ethylene glycol) with high molecular weight and high purity. The strategy for synthesis of this material relied on an anionic polymerization process with potassium naphthalene as an initiating system under mild conditions.

Since potassium naphthalene and methanol was used as an initiating system, the transition of methanol could be determined easily through the change of the color of the reaction system. At the same time, a slight excess of potassium naphthalene appreciably more than stoichiometric amount can efficiently get rid of trace of impurities in the reaction system. Moreover, in the polymerization process at ambient temperature using THF as a solvent, about quantitative yields were obtained and the decomposition of mPEG could be effectively prevented, which often occurred at high temperature or when other kinds of catalysts are used.

It was concluded that the process developed in this study provided mPEG quantitatively (yield  $\geq 94.8\%$ ) with high molecular weight ( $M_n$ : 1–30 kDa) and low polydispersity ( $P_d \leq 1.07$ , GPC) by using potassium naphthalene and methanol as the initiating system, and the product showed satisfied high purity almost without diol PEG.

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