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Separation of polyethylene glycols and amino-terminated polyethylene glycols by high-performance liquid chromatography under near critical conditions

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ABSTRACT

The separation and characterization of polyethylene glycols (PEGs) and amino-substituted derivatives on common silica-based reversed-phase packing columns using isocratic elution is described. This separation is achieved by liquid chromatography under the near critical conditions (LCCC), based on the number of amino functional end groups without obvious effect of molar mass for PEGs. The mobile phase is acetonitrile in water with an optimal ammonium acetate buffer. The separation mechanism of PEG and amino-substituted PEG under the near LCCC on silica-based packing columns is confirmed to be ion-exchange interaction. Under the LCCC of PEG backbone, with fine tune of buffer concentration, the retention factor ratios for benzylamine and phenol in buffered mobile phases, α(benzylamine/phenol)-values, were used to assess the ion-exchange capacity on silica-based reversed-phase packing columns. To the best of our knowledge, this is the first report on separation of amino-functional PEGs independent of the molar mass by isocratic elution using common C18 or phenyl reversed-phase packing columns.

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1. Introduction

There has been a great interest in polyethylene glycol (PEG) and their conjugates of biologically active molecules in recent years [1–5], because PEG is one of the few polymers approved for clinical internal applications in humans by FDA (US Food and Drug Administration) [6]. The first step in preparation of functional PEGs is the chemical derivatization of end groups. Amino-substituted PEG derivatives are widely used among the various functionalized PEGs [7–9]. As the non-functionalized portion is an impurity that will not be reactive towards the target PEGylation, the fractions of non-, mono- and bi-functional PEGs need to be determined for raw materials in the production of polymer conjugates for pharmaceutical applications. However, there are limited numbers of publications on separation and characterization of PEGs and functional PEGs [10]. For example, separation of modified PEG methyl ether on a cyanopropyl column was reported with a gradient elution, with low detection sensitivity and poor shapes of peaks [11]. Barman et al. [12] described very good reversed-phase high-performance liquid chromatography (RP-HPLC) method for separation and quantitative determination of PEG impurities in two monofunctional PEG types, PEG methyl ether and PEG vinyl ether. Separation of PEGs and amino-substituted PEGs on a TSK-GEL G4000PW$_{XL}$ column was described, but molar mass effect of PEG backbone was not mentioned [13]. Tang et al. [14] demonstrated the separation and detection of bi-maleimide-PEG and mono-maleimide-PEG by RP-HPLC gradient elution, although molar mass effect of PEG backbone was observed clearly.

LCCC (liquid chromatography at critical conditions) has been proven to be especially effective in the analysis of functional polymers. In this mode, the retention volume of non-functional polymer becomes independent of molar mass, and this offers the opportunity to separate polymers with respect to their functionality [15–22]. However, the exact LCCC is not easy to obtain experimentally, and the LCCC conditions do not necessarily provide good separation of polymers with different end-groups [20]. RP-HPLC is by far the most widely used mode of HPLC, in which, stationary phases based on modified silica are most frequently used [23,24]. This kind of modification is seldom completed, the residual silanol groups may affect separation, especially in the analysis of basic compounds [25,26]. Cox and Stout had investigated the retention mechanisms of basic compounds on silica and C_8 reversed-phase packing, which were shown to be mainly ion exchange mechanism

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Table 1
Structures and abbreviations of the studied PEGs (RCH_2CH_2O-$[CH_2CH_2O]_n$-CH_2CH_2R').

Compound	R	R′	Avg. molar mass	Abbreviations
1	OH	OH	5500–7000	PEG 6k
2	NH_2	NH_2	6120 (M_w), 5828 (M_n)	$PEGDNH_2$ 6k
3	CH_3O	OH	5272 (M_w), 4677 (M_n)	MPEGOH 5k
4	CH_3O	NH_2	4957 (M_w), 4812 (M_n)	$MPEGNH_2$ 5k
5	OH	OH	3500–4500	PEG 4 K
6	OH	NH_2	4120 (M_w), 4079 (M_n)	$PEGNH_2$ 4k
7	NH_2	NH_2	4312 (M_w), 4106 (M_n)	$PEGDNH_2$ 4k
8	OH	OH	1900–2200	PEG 2k
9	NH_2	NH_2	2090 (M_w), 2029 (M_n)	$PEGDNH_2$ 2k

M_w: weight-average molar mass, M_n: number-average molar mass.

[24,27,28]. Euerby et al. [29] had evaluated a range of commercially available reversed-phase liquid chromatographic columns containing phenyl moieties and C18 moieties, using ion-exchange capacity (IEC) measured as the retention ratio $\alpha_{A/P}$, $k_{benzylamine}/k_{phenol}$, to assess the active amount of ion exchange sites on silica-based surface in the specific buffered mobile phase. The greater the values of $\alpha_{A/P}$, the larger amount of ion exchange sites, and the smaller the values, the less amount of ion-exchange sites [30–32].

To the best of our knowledge, no detailed studies on separation of amino-functional PEGs independent of the molar mass by isocratic LCCC using reversed-phase packing columns have been published yet. In this work, we describe a simple and suitable LC method for separation of PEGs and amino-functional PEGs independent of the molar mass by isocratic elution, taking advantage of ion exchange sites of common silica-based reversed-phase packing columns at appropriate LCCC conditions. Specially, the optimization of the buffer composition in the mobile phase will be investigated.

2. Materials and methods

2.1. Materials

Characteristics and abbreviations of the polymer samples used in this work are summarized in Table 1. PEG 2k, PEG 4k and PEG 6k were purchased from Sinopharm Chemical Reagent (Shanghai, China). MPEGOH 5k was purchased from Sigma-Aldrich (Steinheim, Germany). $PEGDNH_2$ 2k, $PEGNH_2$ 4k, $PEGDNH_2$ 4k, $MPEGNH_2$ 5k and $PEGDNH_2$ 6k were from Beijing Chemgen Pharma (Beijing, China). Ammonium acetate from Sinopharm Chemical Reagent (AR grade, Shanghai, China) was used. Acetonitrile was purchased from Tedia Company (HPLC grade, Fairfield, USA). Water from an Arium® pro UF purification system (Sartorius, Goettingen, Germany) was used. All chemicals and reagents were used as received.

2.2. Chromatographic separation and detection

Analytical chromatography was performed by an HPLC system assembled with Cometro 6000 LDI pump (South Plainfield, USA), a temperature controller and a Softa (Model 300 S, Burbank, USA) evaporative light scattering detector (ELSD) on a XB-Phenyl column (250 × 4.6 mm I.D., 5 μm particle size, 300 Å pore size, Welch Materials, Shanghai, China), a Shodex-C18 column (250 × 4.6 mm I.D., 5 μm particle size, 100 Å pore size, Showa Denko, Tokyo, Japan), or a TSK-GEL G4000PWXL column (300 × 7.8 mm I.D., 10 μm particle size, 500 Å pore size, Tosoh, Tokyo, Japan). PEGs and amino-substituted PEG derivatives do not possess any structural elements absorbing in the commonly applied UV region. ELSD is especially well-suited for the determination of any nonvolatile analyte [33]. For ELSD, air was used as carrier gas, the temperatures of the drift tube and spray chamber were set at 80 °C and 50 °C, respectively.

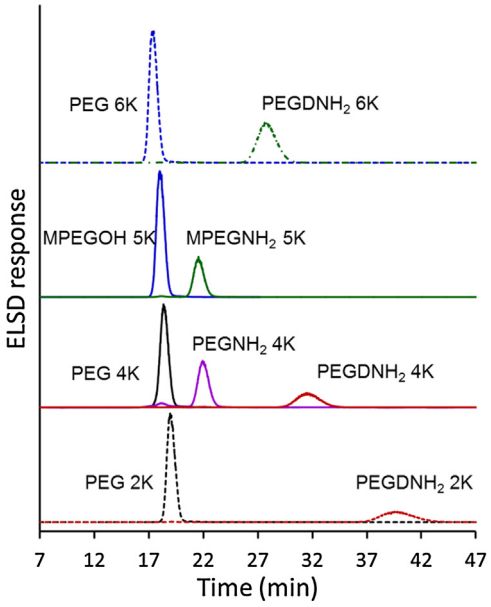

Fig. 1. The chromatograms of PEGs and amino-substituted PEG derivatives obtained on TSK-GEL G4000PW_{XL} column (mobile phase: water with 2.5 mmol/L CH_3COONH_4, flow-rate 0.5 mL/min, polymer concentration in water: 2 mg/mL).

The use of a volatile buffer salt was obligatory because of the ELSD, therefore CH_3COONH_4 was chosen as the buffer salt in this study.

The mobile phases were prepared by first dissolving appropriate amounts of CH_3COONH_4 in water for the desired concentration. The aqueous phase was filtered through a 0.22 μm HPLC filter before mixed with the organic phase and degassed prior to use by ultrasound. Samples were prepared in a 35:65 (volume ratio) mixture of acetonitrile and water at the concentration of 0.2 mg/mL (unless mentioned otherwise) and injected by a loop volume of 20 μL. Acetone was used as a t_0 marker. Data was collected using EZChrom Elite software. Data analysis was performed on a Microsoft EXCEL spreadsheet. Principal component analysis (PCA) was applied to classify the polymers according to the retention times measured for PEGs and amino-substituted PEG derivatives by MATLAB 2011a.

3. Results and discussion

Good separation of PEGs and the amino-substituted PEG derivatives with an average molar mass of 2000 and 3350 according to the ion-exchange interaction between amino-terminated group and the column packing surface was reported [13] on a TSK-GEL G4000PW_{XL} column, which is widely used in aqueous size-exclusion chromatography (SEC). However, the molar mass effect of PEGs was not mentioned. Actually, the size exclusion effect for PEG backbone was clearly observed, just as shown in Fig. 1. It can be seen that sample PEG 6k with higher molar mass was eluted earlier than samples PEG 2k and PEG 4k with lower molar mass. Bi-amino $PEGDNH_2$ 6k also had a shorter retention time compared to other bi-amino-substituted PEGs ($PEGDNH_2$ 4k and $PEGDNH_2$ 2k). Thus separation methods of PEGs and amino-substituted PEG derivatives avoiding the effect of molar mass are worthy to explore.

3.1. Effects of organic solvent composition on the retention of PEG

Liquid chromatography at critical conditions (LCCC) has been proven to be especially effective in the analysis of functional polymers. In this mode, the retention volume of non-functional polymer becomes independent of molar mass, and this offers the opportunity to separate polymers with respect to their functionality

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