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Short communication

Separation of polyethylene glycol oligomers using inverse temperature programming in packed capillary liquid chromatography

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Abstract

Inverse temperature programming in packed capillary liquid chromatography coupled to evaporative light-scattering detection has been used to resolve native polyethylene glycol (PEG) oligomers. The model compound, PEG 1000, was separated on a 300 mm×0.32 mm I.D. capillary column packed with 3 μm Hypersil ODS particles with acetonitrile–water (30:70, v/v) as mobile phase. The retention of the PEG oligomers increased with increasing temperature, different from what is commonly observed in liquid chromatography. The retention times of the oligomers were approximately doubled for each 25°C increment of the column temperature in the temperature range 30–80°C. The oligomers were almost unretained and co-eluted at a column temperature of 30°C. At 80°C a baseline separation of more than 22 peaks was obtained, but the last eluting peaks were severely broadened and all oligomers did not elute. When a negatively sloped temperature ramp from 80 to 25°C at −1.5°C/min was applied, the peak shapes were improved, additional peaks were detected and the analysis time was reduced by 48%. In the temperature programming mode, the intra-day precision of the retention times ranged from 0.5 to 5.8% (n=5).

Keywords: Inverse temperature programming; Packed capillary columns; Poly(ethylene glycol)

1. Introduction

Native polyethylene glycols (PEGs) are water-soluble and non-toxic polymeric compounds consisting of a varying number of repeating ethoxy units between two terminal hydroxyl groups. They are widely used in many technological and scientific fields, primarily in the synthesis of alkyl-substituted PEGs commonly used as non-ionic surfactants and emulsifiers, but also as model compounds for observation of intestinal permeability and adsorption in man [1], in studies of protein pharmacology, immunogenicity and enzymatic actions [2] and also as stationary phases in gas chromatography (GC). From an industrial point of view, chromatographic separation of PEG oligomers is important for product control. There are many applications requiring exact knowledge of the molecular heterogeneity, e.g., when the free hydroxyl groups are to be reacted with appropriate agents during the synthesis of inter-

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mediates [3]. Also, sufficient separation of all oligomers facilitates the calculation of the number-average molecular mass (M_n), the weight-average molecular mass (M_w) and the polydispersity index (M_w/M_n) [4]. Due to the non-volatile nature of native PEGs, liquid chromatography (LC) has been the most widely used technique for PEG characterization. Despite the fact that excellent resolution between PEG oligomers using LC have been reported by a number of authors [4–13], separation by normal-phase (NP) or reversed-phase (RP) LC are generally considered to be difficult due to the high polarity of the solutes [3]. In 1996, Rissler published an excellent review paper on NP- and RPLC separations of native PEGs [3], and concluded that polar stationary phases tend to give too strong solute–adsorbent interactions, necessitating the use of strong solvents like methanol or 2-propanol, which often results in co-elution of the oligomers. On the other hand, more hydrophobic materials tend to give limited retention due to weak solute–stationary phase interactions even when using aqueous mobile phases containing only small amounts of organic modifiers [3]. The low retention of native PEGs on RP materials must be considered in relation to their irregular temperature-dependent retention behavior. More than 20 years ago, Melander et al. observed increased retention of Carbowax 400 at elevated temperatures in RPLC [14]. Later, this irregular temperature-dependent retention behavior has only rarely been exploited for retention control of PEGs. For instance, Escott and Mortimer used an acetonitrile–water gradient and two serial-coupled 250×4.6 mm octadecyl-bonded silica (ODS) columns at 80°C for separation of a blend of PEGs ranging from M_w 200 to 3400 [7]. At elevated temperatures, the authors reported that the oligomer resolution was improved due to increased interaction between the oligomers and the stationary phase material, in addition to the reduced viscosity of the aqueous mobile phase, which allowed increased column length.

The normally broad molecular mass distribution in PEG products is challenging with regard to LC separations, often requiring gradient methods. Temperature programming has in several recent studies proven to be a suitable tool for gradient elution in LC [15–22]. The highly temperature-dependent retention behavior of PEGs on RP materials makes them in principal especially well suited for temperature programming. However, their irregular increased retention at elevated temperatures requires a negatively sloped temperature gradient for successful gradient action. To the authors' knowledge, Lochmüller et al. were the first to manipulate the column temperature actively for retention control of PEGs in RPLC [23]. They used a temperature-controlled water bath to produce a negatively sloped thermal gradient for the separation of PEG 26 000, PEG 46 000 and PEG 95 000 (each eluted as one peak) on a conventional-sized ODS column. However, the use of water baths to control the mobile phase temperature has limitations with regard to the steepness of the gradients, only allowing slow temperature gradients. The interest in miniaturized LC has increased recently, and packed capillary columns are highly suited for temperature programming due to their low heat capacity and negligible radial temperature gradients. Moreover, the small volumetric flow-rates and isocratic mobile phases utilized in temperature-programmed packed capillary LC facilitates the coupling to detection methods requiring effluent elimination, such as evaporative light-scattering detection (ELSD) and mass spectrometry (MS). The aim of the present study was to investigate the potential of using inverse temperature programming in packed capillary LC for the separation of PEG 1000 oligomers.

2. Experimental

2.1. Materials

The PEG 1000 standard was obtained from Macherey-Nagel (Düren, Germany). HPLC-grade acetonitrile was purchased from Acros Organics (Geel, Belgium), while water was deionized and glass-distilled in the laboratory. Hypersil ODS particles (3 μm, 100 Å) were obtained from Hypersil (Shandon, UK), and fused-silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA). Nitrogen and helium (99.998%) were purchased from AGA (Oslo, Norway).

2.2. Preparation of mobile phases and columns

The mobile phase (acetonitrile–water, 30:70, v/v)

was filtered through a 0.45-μm Minisart-RC25 filter from Sartorius (Gottingen, Germany) and degassed with helium for 10 min daily. The ODS particles were packed in a 30 cm long fused-silica capillary (320 μm I.D.×450 μm O.D.), using supercritical carbon dioxide as the slurry medium [24]. The column was conditioned with mobile phase for approximately 1 h at 80°C.

2.3. Instrumentation

A Merck–Hitachi LaChrom L-7100 pump (Merck, Darmstadt, Germany) was used to deliver a flow-rate of 3 μl/min throughout the study. Electrically actuated timed-split injection was performed with a Valco six-port Model C2 injection valve (Valco Instruments, Houston, TX, USA) located in a Famos Well Plate micro autosampler (LC Packings, Amsterdam, The Netherlands). The switching time was set to 11.0 s, giving an injection volume of 550 nl. A prototype Mistral 880 oven (Spark Holland, Emmen, The Netherlands) was used to control the temperature of the column. Detection was accomplished using a modified Varex Mark III ELSD system from Alltech Associates (Deerfield, IL, USA) [24–26]. The column inlet was connected to the injector with a 25 cm×50 μm I.D. fused-silica capillary, while the column outlet was connected directly to the nebulizer of the ELSD system with a 40 cm×20 μm I.D. fused-silica capillary. A Shimadzu C-R5A integrator

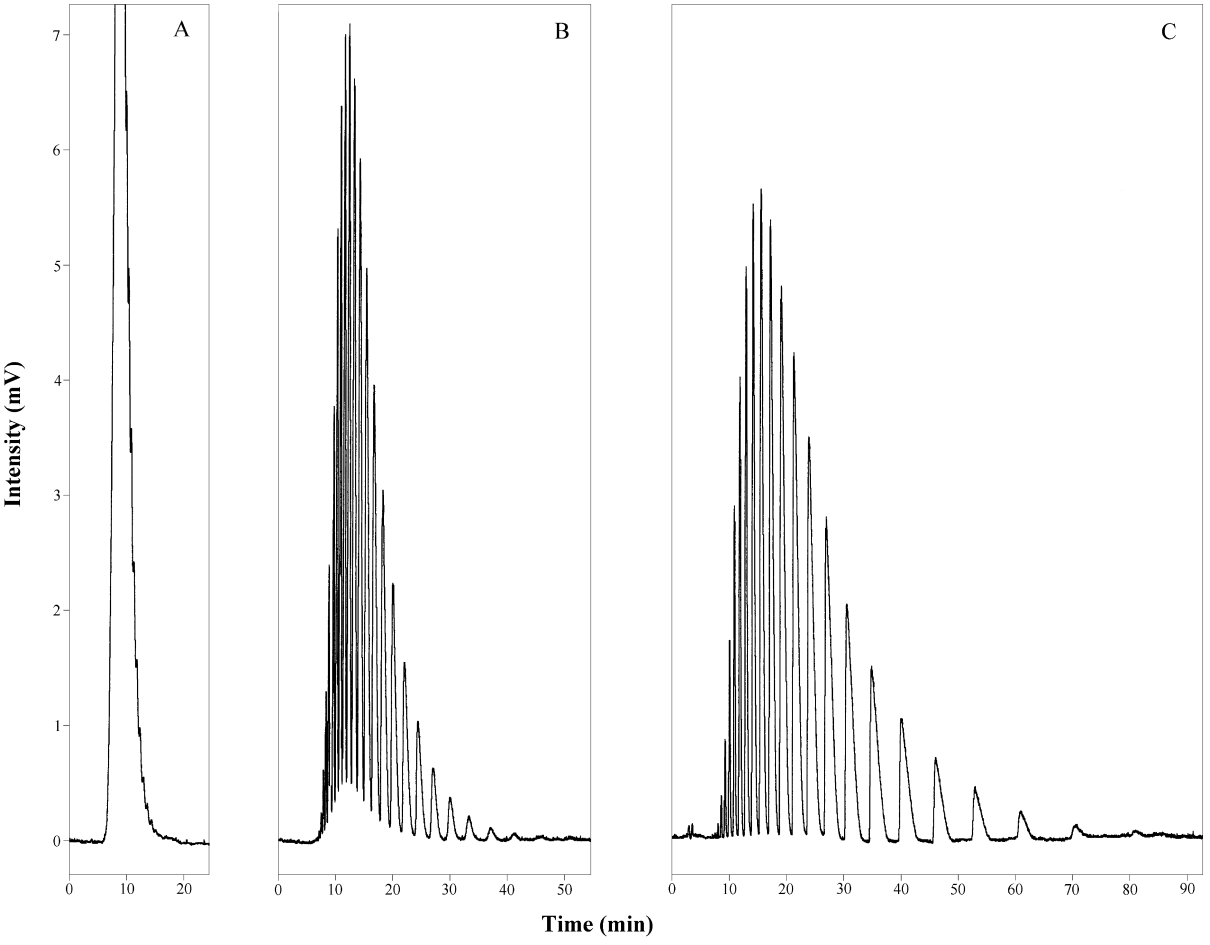

Fig. 1. Isothermal separation of 11 μg PEG 1000 (dissolved in mobile phase) at 30°C (A), 55°C (B) and 80°C (C). Column: 3 μm Hypersil ODS (30 cm×0.32 mm I.D.). Mobile phase: acetonitrile–water (30:70, v/v). Flow: 3 μl/min. Drift tube temperature: 55°C. Nebulizing gas: 2.20 l/min N_2.

(Shimadzu, Kyoto, Japan) was employed for data sampling.

3. Results and discussion

The effect of using isothermal higher temperatures for the separation of PEG 1000 on the Hypersil ODS column was initially examined in the temperature range from 30 to 80°C. Fig. 1A–C shows three isothermal separations of PEG 1000 at 30, 55 and 80°C, respectively, and the retention times of the PEG 1000 oligomers increased markedly with temperature. When using a column temperature of 30°C, all oligomers co-eluted with retention times less than 15 min, while a baseline separation was achieved when the temperature was increased to 80°C. In the latter, separation of at least 22 peaks was obtained, but the analysis time exceeded 80 min and the last eluting peaks were severely broadened and merged more and more with the baseline. Therefore, it was assumed that the high-M_w oligomers were not completely eluted from the column, revealing the need of a gradient LC method. In addition, the retention times of the PEG oligomers were approximately increased by a factor of two for each 25°C increment in the investigated temperature range. This is the opposite of what is normally observed when varying the column temperature in RPLC [27]. The increased retention of native PEGs at elevated temperatures in RPLC has previously been attempted explained in the literature by a combination of two factors. Primarily, native PEGs are water-soluble polymers, but the solubility decreases markedly with the temperature of the aqueous solution [28]. This inverse solubility-temperature relationship correlates to the inverse retention behavior observed in RPLC. Secondly, PEG molecules containing more than nine repeating units ($M_w > 500$) are coiled in solution, and the degree of coiling is temperature-dependent. An increase in temperature will therefore enlarge the three-dimensional structure of the molecules and most likely increase the interaction between the PEGs and the stationary phase [14].

In the case of isocratic LC separations of oligomeric compounds, as used in the present investigations, Martin's rule states that the natural logarithm of the retention times of individual oligomers is proportional to the degree of polymerization [29]. Likewise, the retention times of the PEG 1000 oligomers in Fig. 1C increased exponentially with size, resulting in long analysis time and broad peak shapes of the latest eluting peaks. However, when a negatively sloped temperature ramp from 80 to 25°C at −1.5°C/min was applied, more than 26 peaks were detected in approximately 43 min (Fig. 2), which corresponds to a 48% reduction in analysis time. In addition, the peak shapes were markedly

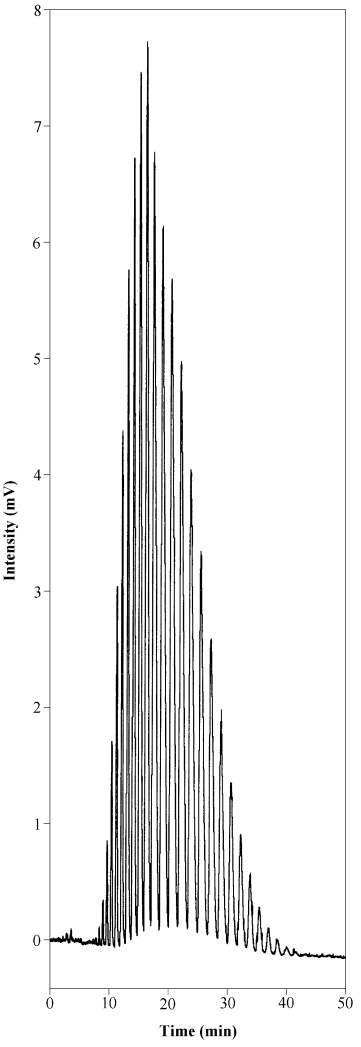

Fig. 2. Temperature-programmed separation of 11 μg PEG 1000 (dissolved in mobile phase). Temperature program: 80°C for 6 min, then −1.5°C/min to 25°C, otherwise the same conditions as in Fig. 1.

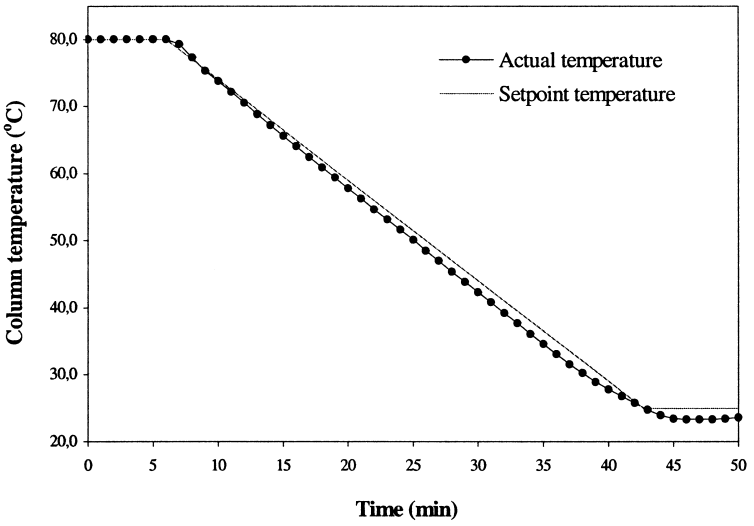

Fig. 3. The average oven temperature of three subsequent programs produced by the prototype column oven (RSD<0.2%) versus the setpoint values.

improved. The intra-day precision of the retention times of the different PEG oligomers ranged from 0.5 to 5.8% ($n=5$) in temperature programming mode. In Fig. 3, the average oven temperature of three subsequent programs produced by the prototype oven is depicted. Although the intra-day precision was less than 0.2% ($n=3$) the actual temperatures deviated slightly from the setpoint values.

Unfortunately, the combination of elevated temperatures and aqueous mobile phases reduces the lifetime of silica-based stationary phases markedly. Nevertheless, the Hypersil ODS column was used in the temperature range from 30 to 80°C for approximately 1 month without a significant decrease in the retention times of the PEG 1000 oligomers. This indicates relatively slow dissolution of the stationary phase even at the highest temperatures applied.

4. Conclusions

The potential of using inverse temperature programming in packed capillary LC for the separation of PEG oligomers has been demonstrated. Inverse temperature programming was found to be superior to isothermal separations in terms of speed of analysis, peak shapes and number of peaks detected. In addition, it is important to note that the small volumetric flow of isocratic mobile phase utilized in the developed chromatographic method is expected to be highly compatible with MS and thus exhaustive oligomer characterization with respect to their structural features is achievable.

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