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Determination of polyethylene glycol in low-density polyethylene by large volume injection temperature gradient packed capillary liquid chromatography

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Abstract

Polyethylene glycol (PEG) 20 000 in low-density polyethylene has been determined using column switching and inverse temperature programming in reversed-phase packed capillary liquid chromatography with evaporative light scattering detection. PEG 20 000 was extracted into water from the polyethylene dissolved in toluene and PEG 35 000 was added as an internal standard (I.S.). The samples in aliquots of 100 μl were reconcentrated on the enrichment column using a loading mobile phase of acetonitrile–water (3:97, v/v) at a flow-rate of 75 μl/min for 3 min, then back-flushed and separated on the analytical column with acetonitrile–THF–water (40:5:55, v/v) as mobile phase. The column temperature was reduced from 68 to 55 °C with a ramp of −1.5 °C/min, held constant for 3 min and then reduced further to 45 °C with a −1.5 °C/min ramp and kept constant for 1 min. The analysis runtime was 20 min. The recovery of PEG 20 000 was determined to 65.1% with 2.8% RSD and the mass limit of detection of PEG 20 000 was 1.25 μg. The within-assay and between day precision of the retention times of both PEG 20 000 and PEG 35 000 displayed RSD of less than 1.1% (n=9), while the overall area ratio RSD of 100 μg/ml PEG 20 000 over PEG 35 000 was 1.3% (n=9). The method was linear within the investigated concentration range 25–125 μg/ml (R^2=0.9983). In addition, a mixture of PEG 4000, 8000, 10 000, 20 000 and 35 000 was analysed on the system to demonstrate the possibility of analysing several PEGs in a sample with the use of temperature gradient elution.

Keywords: Injection methods; Large-volume injection; Temperature gradients; Column switching; Packed capillary column; Poly(ethylene glycol); Polyethylene

1. Introduction

Most synthetic polymeric materials contain additives in order to improve the physical properties and extend the lifetime of the polymers. Additives such as UV stabilizers, antioxidants, antistatic agents, metal deactivators and antiblock agents are often used [1]. Polyethylene glycols (PEGs) are compounds consisting of a varying number of polymerised ethylene oxide (EO) units with two free α,ω-

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hydroxyl end groups. Native PEGs are non-toxic and water-soluble and have been widely used in technical as well as pharmaceutical and biochemical industries. PEGs and their derivatives have been used as non-ionic surfactants, wetting agents in laundry and industrial cleaners and as emulsifiers in pharmaceutical preparations. Low-molecular-mass PEGs have been used as intestinal permeability probes [2], mainly due to their low toxicity and the facts that they are not metabolised by intestinal bacteria and almost completely excreted in urine [3]. PEGs and their derivatives also serve as important ingredients in the food and perfume industries. In the polymer industry PEGs of various sizes have been employed for their properties as water treeing retardant in insulation materials for electric cables, to avoid water to breakthrough the insulation material and cause degradation of the polymer in contact with the electrical field [4].

Traditionally, PEGs have been chromatographed by size exclusion chromatography [5–7], but gradually reversed-phase systems have taken over, particularly for oligomer separation of the lower molecular masses [8–18]. In general, PEG 3000 marks the upper limit for oligomer resolution [12], although capillary electrophoresis have separated PEGs up to M_w=5000 after derivatization [19]. One of the few examples of separation of larger molecular mass PEGs was demonstrated by Lochmüller et al. [11], which showed that the retention process for PEGs is endothermic, with positive changes in enthalpy. This fact was utilized later to obtain baseline separation of all the oligomers of PEG 1000 by a negative temperature gradient, with improved resolution compared to the resolution obtained by a solvent gradient [17]. In a recent paper, Cho et al. [20] studied the retention mechanism of poly(ethylene oxide) in reversed-phase and normal-phase chromatography and concluded that a hydrophobic entropy effect was responsible for the increase in retention with increasing temperature.

The purpose of this study was to develop a model for the quantitative determination of PEGs in low-density polyethylene (LDPE). The model would include extraction of PEGs into water and loading the extract onto a microbore reversed-phase enrichment column. Combining preconcentration using a column switching system and separation by a temperature gradient on a packed microbore reversed-phase analytical column and detection by evaporative light scattering would allow method automation in the future. To illustrate the possibility of analysing samples with varying PEG sizes, a mixture of five different PEGs with molecular masses of 4000–35 000 were examined. The emphasis has been on the reconcentration and separation of groups of relatively high-molecular-mass PEGs, not separation of individual oligomers.

2. Experimental

2.1. Materials and chemicals

The PEG 4000, 8000 and 10 000 standards were obtained from Clariant (Gendorf, Germany) and the PEG 20 000 (with the molecular mass (M_r) of 16 000–24 000 Dalton) and 35 000 (M_r~35 000 Dalton) were purchased from Fluka (Buchs, Switzerland). Tetrahydrofuran (THF) (p.a. grade) was purchased from SDS (Peypin, France) and the toluene (p.a. grade) was obtained from Merck (Darmstadt, Germany). The water used was purified on a Milli-Q system from Millipore (Billerica, MA, USA). HPLC-grade acetonitrile was purchased from Rathburn (Walkerburn, UK). The columns used were packed in house in glass-lined stainless steel obtained from SGE (Kiln Farm, Milton Keynes, UK) with Kromasil C_{18} 100 Å 3.5-μm d_p particles purchased from Eka Chemicals (Bohus, Sweden). The LDPE sample containing 2500 ppm PEG 20 000 was prepared by Borealis (Stathelle, Norway).

2.2. Chromatographic system

Glass-lined stainless steel enrichment- and analytical-columns of 0.5 mm I.D. and lengths of 3 and 10 cm, respectively, were packed with Kromasil C_{18} 100 Å 3.5-μm d_p particles by a liquid slurry technique developed in house. A Merck Hitachi (Darmstadt, Germany) L-7110 isocratic LC pump served as the sample loading pump and a Shimadzu (Kyoto, Japan) LC-10 AD νp isocratic pump was used as the mobile phase delivery system. The Endurance autosampler was equipped with a Rheo-

dyne (Cotati, CA, USA) six-port injector and a 100-μl sample loop, and a modified Mistral column oven was programmed for temperature gradients, both the Endurance and the Mistral were from Spark Holland (Emmen, The Netherlands). Fig. 1 illustrates the schematic set up of the switching system. The analytical column was mounted in the oven, but both valve 1, which was mounted on the Endurance autosampler, and valve 2, a six-port injector from Valco Instruments (Houston, TX, USA) with the enrichment column were placed outside the column oven. An Alltech Varex MKIII (Deerfield, IL, USA) evaporative light scattering detector (ELSD) was used for detection. The detector had previously been modified in order to allow operations at low flow-rates [21,22]. The nebulizer gas flow was kept at 2.2 l/min and the drift tube temperature was 105 °C throughout the study. The loading mobile phase was acetonitrile–water (3:97, v/v) and the analytical mobile phase was acetonitrile–THF–water (40:5:55, v/v). Both the loading mobile phase and the analytical mobile phase were degassed with helium for 5 min prior to use. The loading flow-rate was 75 μl/min and the analytical flow-rate was 20 μl/min.

2.3. Standard solutions

PEG 35 000 was used as an internal standard (I.S.) at a concentration of 100 μg/ml in water. This I.S. solution was used to dissolve and dilute the standard solution of PEG 20 000 in order to maintain a concentration of 100 μg/ml of I.S. in the standards. A stock solution of 4 mg PEG 20 000 dissolved in 4 ml of I.S. solution was prepared in an ultrasonic bath for 5 min. Five concentration levels in the range of 125–25 μg/ml PEG 20 000 were prepared from this stock solution by diluting with the I.S. solution.

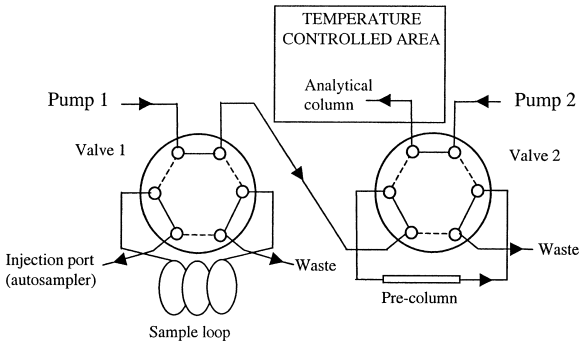

Fig. 1. A schematic diagram of the column switching system.

2.4. Extraction and separation of PEGs

Approximately 1 g of the polyolefin sample was accurately weighed into a 125-ml flask and 2.5 mg of PEG 35 000 was added as the internal standard. Then, 25 ml toluene was added and the sample was heated and refluxed with stirring at 110 °C for 20–30 min until the polymer was dissolved. The solution was allowed to cool while stirring for 5 min before adding 25 ml of water and stirred for an additional 10 min. The mixture was then transferred to a separatory funnel. The toluene and water phases were separated, the water phase was removed, passed through a 0.2-μm filter and 100-μl aliquots of the filtrate were loaded by the autosampler and pump 1 onto the enrichment column, back-flushed with mobile phase from pump 2 into the analytical column and separated with a temperature gradient, either continuous or step-wise.

3. Results and discussion

3.1. Mobile phase and temperature considerations

The fact that, in reversed-phase liquid chromatography, the retention of native PEGs increases with increasing column temperatures, as opposed to the normal trend where the retention of solutes is decreased, was demonstrated by Lochmüller et al. [11]. According to Melander et al. [8] this phenomena can be explained by the conformational changes of PEGs at different temperatures. PEG molecules greater than nine repeating units (M_w>500) are coiled in solution at ambient temperature. This coiling is temperature dependent, and as the temperature increases the degree of coiling will be reduced, the molecular surface area will increase, hence most likely increase the interaction between the C_{18} ligands of the stationary phase and the PEG molecules and therefore increase the retention. Furthermore, the solubility of PEGs in aqueous solutions decreases with increased temperature and this corre-

lates with the increase in retention as the temperature increases [23].

Lochmüller et al. [11] used a temperature controlled circulating water bath with both heating and cooling units for column temperature regulation in their study. Alternatively three individual heated or cooled aluminium blocks were employed as column holders at the inlet, the middle and the end of the column. Thus, a thermal gradient could be generated along the column [11]. By using a column oven with both a heating and a cooling function the analytical system will be much more flexible with regards to temperature programming during the analysis. There will not be a temperature gradient along the column, and the temperature may be used to improve the separation and to some extent the peak shapes of a mixture of different PEGs. From a practical point of view it is known that commonly used silica-based C_{18} phases rapidly degrade in aqueous mobile phases at constant temperatures above 100 °C. At temperatures below 70 °C few problems are experienced with most reversed-phase materials [24]. Andersen et al. [17] used temperature gradients up to 80 °C for a month without significant changes of the t_R of the PEG 1000 oligomers. Therefore, to obtain optimum selectivity and at the same time avoid extensive degradation of the silica-based stationary phase the column temperatures used did not exceed 75 °C.

The enrichment column was placed outside the column oven. Due to the fact that the sample extract was dissolved in water, it was easy to reconcentrate the solutes on the enrichment column without the use of temperatures different from ambient. To avoid collapsing of the C_{18} surface groups of the stationary phase in pure water, the aqueous loading mobile phase contained 3% acetonitrile (v/v).

The loading flow-rate was tested at 20, 50, 75 and 100 μl/min ($n=3$) without any sign of sample breakthrough. Flow rates greater than 100 μl/min were not tested due to the pressure limitations of the loading pump. Even though using a loading flow-rate of 100 μl/min would mean a loading time of 2 min, the sample was loaded onto the column for 3 min at 75 μl/min throughout the remaining experiments, mainly to avoid working close to the pressure limit of the pump. At a loading flow-rate of 75 μl/min the peak area RSD of PEG 20 000 and PEG 35 000 (I.S.) were 0.5 and 1.4%, respectively. The overall PEG 20 000 area RSD of all the different flow-rates ($n=12$) was 2.2%

By using elevated column temperatures for the chromatographic analysis, the viscosity of the mobile phase was reduced, allowing the use of a higher analytical flow-rate; hence the flow-rate was set to 20 μl/min, the column backpressure varied from 11 to 25 MPa from the start to the end of the analysis.

3.2. Separation of a mixture of different sized PEGs

In order to demonstrate the possibility of identifying PEGs of different sizes in the same sample, a standard mixture of 5 PEGs with M_w ranging from 4000 to 35 000 Dalton was analysed. An inverse temperature program was employed. Fig. 2 displays the separation of PEG 4000, 8000, 10 000, 20 000 and 35 000. The concentrations of the different PEGs

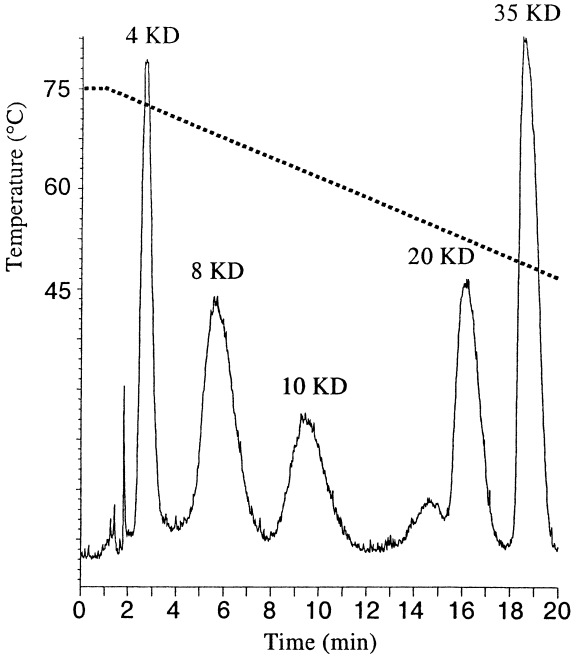

Fig. 2. Temperature programmed separation of PEG 4000, 8000, 10 000, 20 000 and 35 000 Dalton. Temperature program: 75 °C for 1 min, then reduced to 47 °C with a −1.5 °C/min ramp. The flow-rates used were 75 μl/min for the sample loading and 20 μl/min for the chromatographic separation.

were 14, 25, 24, 26 and 28 μg/ml, respectively. The PEG 20 000 peak shows a distinct shoulder on the chromatogram (Fig. 2), this is due to the oligomer distribution of the sample. This shoulder is apparent in both the standard and the sample chromatograms.

3.3. Determination of PEG 20 000 in LDPE

The sample preparation was based on a method used by Borealis, where 2 g of LDPE with PEG 20 000 was dissolved in 100 ml toluene, followed by extraction into 100 ml water in a separatory funnel. The water phase was evaporated to dryness, re-dissolved in 5 ml water and analysed by size exclusion chromatography and refractive index detection.

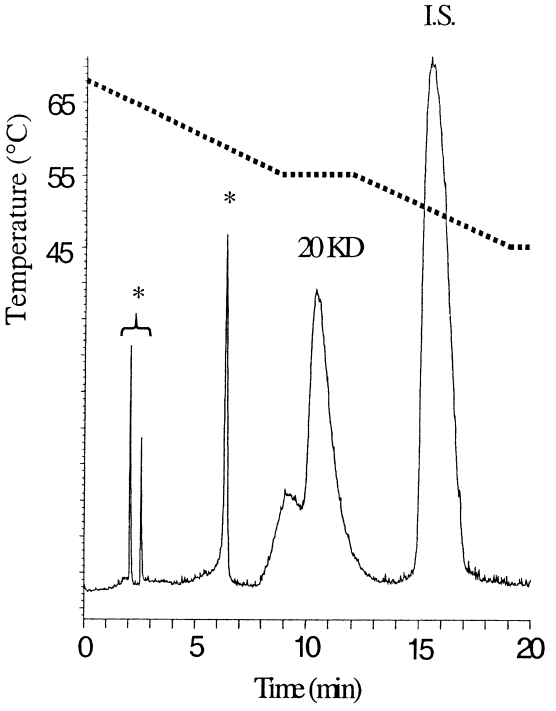

Fig. 3. Chromatogram of the analysis of a 100-μl aliquot of the LDPE sample extract. Separation of PEG 20 000 and PEG 35 000 Dalton (I.S.). Temperature program: the initial temperature was 68 °C then reduced to 55 °C with a −1.5 °C/min ramp, kept constant for 3 min, and reduced further to 45 °C with a −1.5 °C/min ramp and kept constant for 1 min. The flow-rates used were 75 μl/min for the sample loading and 20 μl/min for the chromatographic separation. Peaks marked with asterisks are impurities from the sample.

In the current method the amount of LDPE was 1 gram and the amount of both water and toluene was reduced to 25 ml. With online preconcentration of the solutes on the enrichment column prior to the analysis by micro-LC–ELSD, direct injection of the water phase became possible, avoiding the evaporation step.

The extraction of PEG 20 000 from LDPE was performed with a recovery of 65.1%, with 2.8% RSD (n=12). Aliquots of the extracts were loaded onto the enrichment column at 75 μl/min for 3 min. In order to improve the resolution between PEG 35 000 and PEG 20 000 even more than in Fig. 2, a stepwise temperature program was developed. This provided base line separation of higher concentrations of PEG 20 000 and PEG 35 000. The separation and the chromatographic conditions are displayed in Fig. 3.

The mass limit of detection (mLOD) of PEG 20 000 was determined to 1.25 μg, and the concentration LOD (cLOD) was 12.5 μg/ml. The within-assay and between-day precision of retention times for a standard solution of 100 μg/ml of both PEG 20 000 and PEG 35 000 displayed RSDs less than 1.1% (n=9). The overall RSD of the peak area ratio of 100 μg/ml PEG 20 000 and I.S. was 1.3% (n=9). The method was linear within the investigated concentration range 25–125 μg/ml (R^2= 0.9983).

4. Conclusions

Temperature gradient LC have previously been demonstrated to be able of separating individual oligomers of low-molecular-mass PEGs. This paper has shown that temperature gradients can also be used as an alternative to solvent gradients in group separation of larger molecular mass PEGs. The mass limit of detection obtained with the microbore-LC system (1.25 μg) was improved compared to the 5 μg detection limit (for PEG 1000) reported previously with conventional HPLC–ELSD [10]. After extraction of PEGs from LDPE into water, a combination of automated reconcentration and column switching allowed determination of PEGs at low ppm levels with good precision.

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References

[1] N.S. Allen, Chem. Soc. Rev. 15 (1986) 373.
[2] R.W.R. Baker, J. Ferrett, J. Chromatogr. 273 (1983) 421.
[3] C. Fakt, M. Ervik, J. Chromatogr. B 700 (1997) 93.
[4] Borealis, personal communication (2002).
[5] H. Engelhardt, D. Mathes, J. Chromatogr. 185 (1979) 305.
[6] R. Murphy, A.C. Selden, M. Fisher, E.A. Fagan, V.S. Chadwick, J. Chromatogr. 211 (1981) 160.
[7] O. Güven, Br. Polym. J. 18 (1986) 391.
[8] W.R. Melander, A. Nahum, C. Horváth, J. Chromatogr. 185 (1979) 129.
[9] T. Meyer, D. Harms, J. Gmehling, J. Chromatogr. 645 (1993) 135.
[10] K. Rissler, H.-P. Künzi, H.-J. Grether, J. Chromatogr. 635 (1993) 89.
[11] C.H. Lochmüller, M.A. Moebus, Q. Liu, C. Jiang, J. Chromatogr. Sci. 34 (1996) 69.
[12] K. Rissler, Chromatographia 49 (1999) 615.
[13] B. Trathnigg, M. Kollroser, A. Gorbunov, A. Skvortsov, J. Chromatogr. A 761 (1997) 21.
[14] B. Trathnigg, M. Kollroser, J. Chromatogr. A 768 (1997) 223.
[15] B. Trathnigg, B. Maier, A. Gorbunov, A. Skvortsov, J. Chromatogr. A 791 (1997) 21.
[16] R.E.A. Escott, N. Mortimer, J. Chromatogr. 553 (1991) 423.
[17] T. Andersen, P. Molander, R. Trones, D.R. Hegna, T. Greibrokk, J. Chromatogr. A 918 (2001) 221.
[18] A. Gorbunov, A. Skvortsov, B. Trathnigg, M. Kollroser, M. Parth, J. Chromatogr. A 798 (1998) 187.
[19] J.P. Barry, D.R. Radtke, W.J. Carton, R.T. Anselmo, J.V. Evans, J. Chromatogr. A 800 (1998) 13.
[20] D. Cho, S. Park, J. Hong, T. Chang, J. Chromatogr. A 986 (2003) 191.
[21] R. Trones, T. Andersen, T. Greibrokk, J. High Resolut. Chromatogr. 22 (1999) 283.
[22] R. Trones, T. Andersen, I. Hunnes, T. Greibrokk, J. Chromatogr. A 814 (1998) 55.
[23] F.E. Bailey, J.V. Koleske, Poly(ethylene Oxide), Academic Press, New York, 1976.
[24] T. Greibrokk, T. Andersen, J. Chromatogr. A (2003) in press.