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Liquid chromatography of polymers under limiting conditions of adsorption IV. Sample recovery

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Abstract

The high performance liquid chromatography of polymers under limiting conditions of adsorption (LC LCA) separates macromolecules, either according to their chemical structure or physical architecture, while molar mass effect is suppressed. A polymer sample is injected into an adsorption-active column flushed with an adsorption promoting eluent. The sample solvent is a strong solvent which prevents sample adsorption. As a result, macromolecules of sample elute within the zone of their original solvent to be discriminated from other, non-adsorbing polymer species, which elute in the exclusion mode. LC LCA sample recovery has been studied in detail for poly (methyl methacrylate)s using a bare silica gel column and an eluent comprised toluene (adsorli) and tetrahydrofuran (desorli). Sample solvent was tetrahydrofuran. It was found that a large part of injected sample may be fully retained within the LC LCA columns. The amount of retained polymer increases with decreasing packing pore size and with higher sample molar masses and, likely, also with the column diameter. The extent of full retention of sample does not depend of sample volume. An additional portion of the injected desorli sample solvent (a tandem injection) does not fully eliminate full retention of the sample fraction and the reduced recovery associated with it. The injected sample is retained along the entire LC LCA column. The reduced sample recovery restricts applicability of many LC LCA systems to oligomers and to discrimination of the non-adsorbing minor macromolecular components of complex polymer mixtures from the adsorbing major component(s). The full retention of sample molecules within columns may also complicate the application of other liquid chromatographic methods, which combine entropic and enthalpic retention mechanisms for separation of macromolecules.

Keywords: Chromatography of polymers; Barrier methods; Limiting conditions of adsorption; Sample recovery

1. Introduction

The liquid chromatography of polymers under limiting conditions of adsorption (LC LCA) belongs to the "barrier" methods of high performance liquid chromatography of polymers (polymer HPLC) [1–8]. It utilizes intrinsic difference between mobilities of small molecules in the HPLC eluents and the sample macromolecules. The former species permeate the packing pores and their transport along the column is slow. On the contrary, macromolecules travel along the column much faster because they are partially or fully excluded from the packing pores. If the eluent promotes adsorption of macromolecules on an active column packing, it is 'an adsorli', and sample is injected in a strong solvent, which prevents its adsorption, in 'a desorli', the system may exhibit the LC LCA behavior. Fast moving macromolecules accumulate on the leading edge of the slowly moving desorli zone without breaking into the bulk volume of adsorli eluent. As result,

Abbreviations: ELSD, evaporative light scattering detector; HPLC, high performance liquid chromatography; *M*, molar mass; PMMA, poly (methyl methacrylate); PS, polystyrene; LC LCA, liquid chromatography of polymers under limiting conditions of adsorption; RI, refractive index; SEC, size exclusion chromatography; THF, tetrahydrofuran; UV, ultraviolet; v_i, injected volume; $v_{i,max}$, maximum injected volume; $v_{i,min}$, minimum injected volume; $v_{i,s}$, safe injected volume; V_0, interstitial volume of column; V_M, volume of mobile phase within column; V_R, retention volume

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polymer species elute from the column irrespectively of their molar mass in the form of a narrow, focused peak. If the sample contains both adsorbing and non-adsorbing species, the latter leave the zone of their original solvent and elute in conventional size exclusion chromatography (SEC) mode at lower retention volume. In this way, polymer species possessing different adsorption properties can be discriminated, and independently characterized, for example using an on-line SEC column.

The role of experimental conditions in LC LCA was studied in several different systems [3–6]. When an appropriately chosen column packing, eluent and sample solvent for a given polymer were combined, the following behavior was observed:

(a) Retention volumes of macromolecules were independent of their size in a very broad molar mass range.
(b) Narrow, focused peaks with similar retention volumes were generated for different injected sample volumes and sample concentrations. LC LCA columns packed with common silica gel sorbents tolerated large sample sizes. Up to about 40% of the total column volume and at least up to 100 mg ml^{-1} concentration could be injected into the column of 250 mm × 4 mm size. This is important for tracing and characterization of minor components (even below 1%) in polymer blends applying two-dimensional liquid chromatography.
(c) Narrow-bore, long and efficient columns gave the best results.
(d) The effect of temperature was insignificant for poly (methyl methacrylate)s (PMMA) eluted from bare silica gel in the zone of tetrahydrofuran applying tetrahydrofuran/toluene 35/65 (w/w) eluent.

However, reduced sample recovery was observed in some LC LCA systems using a narrow pore column packing [7]. It was, therefore, of interest to study in detail the problem of sample recovery in LC LCA.

2. Experimental

2.1. Chromatograph

The chromatograph consisted of following components. A Model 510 isocratic pump (Waters, Milford, MA, USA) was employed at the flow rate of 1 ml min^{-1} in most experiments. For extremely low flow rates the isocratic pump Model 64 was used (Knauer, Berlin, Germany) equipped with a micro pump head. The actual flow rate was checked by a burette. An autosampler MIDAS (Spark Holland, Emmen, The Netherlands) was applied for sample injection. Columns were kept at constant temperature in an air oven (Knauer, Berlin, Germany) or in a custom made oven with a duplex wall connected to a water thermostat. The temperature of most experiments was 30 °C. The experimental arrangement of the chromatograph is shown in Fig. 1. For selected recovery

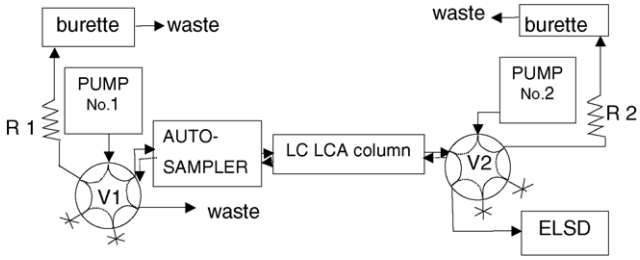

Fig. 1. Experimental arrangement of the LC LCA chromatograph employed. R1 and R2 are hydrodynamic resistors (capillaries), V1 and V2 are the switching valves, ELSD is an evaporative light scattering detector. For details see the text.

assessments, the backflush experiments were applied. Using valves V1 and V2 (Institute of Chemical Technique Fundamentals, Academy of Sciences of Czech Republic, Prague, Czech Republic), after a standard LC LCA experiment had been completed, the column was slowly filled with certain volume of tetrahydrofuran (THF) desorli from the pump no. 2. After a pre-selected time of desorption, the valves V_1 and V_2 were operated again and elution in the original direction was restarted. Evaporative light scattering detectors (ELSD) (Models PL 960 and PL 1000 from Polymer Laboratories, Shropshire, Church Stretton, UK) were used for detection of polymer probes. PMMA eluted within the sample solvent zone and therefore the conventional refractive index (RI) detector was inapplicable. Due to the problems with the quantitative processing of the ELSD response [9,10], the experiments were evaluated only semi-quantitatively. An ultraviolet (UV) detector (Laboratory Instruments Works, Prague, Czech Republic) functioning at 254 nm wavelength was applied for detection of polystyrenes (PS) in a selected series of experiments. Peaks of *n*-hexane used for determination of the column efficiencies and the volume of the mobile phase within columns were monitored by an RI detector Model 198 (Knauer, Berlin, Germany). Experimental data were processed with Baseline (Waters, Milford, MA, USA) or Chroma (Chroma, Graz, Austria) PC softwares.

2.2. Stationary and mobile phases

Bare silica gels were chosen for this study to avoid extensive enthalpic partition and interphase adsorption of macromolecules in favor of bonded stationary phases [11]. Thus, adsorption of macromolecules onto surface silanols of silica gel was anticipated the main enthalpic retention mechanism coupled with the exclusion retention mechanism. Narrow pore (6 nm) spheroidal silica gels Silpearl (Glasswork, Votice, Czech Republic) with particle diameters 7 and 10 μm, were applied in most experiments. Macroporous 10 μm spherical silica gels with pore diameters 10, 30 and 60 nm (Biospher from Labio, Prague, Czech Republic), as well as 100 nm (Separon SGX 1000 from Tessek, Prague, Czech Republic) were utilized for evaluation of the