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Retention of small molecules on polymethacrylate monolithic capillary columns

Michaela Chocholoušková, Martina Komendová, Jiří Urban*

Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Studentská 573, Pardubice 532 10, Czech Republic

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ABSTRACT

In this paper, the concentration of N-isopropylacrylamide in the polymerization mixture has been varied to prepare several polymethacrylate monolithic capillary columns. Polymer monoliths combining N-isopropylacrylamide with zwitterion monomer, as well as various dimethacrylate crosslinking monomers have been prepared and characterized. Uracil, thiourea, phenol, toluene, ethylbenzene, propylbenzene, and butylbenzene have been used to characterize retention of prepared capillary columns in the mobile phases with 40–95% of acetonitrile and at working temperatures ranging from 25 to 60 °C. By an optimization of six-parameter polynomial models we have found that the retention of small molecules is affected mainly by the concentration of the acetonitrile in the mobile phase with very low contribution of working temperature and combined effect of acetonitrile concentration and temperature. Concentration of the mobile phase controlled also enthalpy of the retention. On the other hand, entropic contribution was almost insensitive to the change of the mobile phase composition, especially for mobile phases containing more than 60% of acetonitrile.

1. Introduction

One of the advantages of polymer monoliths is versatility of their properties that can be controlled by polymerization mixture composition, polymerization reaction conditions, and post-polymerization modification [1,2]. A selection of functional monomer in the polymerization mixture is the easiest way how to control surface chemistry and retention properties of prepared stationary phases [3]. N-isopropylacrylamide (NIPAAm) belongs to the family of monomers for the preparation of thermally responsive materials that change their solvation and associated polarity based on external temperature: when heated in an aqueous solution over 32 °C (lower critical solution temperature, LCST) it reversibly loses over 90% of volume and changes from a swollen hydrated (hydrophilic) monomer to a shrunken dehydrated (hydrophobic) one [4]. Unfortunately, this behavior is observed only in pure water and presence of organic solvent eliminates the effect. Nevertheless, N-isopropylacrylamide has been used to prepare stationary phases with retention controlled by an external temperature [5–13], thermally responsive extraction units [14,15] or drug delivery devices [16].

The internal pore surface of rigid polymer monoliths has been modified by poly(N-isopropylacrylamide) and used for an isocratic hydrophobic interaction chromatography of proteins controlled by an external temperature [5]. Poly(N-isopropylacrylamide) grafted silica has been used for the separation of peptides using 0.2 M NaCl solution as a mobile phase [6]. While at 10 °C the peptides were not resolved, baseline separation was achieved at 50 °C because of enhanced hydrophobic interaction in between peptides and stationary phase. An effect of elevated temperature on the retention of small molecules on aminopropyl silica with attached poly(N-isopropylacrylamide) has been studied. Compounds containing a longer hydrophobic chain showed higher increase in retention as a function of the increase in working temperature from 25 to 55 °C when compared to more polar compounds [7]. Poly(N-isopropylacrylamide) polymers differing in length and terminal functional group were grafted onto aminopropyl silica using an activated ester-amine coupling method [8]. The retention factor of steroids became smaller for polymers with higher polarity of terminal group. When analyzed below the lower critical solution temperature the retention factor of steroids was larger on short poly(N-isopropylacrylamide) chains than that on longer chains confirming that length of the polymer and polarity of terminal group have a large impact on the retention properties of prepared stationary phases.

Friedel–Crafts alkylation followed by a surface-initiated ATRP polymerization have been used to modify surface of porous

* Corresponding author. Current address: Department of Chemistry, Faculty of Science, Masaryk University, Brno 625 00, Czech Republic.
E-mail address: urban@chemi.muni.cz (J. Urban).

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polystyrene beads by N-isopropylacrylamide. Optimization of modification reactions provided increase in N-isopropylacrylamide surface coverage and therefore more dominant hydrophobic partitioning interactions with peptides [9]. Surface grafting of N-isopropylacrylamide on poly(vinylbenzylchloride-co-divinylbenzene) monolith allowed separation of steroids in aqueous mobile phase that was controlled only by an external temperature [10].

Silica monoliths have been modified by a thermally responsive brushes of either poly(N-isopropylacrylamide-*co*-n-butyl methacrylate) [11] or poly(N-isopropylacrylamide-co-acrylic acid-*co*-tert-butylacrylamide) [12]. Chromatographic analysis using benzoic acids and insulin peptides [11] or catecholamine derivatives and angiotensin subtypes [12] showed that the brush-modified monolithic silica columns were able to separate these analytes with high resolution and in short analysis times.

Poly(trimethylol propane triacrylate-*co*-N-isopropylacrylamide-*co*-ethylene dimethacrylate) monolithic columns have been prepared by radical polymerization and used for the reversed-phase separation of small molecules. As expected, higher temperature improved peak shape and decreased retention. However, column efficiency has to be further improved to allow proper chromatographic separation [13].

An effect of working temperature, T, on retention factor, k, is in liquid chromatography generally described by van't Hoff equation (1) [17]:

$$\ln K = \frac{\Delta S^0}{R} + \ln \frac{V_S}{V_M} - \frac{\Delta H^0}{RT} = A + \frac{B}{T} \quad (1)$$

where ΔS^0 is the standard partial molar entropy connected with the transfer of the solute from the mobile phase to the stationary phase, ΔH^0 is the standard partial molar enthalpy of transfer of the solute from the mobile phase to the stationary phase, V_S/V_M is phase ratio – the ratio of the volumes of the stationary, V_S, and of the mobile, V_M, phases, and R is the gas constant.

In case, where the retention is controlled by a single retention mechanism, the ln k versus $1/T$ plots are linear lines where intercept A includes contribution of standard partial molar entropy, ΔS^0, and the phase ratio, and slope B is proportional to the standard partial molar enthalpy, ΔH^0. Deviations of the experimental data from the linearity suggest for example changing retention mechanism in the investigated temperature range [18,19] or stationary phase transition [20].

When entropic contribution to the retention is calculated from the intercept of ln k vs $1/T$ lines, it is necessary to determine value of phase ratio which might be difficult due to the inaccurate determination of volumes of mobile and stationary phases [21]. In this work, we have approximated phase ratio (Eq. (2)) by using the value of total porosity, ε_T, (Eq. (SI-1)), determined with non-retained compound that penetrates to all available pore volume and corresponds to volume of the mobile phase [22]:

$$\frac{V_S}{V_M} = \frac{V_C - V_M}{V_M} = \frac{1 - \varepsilon_T}{\varepsilon_T} \quad (2)$$

Several mathematical models have been used to describe combined effect of mobile phase composition and working temperature on chromatographic retention [23,24]. In case that temperature and mobile phase effects are independent of each other, a simple semi-empirical three or four-parameter models can usually describe simultaneous effects of these two variables [22,25,26]. Here, we applied six-parameter polynomial model (Eq. (3)) that includes constant term, two linear terms related to concentration of acetonitrile in the mobile phase and working temperature, one interaction term accounting for combined effect, and two quadratic terms [24]. Value of individual parameters correspond to their effect on response and can be either synergistic (increases response) or antagonistic (decreases response). The parameters with the highest absolute value have the highest effect on the response.

$$y = p_0 + p_1 \cdot \varphi_{ACN} + p_2 \cdot T + p_3 \cdot \varphi_{ACN} \cdot T + p_4 \cdot \varphi_{ACN}^2 + p_5 \cdot T^2 \quad (3)$$

It should be noted, however, that good fit of experimental data does not guarantee the physical correctness of the model and physical meaning of some parameters may not be clearly defined.

In this work, we have explored effect of N-isopropylacrylamide concentration in the polymerization mixture, together with composition of the mobile phase and working temperature on the isocratic retention of small molecules differing in polarity. We have used multivariate analysis to extract parameters corresponding to individual effects controlling retention and used them to characterize retention mechanism. Enthalpy and entropy of the retention of small molecules on the prepared monolithic capillary columns is also discussed.

2. Experimental part

2.1. Materials and chemicals

Polyimide-coated 320 μm i.d. fused silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA). 3-(trimethoxysilyl) propyl methacrylate, sodium hydroxide, hydrochloric acid, trifluoroacetic acid (TFA), 1,4-butanediol, 2,2′-azobisisobutyronitrile (AIBN), toluene, ethylbenzene, propylbenzene, and butylbenzene were purchased from Fluka (Buchs, Switzerland). N-isopropylacrylamide (NIPAAm), N,N-dimethyl-N-metacryloxyethyl-N-(3-sulfopropyl)ammonium betaine (MEDSA), ethylene dimethacrylate (EDMA), tetraoxyethylene dimethacrylate (TeEDMA), bisphenol A glycerolate dimethacrylate (BiGDMA), 1-propanol, acetone, uracil, phenol, and thiourea were obtained from Sigma–Aldrich (St. Louis, MI, USA). Acetonitrile for gradient HPLC were from Merck (Darmstad, Germany). Distilled water was purified in a Milli-Q Reference Water Purification System (Merck Milipore, Darmstadt, Germany).

2.2. Preparation of monolithic capillary columns

The inner wall of the capillary surface has been first modified by 3-(trimethoxysilyl) propyl methacrylate prior the polymerization reaction. Then, monoliths were prepared in capillaries using in situ radical reaction of particular polymerization mixture listed in Table 1. Prepared polymerization mixtures were sonicated for 10 min and filled in the vinylized capillaries with lengths of 150–170 mm. Both ends of the capillary were sealed with stoppers and the capillary was placed in a thermostated bath where polymerization reaction proceeded at 60 °C for various amount of time as shows Table 1. After that, monolithic capillary columns were flushed with acetonitrile and mobile phase. Chromatographic characterization of prepared columns is described in more detail in Supporting information.

2.3. Instrumentation

A modular micro liquid chromatograph was assembled from an LC10ADvp pump (Shimadzu, Kyoto, Japan), a micro valve injector with a 60-nL inner sampling loop (Valco, Houston, USA) controlled using an electronic actuator, a restrictor capillary inserted as a mobile phase flow splitter before the injector, a thermostated column compartment LCO 101 (ECOM, Prague, Czech Republic), a variable wavelength UV detector Saphire operated at 214 nm, adapted for capillary electrophoresis with a 75 μm ID fused silica capillary flow-through cell (ECOM, Prague, Czech