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Comparison of isocratic retention models for hydrophilic interaction liquid chromatographic separation of native and fluorescently labeled oligosaccharides

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ABSTRACT

In this work, we have investigated retention of maltooligosaccharides and their fluorescent derivatives in hydrophilic interaction liquid chromatography using four different stationary phases. The non-derivatized maltooligosaccharides (maltose to maltoheptaose) and their derivatives with 2-aminobenzoic acid, 2-aminobenzamide, 2-aminopyridine and 8-aminonaphthalene-1,3,6-trisulfonic acid were analyzed on silica gel, aminopropyl silica, amide (carbamoyl-bonded silica) and ZIC-HILIC zwitterionic sulfobetain bonded phase. The partitioning of the analytes between the bulk mobile phase and adsorbed water-rich layer, polar and ionic interactions of analytes with stationary phase have been evaluated and compared. The effects of the mobile phase additives (0.1% (v/v) of acetic acid and ammonium acetate in concentration range 5–30 mmol L^{-1}) on retention were described. The suitability of different models for prediction of retention was tested including linear solvent strength model, quadratic model, mixed-mode model, and empirical Neue–Kuss model. The mixed-mode model was extended to the parameter describing the contribution of monomeric glucose unit to the retention of non-derivatized and derivatized maltooligosaccharides, which was used for evaluation of contribution of both, oligosaccharide backbone and end-group to retention.

1. Introduction

The analysis of saccharides and their conjugates with peptides and proteins is difficult due to the wide range of physico-chemical properties and low concentrations of such compounds presented in biological systems. Native oligosaccharides lack chromophore groups, so a derivatization procedure usually has to be applied prior to the analysis. The separation of biologically important oligosaccharides can be utilized by chromatographic methods based on lectin affinity, porous graphitic carbon, or, because of the strongly polar character of the compounds, by hydrophilic interaction liquid chromatography (HILIC) [1].

The retention of compounds in HILIC, which is sometimes also called aqueous normal-phase liquid chromatography, is generally due to three mechanisms—partitioning of analytes between organic-rich mobile phase and water layer adsorbed on stationary phase, polar and ionic (ion-exchange) interactions. Different stationary phases available for HILIC separations, including bare silica, silica gel phases with chemically bonded polar groups (amino-, nitrile-, diol-, pentafluorophenyl-), carbohydrate based phases, mixed-mode, and zwitterionic phases, may enhance particular type of interaction. The stationary phases used in HILIC have been summarized in several review articles [2,3]. The type of packing together with the composition of mobile phase affects the thickness of the adsorbed diffuse water layer on surface of silica-based stationary phase, which influences the retention of polar analytes [4,5].

The fundamental description of retention in HILIC under isocratic conditions can be provided using several models. Unlike in reversed-phase LC, the linear solvation strength model (i.e. the linear dependency of the logarithm of retention factor on concentration of stronger elution solvent; Eq. (1) [6]) is generally not well suitable for description of retention in HILIC although it is considered a partition mechanism of separation. The quadratic model (Eq. (2)) is more suitable for description of retention for mobile phases used over wide range of concentration of organic modifier [7]:

$$\ln k = \ln k_0 - S \times \varphi \quad (1)$$

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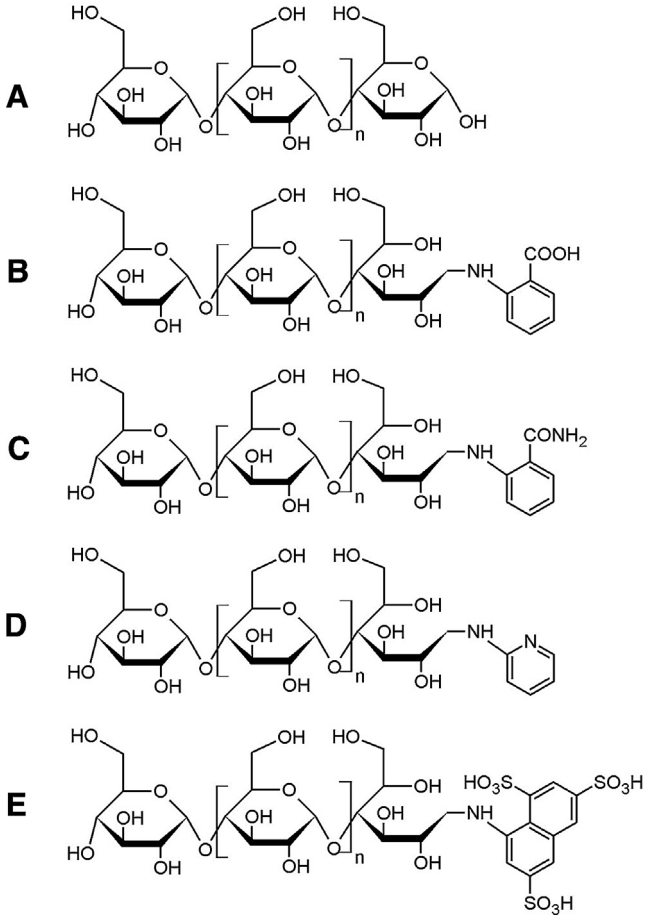

Fig. 1. Structures of non-derivatized maltooligosaccharides (**A**) and their derivatives with 2-aminobenzoic acid (**B**), 2-amino benzamide (**C**), 2-amino pyridine (**D**) and 8-aminonaphthalene-1,3,6-trisulfonic acid (**E**). n = 0–5 in oligomeric series from maltose to maltoheptaose.

$$\ln k = \ln k_0 + S_1 \times \varphi + S_2 \times \varphi^2 \quad (2)$$

where k_0 represents retention factor of an analyte in weaker eluent; φ is volume fraction of stronger eluent in mobile phase and S, S_1 and S_2 are parameters describing elution strength of the eluent used.

For prediction of retention in both isocratic and gradient RP-LC, empirical model introduced by Neue and Kuss can be used, which provide easy analytical solution of integration of gradient equation [8]:

$$\ln k = \ln k_{00} + 2\ln(1 + a \times \varphi) - \frac{B \times \varphi}{1 + a \times \varphi} \quad (3)$$

where k_{00} is the extrapolated intercept (retention factor in pure weak eluent), B is slope of the relationship and a is the coefficient responsible for curvature of the dependency.

The description of retention based on localized surface adsorption used in normal-phase chromatography can be usually applied in HILIC over a limited mobile phase composition range [9]:

$$\ln k = \ln k_0 - n \ln \varphi_{H_2O} \quad (4)$$

where n is the ratio of cross-sectional areas occupied by analyte molecules and by water molecules.

Mechanism of retention (adsorption vs. partitioning) using Eqs. (1) and (4) was compared by McCalley [10]. The studies determining relative importance of both mechanisms were inconclusive as the retention of compounds in HILIC is generally multiparametric process yielding deviations from linearity for both equations [11].

Similarly to RP-LC, the retention of analytes can exhibit non-linear behavior over a wide range of concentration of stronger eluent in mobile phase. This non-linearity can be attributed to the interactions between solutes and solvent, which are not considered in aforementioned equation (Eq. (4)). Therefore, several mixed-mode models were recently introduced, which allows precise characterization of retention in dependency on wide concentration range of solvents in mobile phases [12,13]:

$$\ln k = a + b \ln \varphi_{H_2O} + c\varphi_{H_2O} \quad (5)$$

where a is parameter related to interaction energy between solutes and stationary and mobile phase, b is coefficient related to the direct analyte-stationary phase interaction and c is coefficient related to the interaction energy between solutes and solvents.

Some of the stationary phases used in HILIC can exhibit different retention behavior in low and high-water concentration range in mobile phase. In order to better describe the retention in low-water concentration mobile phases, Jandera and Hájek have introduced a four parameter equation, which can be successfully applied for description of dual HILIC-RP retention mechanism [14]:

$$\ln k = a_2 + m_{RP} \times \varphi_{H_2O} - m_{HILIC} \times \ln\left[1 + b_2 \times \varphi_{H_2O}\right] \quad (6)$$

where the parameters m_{RP} and m_{HILIC} have similar meaning as parameters b and c of Eq. (5) and parameter b_2 corrects the retention in very low water concentration region.

Both native and fluorescently labeled oligosaccharides can be successfully separated in HILIC using various stationary phases. Separation of neutral oligosaccharides has been reported on cyclodextrin bonded phases [15]. Retention of neutral saccharides is usually controlled by partitioning mechanism, but ionic carbohydrates are affected by electrostatic attractive or repulsive interactions, which weakened with increasing concentration of buffer in mobile phase and thus increased ionic strength [16]. When acidic additives to the mobile phase are used, splitting of the peaks can be observed corresponding to the separation of α- and β-anomers [16,17]. For separation of derivatives of oligosaccharides, bonded amide phases provide better selectivity in comparison to the bare silica stationary phase [18,19]. In this work, we have investigated retention of native maltooligosaccharides and their fluorescent derivatives using four different silica-based stationary phases including neutral (silica, amide), charged (amino) and zwitterionic sulfobetaine (ZIC-HILIC) phases. Retention of four types of commonly used derivatives of oligosaccharides with 2-aminobenzoic acid (2-AA), 2-aminobenzamide (2-AB), 2-aminopyridine (2-AP) and 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS) was studied under isocratic conditions and evaluated using above mentioned retention models. The effects of concentration of water in mobile phase and addition of ionic compounds on retention and on contributions of oligosaccharide structural units to the retention were assessed.

2. Experimental

2.1. Materials and reagents

The standards of maltooligosaccharides (maltose to maltoheptaose) as well as acetonitrile, acetic acid, ammonium acetate used for preparation of mobile phases and acenaphthene used for the determination of columns hold-up volume were purchased from Sigma–Aldrich (St. Louis, MI, USA). Deionized ultra-pure water with resistivity 18.2 MΩ cm^{-1} used for preparation of mobile phases was purified using Milli-Q Reference system (Merck Millipore, Billerica, MA, USA). Chemicals used for derivatization of oligosaccharides, 2-aminobenzoic acid, 2-aminobenzamide, 2-aminopyridine, 8-aminonaphthalene-1,3,6-trisulfonic acid, dimethylsulfoxide, and sodium cyanoborohydride