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Investigation of retention mechanism of resorcinarene based cavitands by linear and nonlinear chromatography

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ABSTRACT

Cavitands are cavity-shaped cyclic oligomers and they can create host–guest interactions with various analytes, therefore they have applications in supramolecular chemistry, nanoscale reactions, chromatographic separations, drug encapsulation and delivery, biochemistry. The investigation of the chromatographic behavior of large molecules, such as resorcinarenes and cavitands is meager up to now. To understand the retention of resorcinarenes and cavitands in liquid chromatography, we studied their retention mechanism by the thermodynamic parameters calculated from the van't Hoff equation and by generation of an adsorption isotherm, which can describe the adsorption of the solute on the stationary phase surface. We compared the thermodynamics of the retention for cyclic oligomers in acetonitrile:water and methanol:water mobile phases. Furthermore, we determined the equilibrium adsorption isotherm by inverse method and we made an error analysis of the estimation obtained with the inverse method to ascertain the validity of the obtained isotherm parameters over a broader concentration range.

1. Introduction

Recently, the cavity-shaped resorcinarenes and cavitands have attracted worldwide interest because they can create host–guest interactions, thus they can take part in supramolecular chemistry [1]. Although one of the applications of these cyclic oligomers is their use as selectors in liquid chromatography covalently bonded on silica particles [2–4], the chromatographic behavior of large molecules such as resorcinarene based cavitands is an unexplored field of liquid chromatography. In our previous work – applying the classical van't Hoff law – the retention mechanism of C-tetra-methylcalix[4]resorcinarene, 2-methyl-C-tetra-methylcalix[4]resorcinarene, cavitand and methyl-cavitand (Fig. 1) was investigated on two different types and chain-length stationary phases [5]. Dong found that methods using a C_{18} and a polar embedded column (XTerra) are expected to yield very dissimilar chromatographic profiles because their selectivity differences lead to very scattered correlation of their respective retention data [6]. Although the retention factors obtained on C_8 and C_{18} stationary phases are well correlated for most analytes, they also give the opportunity to study the effect of the chain length on the retention.

Previous measurements – that were performed on BDS Hypersil and XTerra C_8 and C_{18} stationary phases – provided linear van't Hoff plots in the examined temperature range (15–45 °C) in acetonitrile:water mobile phase [5]. Surprisingly, we observed a huge difference between the retention factors of resorcinarenes and cavitands on every column. In the case of BDS Hypesil C_{18} we found a hundredfold difference for the two analytes. The structural difference between the cavitands and resorcinarenes is that the hydroxyl groups of resorcinarenes are in ether linkage *via* methylene-bridges in the cavitands. Besides – within a family of compounds – the presence of methyl groups on the upper rim in the molecules caused a large increase of the retention, too. The transfer of the analytes from the mobile phase to the stationary phase was enthalpy controlled process; the typical enthalpy change ranged between $\Delta H = -6$ to -15 kJ/mol on every column. The entropic contributions of the analytes obtained from the intercepts of the van't Hoff plots changed in wider range ($T\Delta S = -0.5$ to -9.7 kJ/mol) than the enthalpic contribution, so the difference of entropy changes plays an important role in the different retention behavior of cyclic oligomers in addition to other effects. We observed a strong linear relationship between the calculated log *P* values and the Gibbs free energy changes of the

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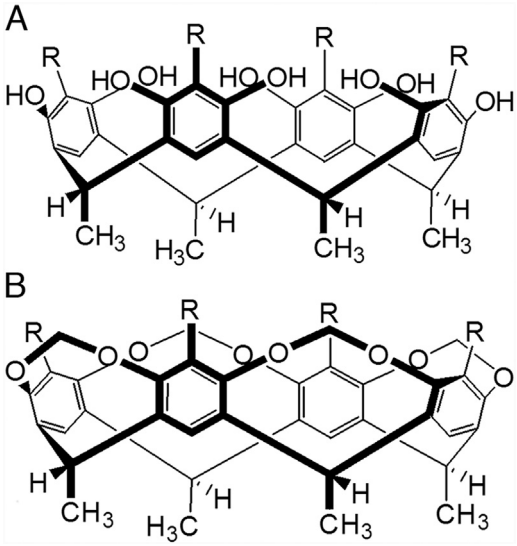

Fig. 1. Structures of resorcinarenes and cavitands. The ligands are (A) R = H in HRM and (B) R = CH_3 in MRM; R = H in HCM and R = CH_3 in MCM.

analytes on every column [5]. In this study we extend the above results with measurements carried out in methanol:water mobile phase to point out the effect of the mobile phase on the retention mechanism. In the present work the same analytes and stationary phases were used, furthermore the same temperature range was applied as in our previous experiments. Thus, we could investigate the retention and thermodynamic of the resorcinarenes and cavitands in methanol containing mobile phase in a comparable manner.

The classical van't Hoff plot (Eq. (1)) provides information on the retention mechanism by thermodynamic properties for the transfer of the analyte from the mobile phase to the stationary phase. In reality, the retention mechanism is very complex, which originates from the complexity of the structure of the alky bonded stationary phase and analytes, furthermore from the nature of interface of RPLC systems. Linear or analytical chromatography does not give detailed information on the retention mechanism because it merely measures the retention time, which is simply the combination of the various contributions of the different interactions between the analytes and the chromatographic phases. With nonlinear chromatography, the adsorption isotherm of the analyte can be determined by the overloading experiments. From the isotherm data, we are able to assess the amount of the various types of adsorption sites present on the surface and the individual equilibrium constants can also be calculated. The advantage of this method is that it can distinguish between the contributions of the saturation capacity and the adsorption–desorption equilibrium constant to the retention factor.

For the determination of the adsorption isotherms, the most commonly used methods are the frontal analysis, the elution by characteristic point, the perturbation method, the pulse methods, and the inverse method. The advantages and drawbacks of all these methods are discussed in detail elsewhere [7]. The main advantages of the inverse method over the most accurate frontal analysis (which was first developed and used independently by James and Phillips [8] and by Schay and Székely [9]) are the lower cost due to the lesser solute consumption and reduced experimental time.

The inverse method (IM) is a relatively recent numerical method for isotherm determination [10–17]. In 1991 Dose et al. used a modified simplex algorithm to obtain a better approach for the determination of equilibrium isotherms parameters of *N*-benzoyl-(D,L)-alanine and *N*-benzoyl-(D,L)-phenylalanine and found a good agreement with the isotherms resulted by frontal analysis [10]. In 1994 James and Sepúlveda developed a more sophisticated method to determine the best numerical estimates of parameters of an isotherm model from individual elution profiles of binary mixtures [11,12] and Juza determined the isotherms of cycloheptanone and cyclopentanone by IM [13]. Antos et al. applied the IM for the estimation the isotherm of methyl deoxycholate [14]. Moreover, the IM was applied a number of times for determining the numerical values of isotherm models of enantiomers, such as mandelic acid [15], 2,2,2-trifluoro-1-(9-anthryl)-ethanol [16], 1-indanol [17], 1-phenyl-1-propanol [18], Tröger's base [19], tryptophan [20], and ketoprofen enantiomers [21].

In this work, we followed the exploration of the retention mechanism and we performed nonlinear chromatography experiments to study the adsorption properties of the resorcinarene based cavitands on the C_8- and C_{18}-bonded surfaces of the BDS Hypersil stationary phase. For the determination of the equilibrium isotherm, we can obtain reliable adsorption isotherm data if we use a wide concentration range of the analyte in the mobile phase. For this purpose, the highest possible concentration must be injected in sufficient volume, but the sample size is usually limited by the solubility of the analyte. As we mentioned above, the inverse method offers an attractive solution for this contradiction, because it needs a minimum amount of sample and solvent.

2. Theory

2.1. Relationship between the retention factor and thermodynamic parameters

The thermodynamic parameters of the solute transfer from the mobile to the stationary phase correlate with the retention factor under isocratic conditions. The enthalpy and entropy changes are evaluated by analyzing the temperature dependence of the retention factor according to the van't Hoff equation:

$$\ln\ k = \frac{-\Delta G^\circ}{RT} + \ln\beta = \frac{-\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln\beta \qquad (1)$$

where k is the retention factor, ΔH°, ΔS° and ΔG° are the enthalpy, entropy and Gibbs free energy change of the reversible binding of the analyte, respectively, β is the phase ratio (the volume of the stationary phase divided by the volume of the mobile phase) T is the absolute temperature and R is the universal gas constant. It is well-known that the phase ratio strongly depends on the structure of the stationary phase – the nature of the bonded ligand strongly influences the volume of the bonded phase, thus the phase ratio – but it is often assumed to be constant at different temperatures and pressures [22].

2.2. Nonlinear chromatography

As a very rough approximation, the retention process can be described on the basis of single equilibria of the analyte distribution between the mobile and stationary phases. The Henry constant (a) is related to the retention factor (see Eq. (2)). In nonlinear chromatography, one can distinguish the contribution of the saturation capacity (q_s) and the adsorption–desorption equilibrium constant (b) to the retention factor. The saturation capacity is the amount of an analyte forming a monolayer on the surface. In the case of homogeneous surface, we can write the retention factor (k) as:

$$k = \beta a = \beta q_s b \qquad (2)$$