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High-resolution magic angle spinning description of the interaction states and their kinetics among basic solutes and functionalized silica materials

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ABSTRACT

Modeling of the interaction is crucial to understanding and predicting chromatography. However, the complexity and variety of the grafted motifs render the creation of an accurate model overwhelmingly challenging, so that most often the classification of column separation properties is described by monitoring the retention times of carefully selected control molecules. We analyzed here the characteristics of the interplay of compounds of basic nature by ^{1}H HRMAS NMR, which provide relevant descriptors for products with pharmaceutical properties, with chromatographic phases for Reversed Phase Liquid Chromatography. Eight grafted silica phases were selected, differing to enhance specific structural properties (monomeric and polymeric grafts, endcapping or not, carbon content, alkyl with polar embedded group or alkyl bonded chain, chemical nature of end capping, native silica). These materials were put in interaction with five basic molecules, previously chosen as probes for the evaluation of efficient base deactivated liquid stationary phases using five theoretical molecular descriptors to cover a large scale of molecular volume, polar surface area, Log *P*, hydrogen-bond donor capacity and finally hydrogen-bond acceptor capacity. ^{1}H HRMAS NMR was capable of describing qualitatively a wealth of interaction states, characterized both thermodynamically and kinetically. In one case (penbutolol) up to five interaction states could be differentiated. Variable temperature experiments revealed the complexity of the retention process on grafted silica as in some cases the kinetics of the interaction is shown to slow down on increasing the temperature.

1. Introduction

With hundreds of dedicated materials, Reverse-Phase Liquid Chromatography provides without a doubt one of the richest database of experimental data on solute/solid interactions, besides being a powerful technique for separating complex mixtures and to identify and quantify their components. It this thus not surprising that modeling of the chromatographic process has attracted much attention. Important factors in this respect are the thermodynamics and kinetics of the interactions of a given molecule with a chromatographic solid [1], for which adapted probes are required capable of following the evolution of the heterogeneous mix at the molecular level.

High-resolution Magic-Angle-Spinning (HRMAS) ^{1}H NMR spectroscopy is a method designed for multiphasic environment, as it could be provided by the mobile/stationary phase ensemble. Thus, this method has found numerous applications in the context of chromatographic or chromatography-inspired analysis. Indeed, NMR techniques have a long history of applications to help understand or model chromatographic phases [2–15]. The group of Klaus Albert has been particularly active in this domain, by either structurally characterizing the grafted moieties in the presence of the mobile phase or by using NMR techniques such as trNOE [16–18] and Saturation Transfer Difference (STD) [19] which allow inferring the contact points between a light and a bulky compound or a solid suspended in solution [2–8,10,11]. By this latter approach, the structural basis of enantiomeric discrimination over a chiral columns has been assessed in two example cases, the separation efficiency of sterols has been studied [7] and the selectivity of molecularly imprinted polymers

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stationary phases has been assessed [6]. Another method that exploited the basics solute/stationary phase thermodynamics is Chromatographic-NMR, a variant of HRMAS NMR diffusometry in which the mobility of a target molecule is modified by the addition of a stationary phase according to their affinity [20]. The technique has been used primarily to understand the fundamentals of the mass transport, and thus of the separation, in chromatographic silica [12–14]. In this study an attempt has been made to qualitatively describe the kinetics of solutes in contact with column material, using different liquid/solid ratios and variable temperature, to reveal if the customary approximations of fast kinetics hold in general and if a correlation between kinetics rates and thermodynamics strength of the interaction, inferred by the LC outcome, exists.

2. Materials and methods

2.1. Samples and chromatographic phases

All the chromatographic phases were kindly donated by Interchim (Montluçon, France), under the commercial acronyms; Uptisphere HDO, HSC, NEC, ODB, TF, PLP and Uptisphere Strategy C18-2 and C18-3. The particle size was 5 μm. The chemical ingredients were purchased from Sigma–Aldrich (Saint Quentin Falavier, France) and the perdeuterated solvents were obtained from Eurisotop (Saint-Aubin, France). Stock solutions of five test basic molecules; benzylamine, penbutolol, carvedilol, quinine and procainamide hydrochloride, were individually prepared at the concentration of 1 mg mL^{-1} in a solvent mixtures of perdeuterated-acetonitrile and phosphate buffer pH 7 in heavy water 40/60 (v/v).

2.2. Experimental

All NMR experiments were performed at 400 MHz on a Bruker Avance (Wissembourg, France) spectrometer equipped with a ^1H HRMAS probe head. HRMAS analysis on each test molecule were performed at two liquid/solid phase ratios, β: low, with 30 μL of solution added to 15 mg of solid; high, corresponding to 40 μL of liquid solution and 5 mg of solid, in a total volume of 50 μL. The first preparation was analyzed at two temperatures (298 K and 320 K) while the second one only at 298 K. In both cases, the test solution was injected in a 4 mm zirconium rotor where roughly half of the solid phase had been introduced, and then the remaining phase was added. The spinning rate was of 4000 Hz.

The pure solutions were analyzed in rotors of a volume of 12 μL.

3. Results and discussion

Table 1 summarizes the chromatographic phases used in this study. While most of them (seven out of eight) are based on C18 grafts, they present a variety of specific properties. Among the C18 bonded silicas the TF phase is the only polymeric one; NEC is not endcapped while HSC is both high density bonded and end-capped, ODB is simply end-capped, HDO had a mixed end-capping treatment and C18-2 and C18-3, which also have a different silica base with respect to all other materials, are multi-step end-capped. Finally, the PLP phase is an original polymeric polar-embedded group stationary phase. It was synthesized for avoiding silanol interactions and thus for giving symmetrical peaks in the analysis of basic solutes. Taking into account the phase stationary evaluation using some general test procedures at which the evaluation with the specific test [7] for basic solutes these eight phases have been classified by ACH analysis, among a group of 79 tested RPLC stationary phases, as lying in five different classes. NEC, TF and PLP lie alone respectively in a first, second and third group the most distant from the nearest forth and fifth groups, C18-3 and HSC in a forth group and the remaining stationary phases, ODB, HDO, and C18-2 in a fifth group [21]. The chromatographic performance of several base-deactivated stationary phases was also evaluated with a specific chromatographic test. In sake of comparison it includes five of present tested stationary phases: HDO, HSC, NEC, ODB and TF [22]. Selected supports were further tested with a pH 7 mobile phase to observe silanophilic interactions. Among all tested supports, best results in term of silanol masking capacity were obtained with embedded polar group supports. By observing asymmetry factors measured with a strong base and a weak base TF, ODB and HDO exhibited lower values (independent of the basic strength of both solutes: 1.1 for TF, 1.2 for ODB and HDO). NEC and HSC showed higher asymmetry factors values as well as different for strong and weak base (NEC: respectively 1.35 and 1.30; HSC: respectively 1.8 and 1.6).

Benzylamine, penbutolol, carvedilol, quinine and procainamide hydrocloride are basic compounds that span over a range of molecular properties: pK_A, volume, polar surface area, octanol–water distribution coefficient at pH 7, hydrogen bond donor and acceptor capacity. This makes them ideal descriptor of interactions with chromatographic phases and they have indeed been used in this sense. Indeed, the choice of the compounds was motivated by previous work in which they have been classified by PCA into three different groups: small basic polar basic compounds (benzylamine), large apolar basic compounds (penbutolol, carvedilol) and large polar basic compounds (quinine and procainamide) for covering the overall the more large as possible behavior of basic solutes on liquid chromatographic stationary phases [23].

Comparing to probes that were used for the assessment of a simplified test methodology for the evaluation of base deactivated supports [24], in the present study, penbutolol and carvedilol were used instead of methadone and fentanyl which are less easily available as these two news probes belong to the same PCA classification group [25].

The chromatographic behavior of the five probe molecules over the eight materials is summarized in Tables 3 and 4 to be used later on as a comparison for the NMR investigation of the basics of the separation process, obtained in the experimental conditions reported by Stella [26].

The five probe molecules present very different retention factors, k, confirming that they are a good selection to cover the diversity of behavior of the eight chromatographic phases under

Table 1
Characteristics of the chromatographic phases.

Phase	Bonding	Bonding technology	End-capping	Carbon content	Pore size (Å)	Surface area, m^2/g
C18-2	C18	Monofunctional	Yes (multi step)	19	100	425
C18-3	C18	Monofunctional	Yes (multi step)	22	100	425
HDO	C18	Monofunctional	Yes (mixed)	17	120	320
HSC	C18	Monofunctional	Yes (multi step)	20	n.c.	n.c
NEC	C18	Monofunctional	No	16	120	320
ODB	C18	Monofunctional	Yes (mono)	18	120	320
PLP	Polar embedded group + Alkyl chain	Polyfunctional	Yes (multi step)	14	120	320
TF	C18	Polyfunctional	Yes (mono)	14	n.c.	n.c