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Hysteresis of retention and enantioselectivity on amylose tris(3,5-dimethylphenylcarbamate) chiral stationary phases in mixtures of 2-propanol and methanol

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ABSTRACT

Seemingly identical chromatographic conditions result in drastically different enantioseparations on amylose tris(3,5-dimethylphenylcarbamate) (ADMPC) columns in mixtures of 2-propanol and methanol. Selectivities of structurally diverse enantiomer pairs depend on the direction from which the composition of the eluent is approached. An alteration in the structure of the chiral stationary phase (CSP) is the only realistic reason behind the dissimilar selectivities in the same eluent. History-dependent retention and recognition mechanisms are indicated by van't Hoff plots and even by a reversal of the enantiomer elution order.

The most notable observation is the easy access to markedly different states of the CSP in the same solvent mixture by a short pretreatment with 2-propanol in one case and with methanol in the other, while the transition between the two states is hindered enough to ensure long-term stability for both. Repeatability strongly depends on the composition of the eluent and it is key to utilization and also to rationalization of the phenomenon.

From a theoretical point of view, this so-called hysteretic behavior poses another challenge to consider when modeling chiral interactions.

1. Introduction

Direct chromatographic separation of enantiomers is an important issue both in collecting analytical information and in preparative scale resolution of racemates. The field had a large expansion in the last decades since the appearance of powerful chiral stationary phases (CSPs) on the market. Polysaccharides cover the majority of chiral separations of nonionic compounds [1] due to their outstanding versatility [2–5]. Amylose tris(3,5-dimethylphenylcarbamate) (ADMPC), a very successful member of this family, was invented by Okamoto et al. 31 years ago [6], but it is still one of the most often used selectors in its coated or immobilized forms [5].

Polysaccharide-based CSPs can be operated in various modes (normal phase, super/subcritical fluids, aqueous-organic solvents, polar organic solvents), depending on the polarity of the eluent used [7]. The mobile phase plays multiple roles in determining the chiral recognition ability of the system. First, it influences which of the numerous available polar and apolar interactions (H-bond, π-π, dipole-dipole) are enhanced [8–13]. Another very important factor is the influence that the eluent exerts on the higher order structure of the stationary phase. Excellent chiral recognition ability of the amylose- and cellulose-based CSPs is to a large extent ascribed to their stereoregularity, which is reflected in the existence of chiral cavities in their helices [5]. Experiments have supported the assumption that these helices respond to the change in the eluent composition by an alteration of their conformation and hence the spatial arrangement of the chiral grooves, which can deeply affect enantioselectivity. Differences in the higher order structure of the CSP, demonstrated by vibrational circular dichroism (VCD) and nuclear magnetic resonance (NMR) spectroscopy, have been associated with a change of the enantioselectivity as radical as the reversal of the enantiomer elution order (EEO) [14,15].

The ability of the polysaccharide derivatives to engage in multiple types of interactions and adopt various conformations render their chiral recognition mechanism intricate to study. Although successful modeling studies have been reported (recent ones are summarized in a review [16]), this tool is still by far immature to provide practically useful predictions. Consequently, chiral method screening in practice involves trial-and-error sequences employing

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a series of columns and eluents. The range of applicable solvents is relatively narrow for the coated polysaccharide-based CSPs, because the selectors are soluble in many solvents. This constraint can be overcome using immobilized CSPs, which are compatible with almost any solvents [17]. The extended solvent range provides many possible conformations of the CSP, which translate to numerous available selectivities. On the other hand, a special difficulty concerning the reproducibility of the results may arise when non-standard mobile phase components like dichloromethane or ethyl acetate (DCM or EtOAc) are used. Switching back to standard solvents, the conformation adopted in DCM or EtOAc can be partially preserved, which leads to substantially changed selectivity compared to the starting one [18]. A long regeneration protocol is necessary to recover the original condition of the column. The whole series of experiments represents a hysteresis, which is the term used in a wider sense when the state of a system is dependent on its history (although the authors did not use this word). More precisely, hysteresis also implies returning to the original state through a different path than the one followed when moving away from the original state, which also takes place in the cited reference. To the best of our knowledge, this is the only example in chiral chromatography where hysteresis of the retention has been reported as a function of the concentration of a major component of the mobile phase.

In the present communication we report the characterization of a hysteretic chiral chromatographic system consisting of an ADMPC selector and various proportions of methanol (MeOH) and 2-propanol (IPA). Practical aspects as well as rationalization of the results are addressed.

2. Material and methods

2.1. Chemicals

HPLC grade 2-propanol and methanol were purchased from VWR (Fontenay-sous-Bois, France). Diethylamine was purchased from LabScan (Dublin, Ireland). The rosuvastatin intermediate (RIN), donepezil, vildagliptin, bisoprolol, quetiapine and the mirabegron intermediate were authentic samples from quality controlled syntheses performed at Egis PLC (Budapest, Hungary). Commercially available compounds were purchased from the following sources: racemic 1,1′-bi-2-napthol and (*R*)-(+)-1,1′-bi-2-napthol (BINOL) from Acros Organics (Geel, Belgium), (*S*)-2-methylpropane-2-sulfinamide from Combi-Blocks (San Diego, CA, USA), (*R*)-2-methylpropane-2-sulfinamide from Fluorochem (Hadfield, United Kingdom), racemic flavanone (FLA), racemic Tröger's base, racemic benzoin and racemic *trans*-stilbene oxide (TSO) from Sigma-Aldrich (St. Louis, MO, USA). Single enantiomers resolved by preparative or semipreparative chromatography are named according to their particular elution order during the preparation at 25 °C as follows: RIN1 before RIN2 on Chiralpak AD (20 μm) in IPA/MeOH = 65/35, TSO1 before TSO2 on Chiralpak AD (20 μm) in IPA, FLA1 before FLA2 on Lux Amylose-1 (5 μm) in MeOH. This naming was kept for identification throughout the study regardless of the elution order in other chromatographic systems.

2.2. Instrumentation

The column comparison experiments were performed on an Agilent 1200 HPLC instrument (Waldbronn, Germany) equipped with G1311 A quaternary pump, G1322 A degasser, G1367B HiP-ALS autosampler, G1316 A TCC column temperature controller and G1315D diode array detector. The ChemStation software (version B.04.01 SP1) was used for instrument control, data acquisition and data processing.

All the other HPLC experiments were performed on a Shimadzu LC-10 HPLC instrument (Kyoto, Japan) equipped with LC-10AD VP pump, DGU-14 A degasser, FCV-10AL VP low pressure gradient mixer, SIL-10AD VP autosampler, CTO-10AS VP column oven and SPD-M10 A VP diode array detector. The chromatograms were acquired and processed by Class-VP software (version 6.14 SP1).

2.3. Chiral stationary phases

Commercially available chiral columns originated from the following sources: Chiralpak AD (20 μm, 250 × 4.6 mm), Chiralpak AD-H (5 μm, 250 × 4.6 mm) and Chiralpak IA (5 μm, 250 × 4.6 mm) were purchased from Chiral Technologies Europe (Illkirch, France), ChiralArt Amylose-C (5 μm, 150 × 4.6 mm) was purchased from YMC (Kyoto, Japan). Lux Amylose-1 (5 μm, 150 × 4.6 mm) and Lux i-Amylose-1 (5 μm, 150 × 4.6 mm) were gifts from Gen-Lab Ltd., Hungarian distributor of Phenomenex (Torrance, CA, USA). The columns experienced normal phase and polar organic eluents before the study except for the new columns in subsection 3.6.

2.4. Chromatographic conditions

Unless otherwise stated, the following parameters were applied: column temperature 25 °C; flow rate 0.2 ml/min for the Chiralpak columns with 5 μm particle size (compeled by high viscosity of IPA, 50 bar pressure limit of the AD-H column, and the desire to use the same flow rate for a given column dimension and particle size) and 1 ml/min for all the other columns; UV detection at 220 nm. The sum of the concentration of the enantiomers was 0.5–1.0 mg/ml. The sample solutions were prepared in IPA except for flavanone which was used directly in MeOH after micropreparative collection. The injected volume was 1 μl. For the basic compounds (donepezil, vildagliptin, bisoprolol, mirabegron intermediate, quetiapine and Tröger's base), 0.1% diethylamine was used as an additive in the mobile phase.

The columns were stored in IPA except when new columns shipped in hexane/IPA = 90/10 were directly rinsed with the solvent of their first experiment. Whenever an experiment required pretreatment with either IPA or MeOH, it was brought about by pumping 10 column volumes (CV) of the corresponding solvent through the column.

All composition values throughout the article are in % (v/v). Mixing of IPA and MeOH was done by the pump of the HPLC system except for cases employing a particular composition throughout a whole experiment series (subsections 3.4.3 and 3.5), when the mixture was manually prepared. We checked that both sorts of mixing provided acceptably similar retention factors (Δk<2%). In subsection 3.4.3 the recycling of the eluent was suspended while peaks eluted from the column.

Dead time was determined by 1,3,5-tri-*tert*-butylbenzene (TTBB) in the concentration of 0.5 mg/ml. Retention volume of TTBB in IPA as the eluent was 1.75 ml on Lux Amylose-1 (150 × 4.6 mm), it ranged from 1.73 ml to 1.93 ml on the columns of same dimensions and from 2.95 ml to 3.11 ml on the 250 × 4.6 mm columns used in subsection 3.6. For the sake of simplicity, 1 CV was rounded as 1.8 ml and 3.0 ml, respectively.

In the temperature study (subsection 3.4), the system was regarded thermally equilibrated when the pressure became constant after setting a new temperature. Two injections were done and the retention factors from the second were used for the van't Hoff plots. The difference from the retention factors obtained in the first injection was <1% in all cases.

A small but sufficient equilibration volume (4 ml, about 2.2 CV) was used for the analytical column in subsection 3.1. Other equilibration volumes as important parameters are specified in the corresponding subsections. The known dwell volume of the sys-

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