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Chiral ligand-exchange chromatography with *Cinchona* alkaloids. Exploring experimental conditions for enantioseparation of α-amino acids

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ABSTRACT

The natural *Cinchona* alkaloid quinidine as chiral selector in chiral ligand-exchange chromatography was systematically studied. Chromatographic conditions for enantioseparation of twenty α-amino acids were first time studied by changing mobile phase parameters such as pH, concentration of organic solvent, type of salt, ligand to metal ratio and also column temperature. Maximum retention and enantioselectivity factors were observed at the region close to pH = 8, since the tertiary amine (the quinuclidinic nitrogen) of the quinidine is protonated only in a small degree, and therefore is available for the chelate formation. Additionally at this pH value there is no other competing ligand for complex the metallic cation. The thermodynamic transfer parameters of the enantiomers from the mobile to the stationary phase from van't Hoff plots within the range of 10–35 °C were estimated. Thus, the differences in the transfer enthalpy Δ(ΔH), and transfer entropy Δ(ΔS) enabled an investigation of the origin of the differences in interaction energies Δ(ΔG). Finally, the molecular geometry of the formed diastereomeric complexes was modelled and energetic differences between both compounds were calculated by a semi empirical method.

1. Introduction

Chiral recognition and enantiomer distinction are fundamental phenomena in nature and chemical systems. They have impact in many chemical fields dealing with bioactive compounds, such as drug discovery, research and development of pharmaceuticals, agrochemicals, food additives, fragrances and pollutants. Enantioseparation represents an important field in analytical science and therefore, the availability of strategies enabling the racemic resolution is a continuously challenging task. In the last decades, a large number of publications about enantioseparation by chromatographic and electrophoretic techniques appeared in literature. Initially, gas chromatography (GC) and high performance liquid chromatography (HPLC) were used for this purpose, but then capillary electrophoresis (CE) was included.

The biological and pharmacological properties of amino acids strongly depend on their stereochemistry and hence amino acids enantiomeric purity is of utmost importance. Several approaches for resolving underivatized amino acids have been successfully applied [1]. Chiral ligand-exchange chromatography (CLEC), firstly proposed by Davankov in the early seventies [2], still represents the elective choice since it does not require any prior sample handling. CLEC consists in the reversible coordination of chelating analyte species from the mobile phase into the coordination sphere of a metal ion that is immobilized by complexation with a chelating chiral selector, forming mixed ternary selector/metal ion/solute complexes. Depending on the steric and functional properties of the analytes, these diastereomeric ternary chelates show different rates of formation and/or thermodynamic stabilities, giving rise to different retention times for the corresponding solute enantiomers. During the chromatographic process, the coordinated ligands are reversibly replaced by other ligands from the mobile phase. Cu(II) is the chelating metal ion of first choice, while Zn(II) and Ni(II) may be proper alternatives [3]. Frequently employed CLEC type selectors include cyclic amino acids such as proline [4] and hydroxyproline [5] as well as sulfur containing amino acids derived from cysteine [6] and penicillamine [7], and also amino alcohols [8].

Applicability of CLEC relies on the presence of metal-chelating functionalities in both the chiral selector (an enantiomeric pure compound) and the analyte. *Cinchona* alkaloids are well known chiral auxiliaries for promotion enantioselective transformations in catalytic processes [9]. Their chemical structure consists of a conjugated heterocyclic quinoline ring linked to a rigid bicyclo heterocyclic aliphatic quinuclidine ring through a carbon atom, C9, linked to a hydroxyl group. In this family of molecules, only C8 and C9 may vary in their configuration resulting

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in the pseudo-enantiomeric quinine and quinidine. *Cinchona* alkaloids especially quinine (QN) and quinidine (QD), became popular in liquid chromatography more recently, when Lindner and co-workers have extensively studied several derivatives, mainly the carbamates as selectors incorporated to chiral stationary phases employed in ion-exchange mode [3]. Although *Cinchona* alkaloids have high potential to form complexes with chiral acidic compounds, they were first time reported as chiral selectors in CLEC recently in our previous work [10]. Now we propose to replace QN by QD as chiral selector and also to extend the study to other transition cations. The liquid-liquid extraction of *Cinchona* alkaloids through the formation of mixed complexes with optically active usnic acids mediated by divalent cations such as Cu(II), Co(II) and Zn(II) [11] has been described.

In this work, chiral separations of α-amino acids using a CLEC system with QD as chiral ligand and a conventional non-chiral octadecylsilica (ODS) column were achieved. To the best of our knowledge, no precedents exist for the systematic study of the influence of the experimental variables over the retention and enantioselectivity for this chromatographic system. In order to study the chemical stability and viability of the aforementioned metallic complexes, the semi-empirical PM6-DH + method to model the complex geometries and to obtain the most stable structures of intermediate coordination complexes presumably mediating the enantioseparations has been employed.

2. Experimental

2.1. Chemicals

The chemicals used were reagent-grade or better. The amino acids (both racemic and pure enantiomers) were purchased from Sigma (St. Louis, MO, US) or from BDH (BDH Ltd., UK); QD was from Fluka (Buchs, Switzerland); the cupric acetate, cupric nitrate, cupric sulfate, cobalt acetate and zinc acetate were from Baker (J.T. Baker Chemical Co., Phillipsburg, NJ, US) and the HPLC-grade methanol (MeOH) from Mallinckrodt (Mallinckrodt Baker Inc., Phillipsburg, NJ, US). Water was purified by means of a Milli-Q Purification System (Simplicity, Millipore, Massachusetts, MA, US).

Mobile phase solutions were filtered through a 0.22 μm Millipore filter and degassed with 10 min sonication before use. Amino acid solutions (7 mg/mL) were prepared in filtered mobile phase and sonicated until completely dissolved.

2.2. Instrumentation

The HPLC experiments were carried out on an Agilent liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with 1100 vacuum degasser, binary pump and column thermostat, 1260 Infinity Autosampler and 1290 Infinity Diode Array Detector. Data acquisition was done by the Open LAB Chromatography Data System (CDS) software (ChemStation C.01.03).

A commercial Eclipse XDB-C18 (Agilent, USA) analytical column (75 mm × 4.6 mm; 3.5 μm) was employed.

2.3. Mobile phase preparation and column equilibration

The mobile phase preparation was described in detail previously [10]. Briefly, the weighed alkaloid (QD) and the metallic salt (in turn $Cu(CH_3COO)_2$, $Cu(NO_3)_2$, $CuSO_4$, $Co(CH_3COO)_2$, $Ni(CH_3COO)_2$ or $Zn(CH_3COO)_2$) were dissolved into a mixture of 20% (v/v) MeOH and 80% (v/v) aqueous 0.1 M NH_40AC/NH_3 buffer or in a 10% (v/v) MeOH and 90% (v/v) 0.1 M aqueous buffer to reach a final concentration of 0.5 mM for the studied divalent cation and 0.5 or 1 mM for QD. The mobile phase $^{s}_{w}$pH was readjusted with either hydrochloric acid or sodium hydroxide to the desired pH value (8.00 or 9.00).

To equilibrate the ODS column, the filtered mobile phase was run at 0.1 mL/min, in an open cycle, until the detector response proved stable (approximately 200 column volumes). The mobile phase flow rate for analysis was set to 0.5 mL/min. After running all the analyses with each mobile phase, the analytical column was always cleaned with a 30:70 (v/v) MeOH: H_2O mixture and then reconditioned by flowing the new mobile phase through the column. These procedures allowed the restoring of the column for the next appropriate ODS surface coating.

The native α-amino acids were detected at 254 nm. KBr detected at 210 nm was used for unretained marker in all analysis. The retention times were taken at maxima of the peaks. The elution order within a racemic pair was determined (when it was possible) by the injection of each pure enantiomer.

2.4. The PM6-DH + computational method

The accuracy of semi empirical quantum method PM6 in predicting formation heats for compounds of interest in biochemistry is somewhat superior to Hartree-Fock (HF) or B3LYP DFT methods, using the 6-31G(d) basis-set. For a representative set of compounds, PM6 gave an average unsigned error (AUE) of 4.4 kcal mol^{-1}; for the same set HF and B3LYP had AUE of 7.4 and 5.2 kcal mol^{-1}, respectively [12]. Hobza et al. have introduced an extension of the semi empirical PM6 method in two directions [13]. The first one includes an empirical correction to the dispersion energy that improves the description of complexes controlled by the dispersion energy. The second one introduces an additional electrostatic term that improves the description of hydrogen-bonded complexes. The resulting method, i.e. the PM6 with corrections for dispersion and hydrogen bonding, was labeled PM6-DH+. This method provides stabilization energies that agree very closely with the benchmark values obtained by much more expensive methods. For the purpose of verifying the stability of this type of complexes, a molecular dynamic simulation during 15 ps with a time step of 0.5 fs for one of the complexes at 900 K, keeping the temperature constant by coupling the system to a Berendsen [14] thermostat with a bath relaxation time of 0.5 ps was performed.

3. Results and discussion

3.1. Chiral separation mechanism

When chiral mobile phase additives are used to regulate analyte retention in reversed phase HPLC, often results in the formation of diastereomeric ion pairs which can be easily separated on conventional reversed phase columns. During passage of a racemic analyte through the HPLC column, diastereomeric mixed-ligand complexes can be formed by a displacement mechanism. Since for the basic concept of CLEC, a transition metal will complex with electron-rich ligands, the complexing between divalent cations and *Cinchona* alkaloids is feasible due to the presence of a hydroxyl and a tertiary amino group situated on adjacent carbon atoms. The difference in the three-dimensional structures between both complexes would potentially lead to the desired enantioseparation. The enantioselectivity depends on the differences in the relative stabilities of that complexes, the energy and their affinity for the stationary phase, thereby resulting in different chromatographic retention.

3.2. Chiral separation conditions

In order to optimize the separation conditions for racemic α-amino acids by using CLEC, some effective factors such as organic modifier content, concentration of chiral ligand, kind of salt (either metallic cation and counter-ion), mobile phase pH and column temperature were investigated.