



Enantioseparation of α -amino acids by means of *Cinchona* alkaloids as selectors in chiral ligand-exchange chromatography



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ARTICLE INFO

Article history:

Received 21 February 2013

Received in revised form 8 May 2013

Accepted 13 May 2013

Available online 20 May 2013

Keywords:

Cinchona alkaloids

α -Amino acids

Chiral ligand-exchange chromatography

Semi-empirical force-field methods

ABSTRACT

A conventional nonchiral column was used for the enantioseparation of several racemic α -amino acids (native and derivatized) through the use of *Cinchona* alkaloids as chiral selectors along with Cu(II) ions in chiral ligand-exchange chromatography. The mobile phase composition (i.e., the organic modifier content and pH) was studied in order to modulate retention and enantioselectivity. Good enantioseparation of many amino acids was obtained using equimolar amounts of Cu(II) and either cinchonidine, quinine or quinidine as chiral selectors in the mobile phase. The molecular geometry of the diastereomeric complexes formed was modeled and energetic differences between both compounds were calculated by methods based on semi-empirical force-field. Good correlations were obtained between experimental enantioselectivity factors and calculated energetic differences.

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1. Introduction

Chiral ligand-exchange chromatography (CLEC), introduced by Davankov and Rogozhin [1] in the early seventies, was the first liquid chromatographic technique successfully used for the enantioseparation of amino acids and other solutes able to form coordination compounds with metal ions, such as α -hydroxy acids and β -amino alcohols. Intensive investigation in this field gave rise to numerous publications in the literature. Research studies were concentrated on the fundamental principles of CLEC, on the chiral recognition mechanism, on the synthesis of stationary phases and on practical applications to chiral separations [2–5].

Separations by means of a CLEC method are based on the formation of labile ternary metallic complexes in the mobile and/or in the stationary phase: one ligand functions as a suitable chiral selector, while the other is the enantiomer, component of the sample. Thus, enantiomers from the racemic sample, acting as ligands, form diastereomeric complexes with the metal ion and the chiral selector. The chiral selector chosen may be present in the mobile phase (chiral mobile phase mode, CMP) or in the stationary phase (chiral stationary phase mode, CSP). Metal complexing for enantiomeric resolution by a chiral mobile phase additive with a nonchiral conventional column was pioneered by Karger et al. [6]. For the CSP

mode, the chiral selector may be a constituent part of the stationary phase either by being covalently bound to the support [4,7] or by being adsorbed hydrophobically onto it [8]. The latter approach with the CSP mode is advantageous since that strategy allows the preparation of the column simply and economically by circulating a solution containing the chiral selector and the metal ion through the column until the detector response has proven stable. This versatile strategy allows a facile change of the chiral selector chosen according to the chemical characteristics of the racemate to be separated. Few disadvantages such as incompatibility with some detection modes (mass spectrometric), system-peaks and tolerance of only little amount of organic modifier can be mentioned. The enantioselectivity capability of these chromatographic systems depends on several variables: the chemical nature and superficial density of the chiral selector, the cation type and concentration, the nature of the metal counter-ion, the pH and the presence of other modifiers in the mobile phase [9–11].

Some of the chiral selectors employed for CLEC mode are N-alkyl-L-hydroxyproline (where alkyl is n-C₇H₁₅-, n-C₁₀H₂₁- and n-C₁₆H₃₃-) [12], N-n-decyl-L-histidine [13,14], which compound has been used to separate racemates of common amino acids mediated by Cu(II). Also, D-penicillamine and (R,R)-tartaric acid derivative have also been used with Cu(II) ion to direct enantioseparation of chiral carboxylic acids and amines obtaining enantioselectivity factors between 1.06 and 2.60. Other chiral selectors that have been used are N-substituted (S)-phenylglycinol derivatives [15], (S)-(N,N)-carboxymethyldeocylleucinol sodium

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salt [8], and L-acetylcarnitine [16], while more recently, (S)-trityl-(R)-cysteine [11] and O-benzyl-(S)-serine [17] were proposed in conjunction with Cu(II) ions to separate racemic amino acids.

Cinchona alkaloids are well known chiral auxiliaries for promotion enantioselective transformations in catalytic processes. The chemical structure of these alkaloids 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 vary in their configuration. In chromatography, quinine acetate dissolved in dichloromethane has been used as ion-pairing agent for the enantioseparation of carboxylic and sulphonic acids [18], although *Cinchona* alkaloids became popular more recently, when the group headed by Prof. Wolfgang Lindner have studied several derivatives extensively, mainly the carbamates formed from the hydroxyl group, as selectors in an ion-exchange mode [19–24].

In the present work, chiral separations of amino acids (both native and derivatized) were achieved by using a CLEC system and a conventional (nonchiral) octadecylsilica (ODS) column. To the best of our knowledge, no precedents exist for the use of *Cinchona* alkaloids as chiral ligands for enantioseparations in the CLEC domain. We have compared the enantioselectivities obtained with quinine (QN), quinidine (QD) and cinchonidine (CD) present in the eluent and have systematically studied the influence of the mobile phase composition, i.e., the pH and content of the organic modifier, in order to modulate the retention and enantioselectivity. In addition, two columns in which the QN and the CD moieties were covalently linked to a silica surface were also tested under otherwise comparable mobile phase conditions.

Finally, the semi-empirical PM6 [25] method was applied to model the complex geometries and obtain the most likely structures of the states of the intermediate coordination complexes presumably mediating the enantioseparations. The accuracy of the PM6 method for predicting heats of formation for compounds of biochemical interest is quite satisfactory. Hobza et al. [26] introduced an extension of the semiempirical PM6 method in two directions. The first included an empirical correction to the dispersion energy that improved the description of complexes controlled by that energy. The second introduced an additional electrostatic term that improved the description of hydrogen-bonded complexes. The resulting method, PM6 with corrections for dispersion and hydrogen bonding, was labeled PM6-DH+. The authors showed that the method provided stabilization energies that agreed quite closely with values obtained by much more operationally expensive methods [26]. The chiral discrimination mechanism is therefore discussed here in terms of calculations for the energy difference between both diastereomeric complexes.

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); the 4-N,N-dimethylaminoazobenzene-4'-sulfonyl (dabsyl) chloride and 2,4-dinitrofluorobenzene (99%) from Aldrich (St. Louis, MO, US); the CD (98%); QN and QD from Fluka (Buchs, Switzerland); the cupric acetate from Baker (J.T. Baker Chemical Co., Phillipsburg, NJ, US); and the HPLC-grade methanol from Mallinckrodt (Mallinckrodt Baker Inc., Phillipsburg, NJ, US). Water was purified by means of a Milli-Q Purification System (Simplicity, Millipore, Massachusetts, MA, US).

2.2. Derivatization procedure

Dinitrophenyl-amino acids (DNP-aas) were synthesized by a previously published procedure [27], and the derivatives were diluted in acetonitrile before injection. The derivatization procedure for dabsyl-amino acids (Dbs-aas) was taken from the literature [28].

2.3. Instrumentation and column preparation

Chromatographic studies were performed on an Agilent 1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with vacuum degasser, binary pump, Rheodyne Model 7010 injection valve (20- μ L sample loop) and variable wavelength detector. Data acquisition was effected by the Clarity software (DataApex, Czech Republic). A commercial Eclipse XDB-C18 (Agilent, USA) analytical column (75 mm \times 4.6 mm; 3.5 μ m) was employed for the CLEC experiments. The mobile phase was prepared by weighing on an analytical balance, the alkaloid and the copper salt (0.5 mM for each one) and then dissolving them into a mixture of 20%(v/v) methanol (MeOH) and 80%(v/v) aqueous 0.1 M $\text{NH}_4\text{OAc}/\text{NH}_3$ buffer. The glass electrode was calibrated with standard aqueous buffer solutions and the $s_w\text{pH}$ of the mobile phase was readjusted with either hydrochloric acid or sodium hydroxide to a desired pH value (between 6.40 and 9.00).

To equilibrate the ODS column, the mobile phase was run at 0.1 ml/min, in an open cycle, until the detector response proved stable (approximately 110–130 column volumes). The mobile-phase flow rate for analysis was set to 1 ml/min. In addition, two chiral columns based on QN and CD, which were covalently linked to mercaptopropylsilica have been tested [27,29]. The mobile phases for these experiments consisted in the mixture of Cu(II) ion, ammonia buffer and methanol in the above-described proportion.

The native α -amino acids were detected at 254 nm. The DNP-aas were monitored at 365 nm and the Dbs-derivatives at 436 nm. Hold-up times were estimated by injection of KBr and detection at 210 nm. The retention times were taken at maxima of the peaks.

3. Results and discussion

From the basic concept of CLEC, a transition metal with a tetra-valent coordination number will complex with electron-rich ligands. Thus the complexing between Cu(II) and *Cinchona* alkaloids is feasible because of the presence in their structure of a hydroxyl and an amino groups located on adjacent carbon atoms. Fig. 1 shows a scheme of the postulated structure for the complexes between each amino acid enantiomer and the *Cinchona* alkaloids mediated by the Cu(II) cation. The difference in the three-dimensional structures between both complexes shown would potentially lead to an enantioseparation. Complexation might possibly have different equilibrium constants and/or the partition equilibria of both diastereomeric complexes with the hydrophobic surface might prove different.

As antecedents, a carbon paste electrode incorporating a mixture of Cu(II) and QN has been developed and subsequently employed for voltammetric iodide determinations [30]. Also, the liquid-liquid extraction of *Cinchona* alkaloids through the formation of mixed complexes with optically active usnic acid mediated by divalent cations such as Cu(II), Co(II) and Zn(II) [31] has been described. No references are available, however, on the formation of coordination complexes between metal ions and both, *Cinchona* alkaloids and amino acids simultaneously.

3.1. CD as a chiral selector

Fig. 2 shows the chromatograms obtained after the injection of Phe (left) and Trp (right) under a CLEC system with a hydro organic

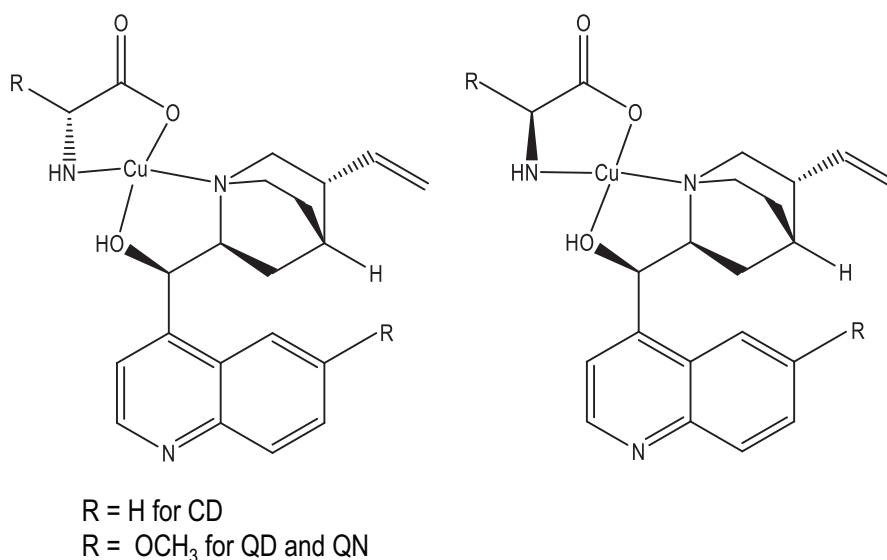


Fig. 1. Proposed structures of the ternary complexes between each *Cinchona* alkaloid, the Cu(II) ion, and amino acids of the D (left) or L (right) configuration.

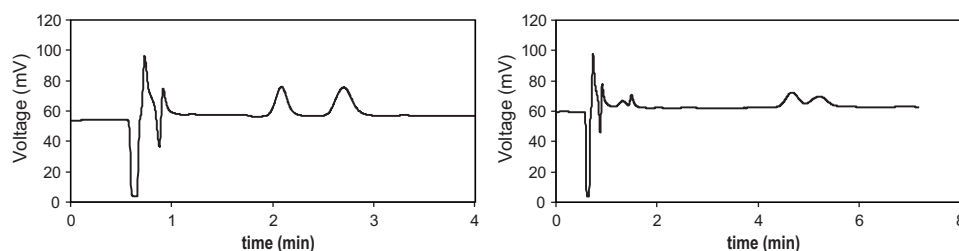


Fig. 2. Chromatograms showing the enantioseparation of Phe (left) and Trp (right). Column: Eclipse XDB-C18. Mobile phase 20:80 MeOH/buffer ammonium acetate, pH = 8.60. Flow-rate: 1 ml/min. Detection wavelength: 254 nm.

mobile phase containing CD and 20%(v/v) MeOH at pH=8.60. The enantioselectivity factors were 1.51 and 1.15, respectively. All the chromatograms obtained under these conditions showed the enantiomer peaks along with unavoidable system-peaks that, in some instances, made the data interpretation difficult. Other amino acids studied (Val, Pro, Met, Nor, Ile, Thr) could not be separated because of their very low retention under these conditions. As the MeOH content was decreased, however, their retention increased and the chiral separation became possible. Table 1 shows the retention factors, enantioselectivities and resolutions for the amino acids studied.

Table 1
Retention, enantioselectivity and resolution factors for amino acids with 0.5 mM CD as chiral selector.

| Amino acid | 10% MeOH | | | 5% MeOH | | |
|------------|----------|----------|-------|---------|----------|-------|
| | k_1 | α | R_s | k_1 | α | R_s |
| Met | 0.64 | 1.20 | <1 | 1.03 | 1.26 | <1 |
| Phe | 5.76 | 1.67 | 4.4 | – | – | – |
| Pro | 0.25 | 1.00 | – | 0.30 | 1.00 | – |
| Trp | 19.05 | 1.68 | 4.0 | – | – | – |
| Nor | 2.22 | 1.00 | – | 2.87 | 1.00 | – |
| Val | n.r. | n.r. | – | 0.61 | 1.21 | <1 |
| Ile | n.r. | n.r. | – | 1.61 | 1.36 | 1.22 |
| Thr | n.r. | n.r. | – | 0.16 | 1.40 | <1 |
| Ser | n.r. | n.r. | – | 1.00 | 1.22 | <1 |

n.r.: not retained.

Column: Eclipse XDB-C18. Mobile phase with two concentrations of methanol at pH=8.60. Flow-rate: 1 ml/min. Detection wavelength: 254 nm.

Another alternative for increasing the analyte hydrophobicity was to derivatize the amino acids, thus also enhancing signal selectivity (detection within the visible wavelengths, where the alkaloids present no absorption background at all) and sensitivity. We first derivatized the amino acids with Sanger's reagent, but the 2,4-dinitrophenyl derivatives obtained could not be separated. We attributed that lack of enantioseparation to the possibility that the deactivation exerted by the nitro groups on the aromatic ring had affected the availability of the free electron pair of the amino group to coordinate with the metallic cation. In contrast, the derivatization of the amino acids with dabsyl chloride produced much more hydrophobic molecules than the native amino acids so that the retention of the Dbs-aas by the ODS column became significantly higher and enantioseparations were achieved under elution gradients. The optimized results corresponded to the following linear gradient: initial 40%B, at 20 min 50%B, at 35 min 60%B (B: MeOH and A: 80%(v/v) buffer 0.1 M, 20%(v/v) MeOH containing 0.5 mM Cu(II) and 0.5 mM CD at pH=8.50). The amino acids chromatographed with this elution gradient (Val, Met, Thr, Phe, Nor and Abu) were strongly retained (retention factors ranging from 11 to 29) but their enantioselectivity factors were very low ($\alpha \sim 1.02$ – 1.06 ; data not shown).

3.2. QN and QD as chiral selectors

3.2.1. Eluent pH

The influence of the mobile phase pH on the retention and enantioselectivity was studied within the interval between 6.50 and 9.00 with QN as chiral selector. At the lower pH limit, the retention of all the amino acids decreased drastically because of the protonation

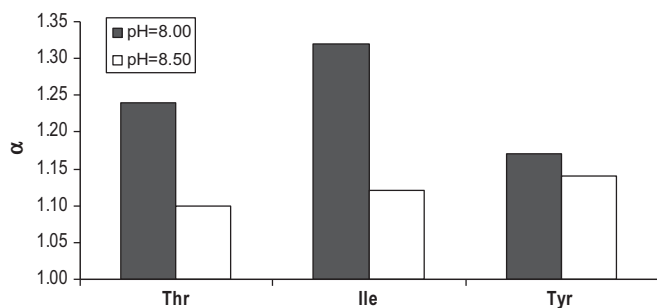


Fig. 3. Influence of the mobile phase pH on enantioselectivity for Thr, Ile and Tyr. Mobile phase 20%(v/v) MeOH with QN as chiral selector. Other conditions are as in Fig. 2.

of the quinuclidinic nitrogen of the alkaloid. The pKa value of the quinuclidinic nitrogen in water is 8.50 [32], whereas in 20%(v/v) MeOH can be estimated to decrease to about to 8.30 [33]. Therefore, at pH 6.50 the ratio $[QN]/[QNH^+]$ is 0.016, that is, less than 2% of the alkaloid molecules are unprotonated, thus preventing the formation of the coordination complex with Cu(II) ion. At pH=9.00, however, close to the silica-based column pH limit, a significant distortion of the peaks profile could be noted, losing enantioresolution capacity. At the intermediate pH (between 8.00 and 8.50), the retention remained practically constant, but the enantioselectivity factors were significantly higher at pH 8.00. Fig. 3 compares the enantioselectivity factors for Thr, Ile and Tyr at pH 8.00 and 8.50.

3.2.2. Comparison between QN and QD as chiral selectors

The influence of the amount of organic modifier was tested with both QN and QD as selectors in the mobile phase along with variable contents of MeOH (10 and 20%). Table 2 compares the retention and enantioselectivity data for several amino acids. A decrease in the amount of organic modifier in the mobile phase leads to an increase in retention times for all the amino acids with either alkaloid in the eluent. The organic modifier affects all the hydrophobic interactions, indicating that the latter are essential for the retention mechanism [5,14]. Table 2 also shows the influence of the variation

in the MeOH concentration on the enantioselectivity: when QD was the chiral selector in the mobile phase, a decrease in the MeOH concentration from 20% to 10% resulted in an increase between 7 and 9% in the enantioselectivity, but with QN as the chiral selector that same decrease produced the opposite pattern.

Fig. 4 shows a comparison between the retention factors and enantioselectivity values for several amino acids chromatographed in a mobile phase of 20%(v/v) MeOH buffered at pH=8.00 and equal quantities (0.5 mM) of Cu(II) and QN or either QD. A higher retention for all the amino acids and usually a higher enantioselectivity for most were achieved with QD, as opposed to QN, in the mobile phase. Furthermore, Nor and Abu displayed only a slight separation when QN was the chiral selector; whereas these two amino acids attained enantioselectivity factors as high as 1.56 and 1.38, respectively with QD in the mobile phase. QN (1S, 3R, 4S, 8S, 9R) and QD (1S, 3R, 4S, 8R, 9S) are referred to as pseudo-enantiomers because they have opposite absolute configuration at both C8 and C9. The stereochemistry of those molecules around these centers is primarily responsible for their enantioselectivity capacity. As a consequence, an inversion in the order of elution of the amino acids stereoisomers was observed, with the L-enantiomer eluting first with QD and the D-enantiomer with QN in the mobile phase. As an example, Fig. 5 shows the chromatograms obtained after the injection of Nor with a mobile phase containing 0.5 mM of QN (left) or QD (right) with 0.5 mM Cu(II) and 10%(v/v) MeOH at pH=8.00.

For most amino acids the retention times and the enantioselectivities as well were much lower for CD as the chiral selector in the mobile phase than for the other two alkaloids (data not shown).

3.3. Chiral recognition mechanism and molecular modeling

Unlike what happens in other chiral chromatographic techniques, in CLEC no direct interaction occurs between the chiral selector and the individual enantiomer, rather the metallic cation interacts with both molecules simultaneously to form a mixed ternary complex. In order to evaluate the role of the Cu(II) ion in the recognition mechanism, all the amino acids that were enantioresolved by having CD in the mobile phase were injected under the same conditions as before but without the Cu(II) ion present. The result was a total loss of the enantioselectivity of the

Table 2
Retention, enantioselectivity and resolution factors for amino acids chromatographed with variable amounts of MeOH at pH=8.00 and with either QD or QN in the mobile phase.

| Amino acids | QD | | | | | | QN | | | | | |
|-------------|-------|----------|-------|-------|----------|-------|-------|----------|-------|-------|----------|-------|
| | 20% | | | 10% | | | 20% | | | 10% | | |
| | k_1 | α | R_s | k_1 | α | R_s | k_1 | α | R_s | k_1 | α | R_s |
| Pro | 1.65 | 1.00 | – | 2.13 | 1.00 | – | 1.21 | 1.00 | – | 1.70 | 1.00 | – |
| Ser | 1.20 | 1.26 | <1 | 2.03 | a.p. | <1 | 0.81 | 1.16 | <1 | 1.18 | 1.19 | <1 |
| Val | 1.84 | 1.26 | <1 | 2.20 | 1.24 | <1 | 1.54 | 1.12 | <1 | 2.46 | 1.00 | – |
| Ala | 0.88 | 1.00 | – | 1.27 | 1.00 | – | 0.86 | 1.00 | – | 0.95 | 1.00 | – |
| Leu | 3.65 | 1.22 | 1.0 | 4.22 | 1.33 | <1 | 2.96 | 1.17 | <1 | 4.47 | a.p. | <1 |
| Phe | 10.21 | 1.62 | 2.2 | 15.28 | 1.75 | 1.2 | – | – | – | 20.01 | 1.13 | <1 |
| Trp | – | – | – | 10.03 | 2.20 | 1.2 | 26.09 | 1.38 | 1.9 | 62.30 | 1.26 | 1.0 |
| Met | 3.22 | 1.00 | – | 4.14 | a.p. | <1 | 2.17 | 1.00 | – | 3.05 | 1.00 | – |
| Nor | 4.06 | 1.56 | 1.7 | 5.22 | 1.66 | 1.1 | 5.50 | a.p. | <1 | 6.42 | 1.20 | <1 |
| Abu | 1.19 | 1.38 | 1.0 | 1.82 | a.p. | <1 | 1.09 | a.p. | <1 | 1.52 | a.p. | <1 |
| Thr | 1.07 | 1.39 | 1.0 | 1.24 | 1.49 | <1 | 0.78 | 1.24 | <1 | 1.02 | 1.25 | <1 |
| Arg | 2.41 | 1.00 | – | 3.07 | 1.00 | – | 1.47 | 1.00 | – | 1.47 | 1.00 | – |
| Ile | 3.53 | 1.12 | <1 | 4.53 | a.p. | <1 | 2.94 | 1.32 | 1.0 | 4.53 | 1.17 | <1 |
| His | 3.66 | 1.00 | – | – | – | – | 1.71 | 1.00 | – | 3.51 | 1.00 | – |
| Asp | 0.66 | 1.00 | – | 0.91 | 1.00 | – | 0.69 | 1.00 | – | 1.06 | 1.00 | – |
| Asn | 1.10 | 1.00 | – | 1.35 | 1.00 | – | 0.70 | 1.00 | – | 1.08 | 1.00 | – |
| Lys | 2.85 | 1.00 | – | 3.18 | 1.00 | – | 1.48 | 1.00 | – | 1.66 | 1.00 | – |
| Tyr | 7.06 | 1.62 | 1.5 | – | – | – | 4.34 | 1.17 | 1.0 | 9.78 | 1.13 | <1 |
| Orn | 4.02 | 1.00 | – | 4.35 | 1.00 | – | 1.69 | 1.00 | – | 2.11 | 1.00 | – |

a.p.: asymmetric peak.

Other experimental conditions are as in Table 1.

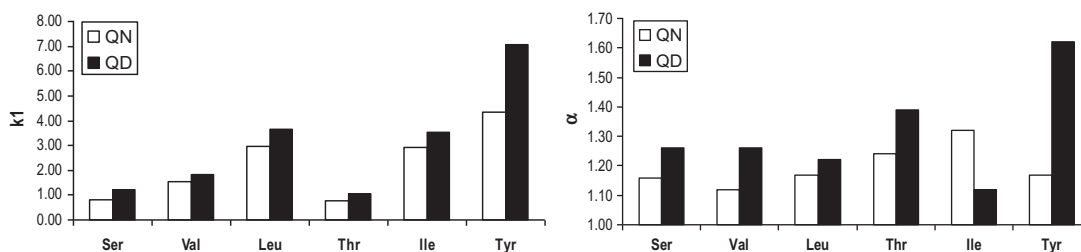


Fig. 4. Retention (left) and enantioselectivity (right) factors for several amino acids chromatographed with a mobile phase containing 20%(v/v) MeOH buffered at pH = 8.00 along with containing equal quantities (0.5 mM) of Cu(II) and either QN or QD.

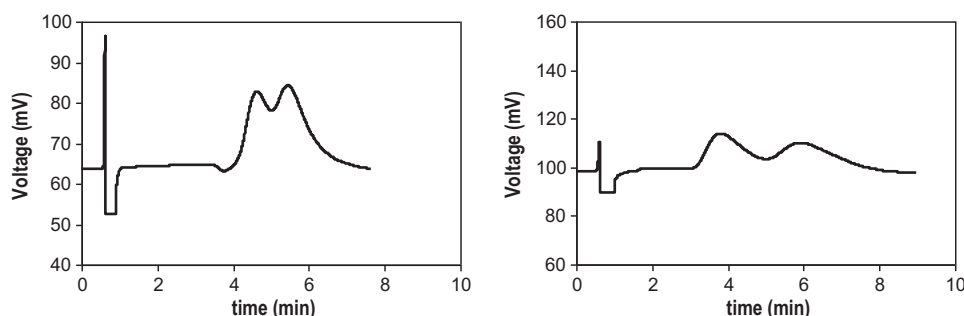


Fig. 5. Chromatograms showing the enantioseparation of Nor. Mobile phase 10%(v/v) MeOH pH = 8.00 and 0.5 mM QN (left) or QD (right). Other conditions are as in Fig. 2.

system confirming that the metal ion is critically involved in the chiral recognition mechanism.

The calculation of heats of formation for compounds of interest in biochemistry by the semiempirical PM6 method is somewhat better than by the Hartree–Fock (HF) or DFT-based methods, such as B3LYP, through the use of a 6-31G(d) basis set. For a representative set of compounds, PM6 has been found to give an average unsigned error of 4.4 kcal/mol, which value is far lower than the average unsigned errors provided by the HF and B3LYP methods [25]. The extended PM6-DH+ method was used in the present study to model the set of ternary complexes formed between the specific alkaloid, the Cu(II) ion and the amino acid. To that end, the complexes were modeled through the use of the free molecule and the visualizer editor Avogadro [34]. The most stable conformation for the alkaloid molecules found by Urakawa et al. [35], referred to as *Open* (3), was used as the starting point for establishing the geometry. Then, the structures of CD, QN and QD were optimized. For the amino acids, both the D and L configurations were considered as starting points. Moreover, a fourfold coordination sphere was considered around the Cu(II) ion formed by the nitrogen and the oxygen atoms from the alkaloid hydroxyl moiety along with a nitrogen and an oxygen atoms from the amino acid. Geometric isomerism was accounted for by allowing the two nitrogen atoms (and, consequently, the two oxygen atoms) to be either *cis* or *trans* to each other. Therefore, four starting structures were constructed for every complex. To find lower-energy conformations of the complexes under study a conformational search was carried out by means of a brute force method in which certain selected dihedral angles not affecting the coordination sphere around the copper ion were modified. All the calculations were performed at the PM6-DH+ level of theory as implemented in the MOPAC program [36]. Solvent effects (water) were taken into account through a polarizable continuum method (PCM). In all the examples the most stable complexes led to a square planar coordination around the Cu(II) ion. The result of the energy calculations are listed in Table 3 and the modeled geometry of the complexes formed by QD-Val and QN-Ser, both mediated by Cu(II), are shown in Figs. 6 and 7, respectively. The figures illustrate that for CD complexes the nitrogen atoms are located in the *trans* configuration from each other for both the D

and L-amino acid isomers (an array also observed for the CD-Ala complexes); but interestingly, for the QN complexes, the configuration of the two nitrogen atoms is *trans* for the D-isomer, but *cis* for the L-isomer. Furthermore, the L-isomer is found to be more stable than the D-isomer by a difference of 2.508 kcal/mol. The large differential in the energy between the two isomers could be attributed to the oxygen of the γ -hydroxyl group in Ser lying in close spatial proximity to the Cu(II) ion in the L-isomer, there forming a pseudo fivefold coordination sphere around the metallic center.

Finally, two chiral columns in which either CD or QN were covalently linked to mercaptopropylsilica have been tested [27,29]. The mobile phases for these experiments consisted in a mixture of Cu(II) ion, ammonia buffer, and methanol in the same composition as described above but without the addition of the alkaloid. No enantioseparation for any racemic amino acid was obtained with the CD-based column, while with the QN-based column, Phe and Trp

Table 3

Calculated energetic differences between both diastereomeric complexes, the more stable diastereomeric form, bond lengths and bond angles for the complexes modeled.

| | CD-Cu-Ala | QD-Cu-Val | QN-Cu-Ser |
|-------------------------------|-----------|-----------|-----------|
| More stable diastereomer | D | L | L |
| Energetic difference/kcal/mol | 0.922 | 0.265 | 2.508 |
| Bond lengths ^a /Å | | | |
| Cu–N(aa) ^b | 1.915 | 1.917 | 1.959 |
| Cu–N(alk) ^c | 1.878 | 1.876 | 1.894 |
| Cu–O(aa) ^d | 1.898 | 1.900 | 1.924 |
| Cu–O(alk) ^e | 1.998 | 1.985 | 1.993 |
| Bond angles ^d /° | | | |
| N(aa)–Cu–N(alk) | 177.5 | 178.5 | 103.8 |
| N(aa)–Cu–O(aa) | 86.7 | 86.7 | 84.5 |
| N(aa)–Cu–O(alk) | 99.0 | 96.8 | 174.9 |
| N(alk)–Cu–O(aa) | 95.2 | 94.6 | 171.0 |
| N(alk)–Cu–O(alk) | 79.2 | 82.0 | 81.2 |
| O(aa)–Cu–O(alk) | 173.2 | 170.0 | 90.6 |

^a For the more stable diastereomeric complex.

^b Nitrogen atom of the amino acid amino group.

^c Nitrogen atom of the alkaloid quinuclidine ring.

^d Oxygen atom of the amino acid carboxylic group.

^e Oxygen atom of the alkaloid hydroxyl group.

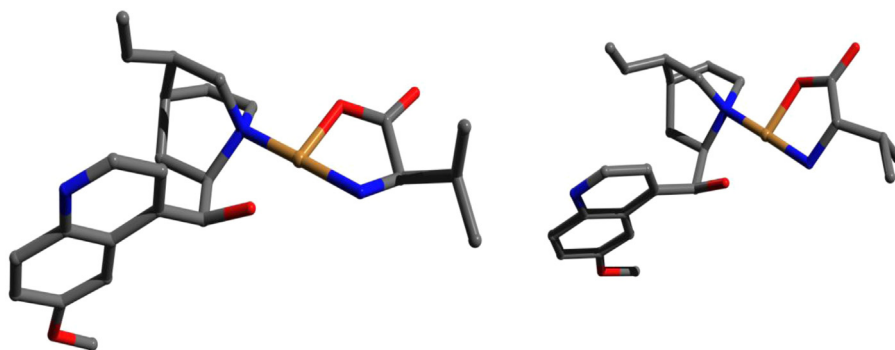


Fig. 6. Molecular geometry of the diastereomeric complexes formed between D-Val (left) or L-Val (right) and QD mediated by Cu(II). The yellow atom represents copper, the gray atoms correspond to carbon, the blue ones to nitrogen, the red ones to oxygen and the white ones to hydrogen atoms. (For interpretation of references to color in this figure legend, the reader is referred to the web version of this article.)

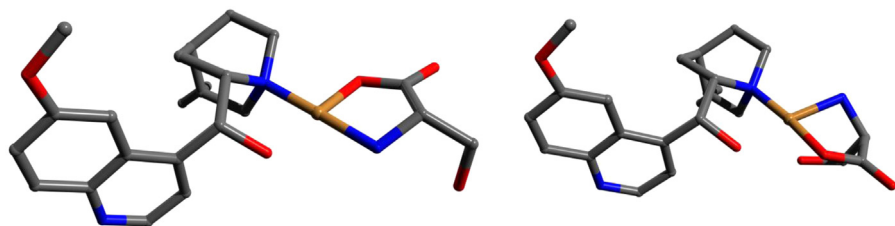


Fig. 7. Molecular geometry of the diastereomeric complexes formed between D-Ser (left) or L-Ser (right) and QN mediated by Cu(II). The color code is the same as in Fig. 6.

were the only two racemic analytes that could be enantioseparated ($\alpha = 1.27$ and 1.29 , respectively).

4. Conclusions

Cinchona alkaloids are for the first time proposed here as chiral selectors in the mobile phase under a CLEC mode. QN, QD, and CD were successfully used for the enantioseparation of several α -amino acids through the diastereomeric complexes formed with Cu(II) ions. In this approach the retention times and enantioselectivity factors can be easily modulated by controlling some of the tuneable mobile phase parameters: pH and MeOH content. The diastereomeric complex geometry was theoretically modeled through the use of the extended PM6-DH+ method and the energetic differences calculated between both diastereomeric forms of a given amino acid with an alkaloid molecule, after taken into account the effects of the solvent. Excellent agreement between the differences in energy of formation and enantioselectivity factors were obtained for two systems (CD-Cu-Ala and QD-Cu-Val). In contrast, with serine the calculated energetic differences did not agree with the enantioseparation observed experimentally.

Acknowledgements

We wish to thank to CONICET (PIP-0777), ANPCyT (PICT2011-1611) and UNLP (Argentina) for the financial support. We are grateful to Prof. T.S. Kaufman (Departamento de Química Orgánica, U.N. Rosario, Argentina) for their valuable contributions. Dr. Donald F. Haggerty, a retired career investigator and native English speaker, edited the final version of the manuscript.

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