Micellar extraction of amino acids using chiral hydrophobic selectors. A comparison with chromatographic procedures

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In this work we study the micellar extraction of two amino acids, namely tryptophan and tyrosine, using *N*-*n*-dodecyl-L-proline (**1**) and *trans*-*N*-*n*-dodecyl-4-hydroxy-L-proline (**2**) as hydrophobic chiral selectors with copper(II) ions. In the first part of our study the solubilities of the two selectors **1** and **2** and their ability to form micelles are examined. Quantum mechanical calculations are performed to access their Gibbs solvation energies in order to explain their different behaviours. Micellar extraction is then studied using both selectors solubilised in non-ionic micelles. The results obtained are discussed and comparisons made with the data reported in the literature for similar selectors deposited onto an octadecyl silylated stationary phase in chromatographic procedures or solubilised in a real biphasic system in solvent extraction experiments.

Introduction

Micelles have demonstrated over many years their ability to solubilise or to bind many different solutes: for instance, the solution properties of phenol derivatives,[1] aliphatic alcohols,[2] dyes,[3] synthetic perfumes,[4] apolar molecules,[5] and ions with ionic surfactant[6] have been shown to be greatly influenced by surfactant aggregates. More recently, the coupling of ultrafiltration to micellar extraction has shown that micellar systems have interesting potential in the analytical chemistry of enantiomeric species[7] or geometric isomer species,[8] or in the removal of polluting species from aqueous waste effluents.[9] It has also been shown that solubilising a complexing hydrophobic molecule allows the selective complexation and extraction of different metal ions.[10] In these cases the stoichiometry and the stability of the organometallic species formed can be comparable to those obtained in real biphasic experiments, demonstrating that the phenomena involved in biphasic and micellar systems are basically the same.[11] In fact, one can consider that the micellar core acts as a micro-organic phase, usually called the micellar pseudo-phase. The limit of this analogy is attained when one considers the stoichiometry of the complex formed in both media: non-neutral complexes can be formed at equilibrium in given conditions in micelles, whereas in biphasic systems, when the complexing agent has no labile proton, a counterion has to be extracted to ensure its solubility in the organic phase.[11,12]

In this work we aim to study the extraction of two different amino acids, using their capacity to form ternary complexes with another ligand and a metal ion in a micellar microenvironment. Moreover, we also examine the resolution feasibility. Our interest in such systems was stimulated by an original paper by Creagh *et al.*[13] In their work, these authors used a cholesterol glutamate derivative as the selector, phenylalanine as the amino acid, and Cu(II) as the metal ion. Ternary complexes were formed by these species in non-ionic micelles with good enantioselectivity. However, cholesterol glutamate is a very expensive selector. Furthermore, as recently discussed by De Bruin *et al.*,[14] its polyfunctionality and the presence of nine stereogenic centres impede understanding of the phenomena governing the resolution of racemics. Thus we decided to check other selectors. Our criteria for a good selector were: i) must be available in great quantities; in other words it has to come from the so-called chiral natural pool, ii) chemical synthesis must be as straightforward as possible, iii) must not be racemisable under usual chemical conditions, iv) and moreover, must be lipophilic enough to ensure a preferential adsorption of the complex onto the micelle.

With this last point in mind, the knowledge brought by studies with chiral aqueous eluents (see, for instance, ref. 15 and references cited therein) or with covalently bonded selectors (see, for instance, ref. 16 and references cited therein) could not be directly applied to this study.

Bearing in mind these four points, we focused on two selectors [namely *N*-*n*-dodecyl-L-proline (**1**) and *trans*-*N*-*n*-dodecyl-4-hydroxy-L-proline (**2**)] that had already been investigated by chromatography,[17] emulsion liquid membranes,[18] in normal biphasic liquid–liquid extraction conditions,[19] or with the help of a hollow-fibre device.[20] These two last points were of course of special importance for us, since we had good reason to expect that the results observed in real biphasic systems should be transferable to micellar systems.[11]

The literature reports that various amino acids can be extracted by these L-proline and L-hydroxyproline derivatives in the presence of cupric ions. The enantioselectivity depends to some extent on the length of the alkyl moieties of the selector, but also on the organic solvent used to solubilise the ternary complex.[19] Enantiomers have also been separated using dynamic coating protocols in HPLC experiments.[17] The overall data indicate that chiral recognition is higher with the *trans*-4-hydroxy-L-proline than with the L-proline derivative, whether in chromatography[17a] or in a biphasic liquid experiment.[19]

This study is restricted to two amino acids, tryptophan (Trp) and tyrosine (Tyr), chosen for their high complexing ability towards copper[21] and for their chromophoric group that allows easier detection.

Experimental

Syntheses

The synthesis of the selectors was achieved according to the

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method proposed by Ding *et al.*,[20] except for the hydrogen pressure, which was raised to 5.0 MPa to ensure a more rapid reduction of the intermediate imine.

***N-n*-Dodecyl-L-proline (1).** From *n*-dodecanal (0.1 mol) and L-proline (0.05 mol), after purification by preparative chromatography on neutral alumina with CH_2Cl_2–EtOH (95 : 5) was obtained **1** (9.7 g, 68%) as a white wax: mp 112–114 °C (Et_2O, Tottoli, uncorrected), $[\alpha]_D$ −40.6° (*c* 1, HCl 1 M); δ_H ($CDCl_3$, 250 MHz) 0.85 (t, 3 H, $C_{10}H_{20}$-C*H*$_3$), 1.15–1.4 (m, 18 H, 9 × C*H*$_2$), 1.75 (m, 2 H, N-CH_2-C*H*$_2$), 1.95 (m, 2 H, N-C*H*$_2$-$C_{10}H_{20}$), 2.2 (m, 1 H), 2.35 (m, 1 H), 2.8 (dd, 1 H), 2.95 (m, 1 H), 3.15 (m, 1 H), 3.65 (m, 1 H), 4.0 (1 H, H-2), 8.3 (br s, 1 H, CO_2H); δ_C ($CDCl_3$, 90 MHz) 170.3 (CO_2H), 70.0 (C-2), 55.9 (N-*C*H$_2$-$C_{10}H_{20}$), 55.0 (C-3), 32.0, 29.7, 29.6, 29.55, 29.4, 29.2, 26.9, 26.0, 23.6, 22.75 (C-4, -5, 8 × *C*H$_2$), 14.2 ($C_{10}H_{20}$-*C*H$_3$); EIMS calcd. for $C_{17}H_{33}NO_2$ 283, found *m*/*z* 284 $[M + H]^+$; anal. calcd. C, 72.0; H, 11.7; N, 4.9; found: C, 71.4; H, 12.0; N, 4.9%.

***trans*-*N*-*n*-Dodecyl-4-hydroxy-L-proline (2).** From *n*-dodecanal (0.075 mol) and *trans*-4-hydroxy-L-proline (0.05 mol), after preparative recrystallisation from H_2O–EtOH (50 : 50) was isolated **2** (9.0 g, 60%) as a white solid: mp 162–164 °C (H_2O, Tottoli, uncorrected), $[\alpha]_D$ −38.2° (*c* 1, HCl 1 M); δ_H ($CDCl_3$, 250 MHz) 0.83 (t, 3 H, $C_{10}H_{20}$-C*H*$_3$), 1.1–1.35 (m, 18 H, 9 × C*H*$_2$), 1.7 (m, 2 H, N-CH_2-C*H*$_2$), 2.0 (t, 2 H, N-C*H*$_2$-$C_{10}H_{20}$), 2.4 (m, 1 H), 3.0 (d, 1 H), 3.1 (m, 1 H), 3.3 (m, 1 H), 3.5 (br s, 1 H, OH), 3.9 (dd, 1 H), 4.0 (m, 1 H, H-2), 4.45 (dd, 1 H); δ_C ($CDCl_3$, 90 MHz) 171.15 (*C*O$_2$H), 69.45, 69.2 (C-2, -4), 61.5 (N-*C*H$_2$-$C_{10}H_{20}$), 57.8 (C-3), 38.65, 31.9, 29.65, 29.6, 29.5, 29.35, 29.25, 26.6, 26.0, 22.7 (C-4, -5, 8 × *C*H$_2$), 14.1 ($C_{10}H_{20}$-*C*H$_3$); EIMS calcd. for $C_{17}H_{33}NO_2$ 299, found *m*/*z* 300 $[M + H]^+$; anal. calcd. C, 68.2; H, 11.1; N, 4.7; found: C, 67.9; H, 11.2; N, 4.9%.

Procedures

Ultrafiltration technique. An 8010 Amicon cell, designed to work with 10 ml of the micellar solution, was used with 10 kd cellulosic membranes (YM 10, Amicon). The working pressure was 0.3 MPa. In all the experiments the pH was adjusted with diluted HCl or NaOH.

Quality control of the ultrafiltration membrane. Before each experiment, after rinsing, a filtration of water was performed and the time required to filter 5 ml of water measured; its reproducibility indicated that there was no fouling or pinhole of the membrane.

HPLC. The device was a Kontron series 300 system, equipped with a 25 cm length × 4.5 mm id Kromasil column. The eluents were of gradient type as follows: H_2O–CH_3CN = 90 : 10 to 50 : 50 in 10 min (1 ml min^{-1}) for Trp and H_2O–CH_3CN = 100 : 0 to 60 : 40 in 10 min (1 ml min^{-1}) for Tyr. Calibration curves were realised using standards in acetonitrile. UV detection was achieved at 274 nm for Trp and 230 nm for Tyr.

Chemicals. Water was purified using an ELIX3 (Millipore apparatus). All other chemicals were of analytical grade, D or L-amino-acids (puriss p.a.) and Triton X-100 (BioChemika) were purchased from Fluka, acetonitrile from Carlo Erba. Surface tension was measured with a Kruss 10 digital tensiometer at 25 °C using the Wilhelmy plate method.

Preparation of the solutions. The selectors were first dispersed in pure TX-100, which is a viscous oil at room temperature. The resulting mixtures were then diluted with the aqueous amino acid solution, and then a copper solution was added. The final solutions were always clear, in the pH range 2–11, with a blue colour becoming more intense with increasing pH.

Table 1 Gibbs free energy of solvation (in kcal mol^{-1})

Molecule	Form	Micelle	Water
1	Neutral	−15.9	−4.1
	Zwitterionic	−42.3	−23.3
2	Neutral	−19.2	−8.7
	Zwitterionic	−43.2	−26.6

Blank experiments. Various blank experiments were performed. There was no copper complexation by TX-100 micelles alone. In the absence of selector and micelles, an insoluble copper complex of Trp is formed, simulating an amino acid membrane rejection. In the presence of selector, much less copper being free in solution and no precipitate being observed, non-selective extraction of amino acids and hydrolysed species was negligible.

Copper concentrations. These were measured using the atomic absorption technique (Varian AA 1275) with an air–acetylene flame. Calibration was carried out using Cu(II) standards in aqueous solution containing the CMC (critical micelle concentration) of Triton X-100.[22]

Results and discussion

Due to a lack of information on the two selectors we decided to study their solution properties in a preliminary step before further investigation.

Solubility and surface properties of the selectors

We first checked the solubilities of our two selectors in water. We were able to prepare 50 mM solutions of **1** at pH > 6. Surprisingly, the hydroxylated molecule **2** is much less soluble in water than **1**, even if the temperature is raised to 50 °C. In order to get a deeper insight into this peculiar behaviour, we performed quantum mechanical calculations to obtain the Gibbs free energy of solvation of both compounds in water. Since these molecules are able to undergo micellisation, they can exist in solution in two forms, either as isolated molecules or as micelles. We decided, for practical reasons, to model these two systems (micelle and aqueous solution) with the solvation models available in Amsol.[23] In these models, a single molecule is surrounded by an infinite polarisable dielectric continuum representing the solvent. The micelle medium is mimicked by the solvent octanol in order to simulate both the hydrophobic and hydrophilic interactions, and of course, the specific solute–solvent hydrogen bond, should this exist. Doing this, we assumed that the hydrogen bonding interactions present in octanol are equivalent to those present in the micellar aggregates, although it has been experimentally proved that hydrogen bonding may play a specific role in particular cases.[24]

All energy calculations were done with the PM3 Hamiltonian[25] in order to keep the computational time within reasonable limits. Geometries were fully optimised for isolated molecules, and also in octanol and in water. The solvent models used were SM3[26] for water and SM5.4P[27] for octanol. As both molecules are substituted amino acids, they exist in aqueous solution in zwitterionic forms. We then computed the Gibbs free energy of solvation for both the zwitterionic and the neutral forms for the two molecules under investigation. The results are gathered in Table 1.

One can see, from Table 1, that in all cases the *trans*-*N*-*n*-dodecyl-4-hydroxy-L-proline (**2**) molecule is slightly more solvated than **1** in aqueous solution and in octanol. It is noteworthy that the differences in Gibbs free energy of

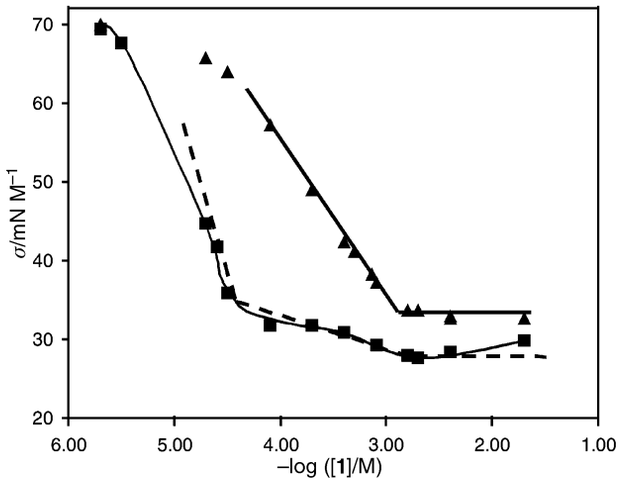

Fig. 1 Plots of the surface tension *versus* the logarithm of **1** concentration. (▲) pH = 6; (■) $[Cu^{2+}]/[\mathbf{1}] = 1$.

solvation between the two molecules are small, but larger than the accuracy limit of the methods used (1–2 kcal mol^{-1}). Nevertheless, the different solubility behaviour cannot be understood from these solvation results.

One can consider the solubilisation process as two steps. The first one transforms the molecules from the solid phase into the gas phase, and the second one solvates the isolated gas phase molecules. From this point of view, the energy variation associated with the solubility process contains two contributions, the opposite of the crystal energy and the solvation energy. It is then clear that the difference in the crystal energy between the two species under study is responsible for the peculiar solubility behaviour of **2**, since our calculations show that the Gibbs free energies of solvation for the molecules are close to each other. Computing this energy difference accurately is beyond our computer facilities. However, since both molecules differ only by a hydroxy group, one can suggest that the difference in crystal energies can be approximated by the potential of the hydroxylated molecule to form intermolecular hydrogen bonds. This is supported by the crystal structure of L-hydroxyproline.[28]

A crude estimate (−15 kcal mol^{-1}) of the specific intermolecular interaction energy is obtained by means of a PM3 quantum chemical calculation of a dimer of *trans*-*N*-*n*-dodecyl-4-hydroxy-L-proline (**2**). This energy contains, in addition to the hydrogen bonding interaction, the attractive interaction between alkyl chains, which is common to both molecules. The same type of calculation on *N*-methyl-L-hydroxyproline dimer, in order to remove the main part of the alkyl chain interaction, gives the stabilisation due to hydrogen bonding as *ca.* 5 kcal mol^{-1}. This contribution is large enough to ensure that the hydroxylated species (**2**) will be less soluble in water than **1**, due to specific intermolecular interactions in the solid phase.

The surface tension *versus* the logarithm of the concentration of **1** is plotted in Fig. 1. In water at pH 6, which is the pH of the isoelectric point of the proline, the variation observed is rather typical of a surfactant: the surface tension decreases until a plateau is reached. The breakpoint of this curve (*i.e.* the CMC) is close to 1 mM, which is comparable to that observed when a zwitterionic surfactant bearing a dodecyl chain is considered.[22] The constant value of the surface tension is also typical of an hydrogenated alkyl chain surfactant, and the absence of a well around the CMC clearly indicates that there is no reason to suspect the presence of hydrophobic impurities. In the presence of copper ions, the curve is much more original. As have other authors who have worked with β-alanine derivatives, we could distinguish two breaks in the curve.[29] In the case of β-alanine the first break was attributed to the formation of a surface-active metal–surfactant complex and the second one to the CMC of the free surfactant. This explanation is not completely satisfying for us: one has to bear in mind that both the aggregation and the complexation are dynamic processes; one cannot strictly distinguish a first step of complexation and a second of aggregation, each phenomenon being related to the other. A more rigorous approach would describe this curve as a continuous decrease of the surface tension resulting from the two simultaneous processes; an entire theoretical description is beyond the scope of this work.

Again, as in the case of β-alanine derivatives, the ratio of the concentration of copper(II) ions *vs.* the selector cannot be varied outside a narrow range of values: for metal concentrations lower than those of the ligand, the formation of complexes involving two selectors for one metal induces a precipitation that could cause the formation of large lamellar aggregates instead of micelles.[29,30]

These solubility problems led us to consider mixed micelles constituted of a neutral surfactant, namely Triton X-100, and a selector, instead of pure selector micelles to perform the amino acid extraction.

Micellar amino acid extraction

Typically, in our experiments of micellar extraction coupled with ultrafiltration, a given volume of the studied micellar solution at thermodynamic equilibrium was transferred into the ultrafiltration cell and a given fraction of this solution was filtered. The usual assumption is to consider that, in the absence of the Donnan membrane effect, the concentrations of the solutes in the filtrate are identical to those in the aqueous pseudo-phase surrounding the micelles at equilibrium before ultrafiltration. Moreover, one has to consider that the equilibrium is not displaced during the ultrafiltration (see ref. 11 and references cited therein). Given the blank experiments described in the experimental part, the mass balance equations are eqns. (1) and (2):

$$[AA]_0 = (V_r/(V_r + V_f))[AA]_m + [AA]_f \quad (1)$$

$$[AA]_r = [AA]_m + [AA]_f \quad (2)$$

AA means amino acid, $[AA]_m$ being the concentration in the micelles, $[AA]_0$ the overall initial concentration, $[AA]_r$ the retentate and $[AA]_f$ the filtrate and water pseudo-phase concentrations, V_f and V_r the filtrate and retentate volumes, respectively. The results obtained here can be presented in two sets: the extraction of the amino acid itself and the co-extraction of copper.

When the extraction of the amino acid is considered, the results are plotted as a partition coefficient Q as a function of pH. Q is calculated according to eqn. (3):

$$Q = \frac{[AA]_r - [AA]_f}{[AA]_f} \quad (3)$$

It can be deduced from eqns. (1) and (2) that Q represents the partition coefficient really observed at the end of the filtration experiment, *i.e.*, when the micelles are concentrated by a factor of two.

When the extraction of Cu(II) ion is considered we choose to present the results in terms of yields, Y%, whose values are calculated as eqn. (4):

$$Y\% = \frac{[Cu]_0 - [Cu]_f}{[Cu]_0} \times 100 \quad (4)$$

$[Cu]_0$ and $[Cu]_f$ are the initial and filtrate concentrations of copper, respectively.

For the sake of clarity we felt it necessary to make explicit the relation between Q and Y. This can be done by calculating Q values for copper distribution, or simply considering the most

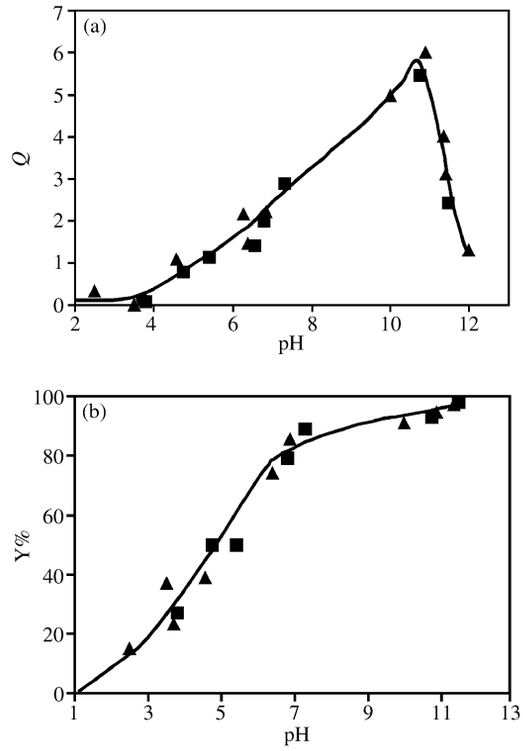

Fig. 2 a) Amino acid partition coefficient, *Q*, *versus* pH plot for Trp extracted by **1**. b) Yields, *Y*%, *versus* pH plot for the extraction of Cu(II) *versus* pH for Trp extracted by **1**. (■) [D-Trp] = 5×10^{-4} M; (▲) [L-Trp] = 5×10^{-4} M; [**1**] = 10^{-3} M; [$CuCl_2$] = 10^{-3} M; [Triton X-100] = 2×10^{-2} M.

simple case, in which an amino acid is extracted simultaneously with a single copper ion. Then, taking into account V_f and V_r, the relationship becomes eqn. (5):

$$Y\% = \frac{100\ QV_r}{V_f + V_r(1 + Q)} \quad (5)$$

In our experimental conditions, $V_r = V_f = 5$ mL; the relationship now simplifies to eqn. (6):

$$Y\% = \frac{100\ Q}{2 + Q} \quad (6)$$

The selectivity between two enantiomers or two amino acids, usually called α, can be calculated directly from the ratio of the *Q* values of the two solutes considered.

Figs. 2 to 5 report the results relative to tryptophan and tyrosine for the two selectors. In these experiments: i) the ratio of the concentration between the selector and Triton X-100 is low enough to avoid any problems of solubility; ii) the ratio of copper to selector is 1, and there is twice the amount of selector compared to amino acid to be extracted. The reason for this is that we wanted to tune experimental conditions to allow a total extraction of the amino acid, assuming a ternary complex having a 1 : 1 : 1 stoichiometry and avoiding as far as possible free species (*i.e.*, copper ion or amino acid) that probably lower the selectivity of such systems.

In all the experiments reported here, the extraction increased continuously for pHs ranging from 2 to 11, and then for higher pHs the amino acid extraction decreased. Another general trend of our results can be stressed: the extraction of tryptophan is more efficient than that of tyrosine, whatever the selector considered. Using the *trans*-4-hydroxy-L-proline instead of the proline derivative as the selector did not improve the *Q* values.

A major difference between copper and amino acid extraction is that for copper the yields did not decrease for pHs above

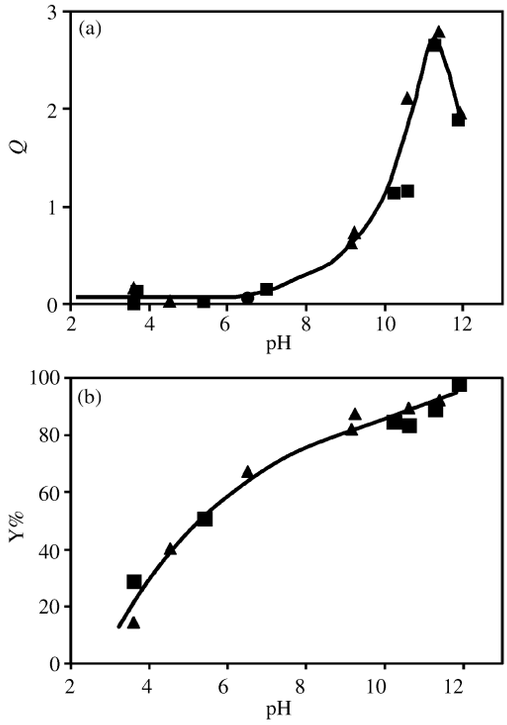

Fig. 3 a) Amino acid partition coefficient, *Q*, *versus* pH plot for Tyr extracted by **1**. b) Yields, *Y*%, *versus* pH plot for the extraction of Cu(II) *versus* pH for Tyr extracted by **1**. (■) [D-Tyr] = 5×10^{-4} M; (▲) [L-Tyr] = 5×10^{-4} M; [**1**] = 10^{-3} M; [$CuCl_2$] = 10^{-3} M; [Triton X-100] = 2×10^{-2} M.

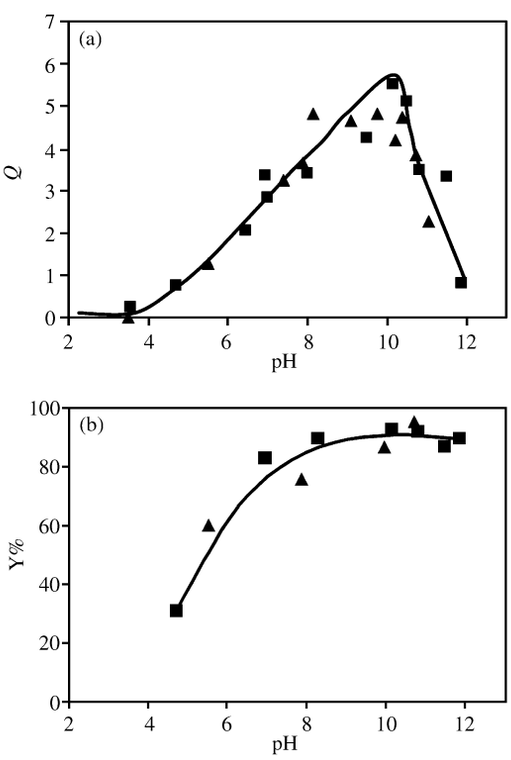

Fig. 4 a) Amino acid partition coefficient, *Q*, *versus* pH plot for Trp extracted by **2**. b) Yields, *Y*%, *versus* pH plot for the extraction of Cu(II) *versus* pH for Trp extracted by **2**. (■) [D-Trp] = 5×10^{-4} M; (▲) [L-Trp] = 5×10^{-4} M; [**2**] = 10^{-3} M; [$CuCl_2$] = 10^{-3} M; [Triton X-100] = 2×10^{-2} M.

11. A second difference is that the yield of copper extraction for a given pH is higher for Trp than for Tyr, whatever the selector considered here. Finally the last general trend for copper extraction behaviour is that at low pH (below 7) the copper ions

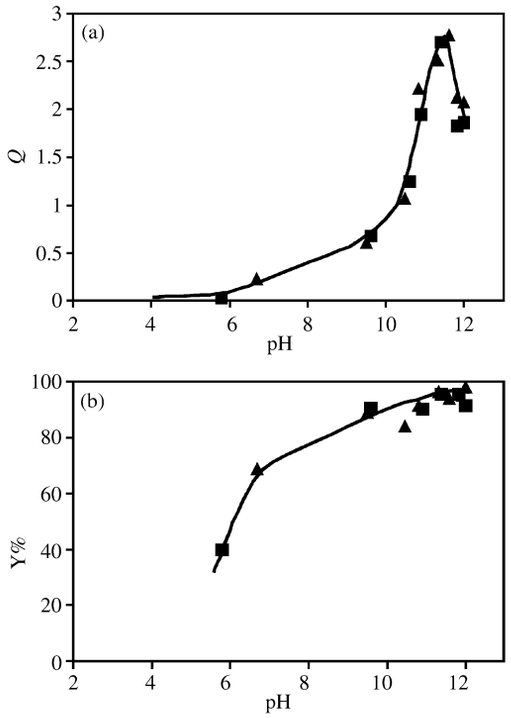

Fig. 5 a) Amino acid partition coefficient, *Q*, *versus* pH plot for Tyr extracted by **2**. b) Yields, *Y*%, *versus* pH plot for the extraction of Cu(II) *versus* pH for Tyr extracted by **2**. (■) [D-Tyr] = 5×10^{-4} M; (▲) [L-Tyr] = 5×10^{-4} M; [**2**] = 10^{-3} M; [$CuCl_2$] = 10^{-3} M; [Triton X-100] = 2×10^{-2} M.

are extracted to a far greater extent than the amino acid. We observed *Q* values for Tyr not exceeding 0.25 below neutrality and copper yields often higher than 50%; this clearly indicates that the complex CuSelAA (*i.e.*, the complex which involves one copper atom, one selector, and one amino acid) is not the only species responsible for copper extraction in our experiments.

From these observations we are able to propose Scheme 1.

$$Cu^{2+} + SelH \rightleftharpoons CuSel^{+} + H^{+}$$

$$SelH + CuSel^{+} \rightleftharpoons CuSel_2 + H^{+}$$

$$CuSel^{+} + AAH \rightleftharpoons CuSelAA + H^{+}$$

Scheme 1

These three equilibria suffice to describe the phenomena at pH below 11. Of course, all the linear combinations of these equations are possible, since we assume the system to be at the thermodynamic equilibrium state.

We observed that the number of moles of copper extracted at low pH is higher than the number of moles of amino acid. This can be explained by a higher affinity of the selector towards copper than towards the amino acids Trp and Tyr. This implies that $SelCu^{+}$ is present at low pH, solubilised in the micelles. The species Sel_2Cu cannot be excluded, even though a 2 : 1 stoichiometry is usually disfavoured in acidic conditions.

The presence in the solution of $AACu^{+}$ and AA_2Cu is possible and even plausible. We performed blank experiments, without selector solubilised in the micelles, to evaluate in what proportion they could exist. A UV band, corresponding to these complexes, in these conditions was observed, and precipitation occurred in neutral conditions, especially with tryptophan, but no extraction was observed. Therefore, we assume that $AACu^{+}$ may be present in water, but neither $AACu^{+}$ nor AA_2Cu allows the extraction of copper with our micellar systems.

The decrease of the *Q* values for amino acids above pH 11 cannot be explained by the preferential formation of a Sel_2Cu complex. Indeed, with $[Sel]_0 = [Cu]_0$, this would have led in our experimental conditions to a maximum value of *Y*% for copper of 50%, whereas we observed values greater than 90%. In fact, the only interpretation that holds is that colloidal species of copper are likely to be retained by the membrane or adsorbed onto the micelles (as we could not observe any precipitate on the ultrafiltration membrane, we are led to think that the latter explanation is the more plausible). For technical reasons, including the stability of the ultrafiltration membrane, one cannot imagine a practical application at pHs above 11, thus we focused our efforts at lower pHs.

Finally, we can assume that the ternary complex AACuSel is solely responsible for the extraction of amino acids in our experiments.

It is obvious that we failed to demonstrate any enantiospecificity of these micellar systems under our conditions. In most of our results, the differences in *Q* values between *R* and *S* enantiomers fell into the experimental error domain. It is worth noting that the experimental errors observed here are mainly due to problems in controlling the pH; pH buffering substances (usually weak acids or bases) were not used here, since they might complex the copper ions and interfere with the formation of the ternary complex.

These results become even more anomalous when one considers that the *trans-N-n*-dodecyl-4-hydroxy-L-proline (**2**) derivative with leucine gives a *Q* value two-times higher for the *S* enantiomer than for the *R* enantiomer in the water–butanol system,[19] still bearing in mind that metal ion extraction properties are very close in biphasic solvent and micellar systems.[11] Moreover, *a* values of 2.52 for Trp and 2.79 for Tyr were measured by chromatography using a C_{18} stationary phase dynamically coated with the corresponding *N*-hexadecyl-L-proline derivative;[17a] we assumed this would mimic in many ways the environment generated by a micellar system.

The separation obtained by chromatographic procedures being sensitive to the enantiomeric purity of the selector we checked the performance of **2** deposited on a C_{18}–silica stationary phase. The experimental chromatograms for Trp and Tyr are reported in Fig. 6. High *a* values were obtained: 2.81 for Trp and 4.31 for Tyr. Despite some differences in experimental conditions (hydrophobicity of the selector, composition of the eluent) the values we obtained here show the same enantioselectivity (*i.e.*, 2.52 and 2.79 for Trp and Tyr, respectively) as those reported by Davankov *et al.*, where they used *N*-hexadecyl-L-hydroxyproline as an immobilised chiral selector with an aqueous eluent containing 15% acetonitrile. The "chromatographic" efficiency of the selector we used in this work is thus well illustrated.

Finally, remembering the *a* value of 4.5 obtained for phenylalanine in micellar solutions of an analogue of Triton X-100 using an amino acid derivative (namely cholesterol glutamate) under very comparable experimental conditions,[13] we definitely do feel that our studies here were worth trying.

We want to underline in the following paragraph what are the directions indicated by our results when compared to those reported in the literature.

The systems we studied may be regarded as the simplest ones which could have led to an enantioselective behaviour: the selectors are composed of a single linear aliphatic chain tethered to a natural chiral amino acid head, solubilised in the most classical type of non-ionic micelles. Even though all the stoichiometric experimental concentrations, all the natural amino acids, and all the transition metal ions (that might possibly be considered for this type of study) have not been checked in this study, we are now convinced that the system we used here suffered from a certain number of flaws and that at least one of them has to be solved to reach enantiospecificity. (i) We suspect that the rigidity and bulkiness of the alkyl chain

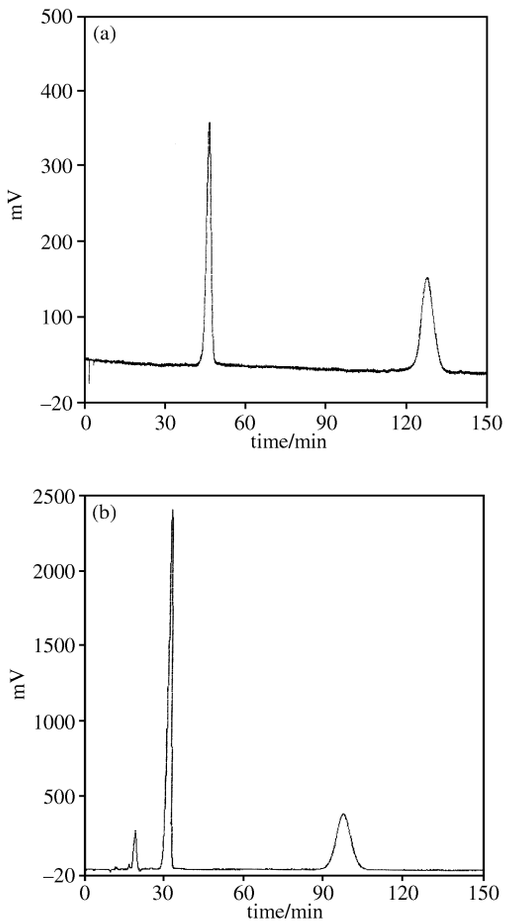

Fig. 6 Chromatograms of racemic Trp (a) and Tyr (b). Eluent: 0.1 mM $(CH_3COO)_2Cu$ in water, pH ~ 5. Selector: **2** (*ca.* 100 mg), 25 cm length column from Eka Nobel (Kromasil, ODS, 5 μ).

of the selector play a major role by enhancing steric hindrance in the ternary complex and thus can generate a difference in the stability between *SS* and *SR* ternary complexes. (ii) Constraint on the polar head of the selector can also be varied by changing functionality while adding one complexing group, *i.e.*, the copper ion being known to coordinate in a square planar manner, one can imagine easily that complexing in a bidentate, instead of monodentate, manner will generate a spatial arrangement of the ternary complex that will differ dramatically for the two enantiomers of the amino acid. These two points constitute the main difference between this work and that of Creagh *et al.*[13]

It is worth noting that rigidity of the alkyl chain of the selector solubilised in the micelles can also influence their dynamic behaviour. This phenomenon can explain the difference in behaviour between the alkyl chain used here and the cholesteryl chain reported elsewhere.[14] This labile character of the micelles is the main difference between micelle and the stationary reversed-phase in which these *N*-alkylproline derivative-selectors succeeded in recognising enantiomers.[17a,31] Some interesting work by Roumeliotis *et al.* stated that the modulation of the length of the spacer between the stationary phase and the polar head of the selector could, in some cases, induce a complete inversion of the eluting order of enantiomers. This implies that for given alkyl chain lengths α-values are equal to unity. The tuning of the lipophilicity of our selectors (*e.g.*, C_8 or C_{16} instead of C_{12}) could bring some light to this hypothesis. Another way by which it might be possibile to circumvent the dynamic behaviour of the aggregates would be to substitute polymeric micelles with normal ones, as has been shown to be possible in some cases.[32] From this point of view, another possibility, *e.g.*, the use of a dialkyl-chain chiral agent solubilised in vesicles, seems rather promising. This kind of system has demonstrated differences in behaviour with micellar systems in the field of metal ion complexation.[33]

Finally it is customary to admit that water impedes the use of polar interaction to obtain molecular recognition, and that this would explain why the proline and *trans*-4-hydroxy-L-proline derivatives would allow acceptable recognition in water–organic solvent systems and not in micellar systems. A strategy to address this problem may be to increase the hydrophobicity of the complexing agent, to cause the diastereoisomeric complex to stay in the micellar oily core, well insulated from the water.

In summary, this work illustrates the possibilities of forming ternary complexes in micellar systems. We are convinced that the diastereoselectivity of such systems can be rationally developed by varying the lipophilicity and the rigidity of the selector and/or of the structure of the microheterogeneous aggregates involved. One has to bear in mind that the separations attempted here depend on very small Gibbs energies of transfer differences between the diastereoisomeric species (typically between 0.2 and 0.8 kcal mol^{-1}). This problem, which has been addressed by another team[34] very recently by using multistage ultrafiltration systems, will be the aim of further work.

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