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# Extraction and Separation of Racemic α-cyclohexyl-mandelic Acid Using Chiral Ligand as Selector

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**Abstract:** Based on chiral ligand exchange, extraction and separation of α-cyclohexyl-mandelic acid (HCHMa) enantiomers in the two-phase system containing copper ion (II) ($Cu^{2+}$) and N-n-dodecyl-L-hydroxyproline (HN) was studied. The influence of pH, concentrations of $Cu^{2+}$ and chiral ligand and solvents on partition coefficients (K) and separation factors (α), were discussed in detail, respectively. The experimental results show that HN forms more stable ternary complexs with S-HCHMa enantiomer than with R-enantiomer. There is a larger influence of pH on K and α. At low pH values (<3.5), formation of binary complexes is thermodynamically unfavourable. K and α are best when pH values are above 3.5 and the molar ratio of chiral ligand to $Cu^{2+}$ is 2:1. Solvents also influence K and α very much.

**Key words:** Chiral ligand, exchange, selector, extraction, separation, HCHMa enantiomers

## INTRODUCTION

It is widely known that the different enantiomers of a drug can have vastly different pharmacological activities to such an extent that, in extreme cases, these effects may even oppose each other. Regulating authorities worldwide are showing increasing concern about the issue of stereospecificity of drugs and in many countries, regulations regarding the chirality of drugs are in place or under consideration. In many cases, it is thus imperative to separate these closely related chiral isomers to obtain stereochemically pure drugs (Cannarsa, 1996; Miriam, 1995; Rosa and Pilar, 2000).

Separation techniques-such as conversion to disastereomers followed by preferential crystallization, kinetic resolution and chiral HPLC-currently in use for the production of stereochemically pure drugs are expensive and sometimes inadequate, hence the interest in the development of less costly, yet evenly effective methods of separation (Abolfazl and Robert, 2002; Charles and Roongnapa, 1996; Vladimir *et al*., 1989)

Chiral extraction for enantioseparation of enantiomers has great potentialities and it has been highly regarded in recent years (Cen and Cai, 2000; Jiao *et al*., 2005; Jérôme *et al*., 2000). Racemic HCHMa serves as a model in this study, which is a kind of important precursor of chiral drugs and can be used to synthesize multiplicate chiral drugs with vital biological activity and well curative effect such as oxybutynin (Vladimir *et al*., 1982). In this study, based on chiral ligand exchange, the distribution behavior of HCHMa enantiomers in the two-phase system containing $Cu^{2+}$ and HN was studied.

## THEORY

In the system of chiral ligand exchange extraction, property of extraction mainly depends on two types of reaction in organic phase. At first, transition metal ions, often $Cu^{2+}$ in aqueous phase, partition into organic phase by forming binary complexes ($CuN_2$) with the free ligands in organic phase at the two phases boundary where the ligand can release one or more protons into the aqueous phase.

$$2\overline{HN} + Cu^{2+} \Leftrightarrow \overline{CuN_2} + 2H^+ \quad (1)$$

Where the overbar notation is used to describe a species in the organic phase. So, pH in aqueous phase plays an important role on the partition coefficients of copper which are defined as the ratio of the total copper concentration in organic phase to that in aqueous phase.

$$K_{Cu} = \frac{\overline{[CuN_2]}}{[Cu^{2+}]} \quad (2)$$

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After $Cu^{2+}$ in aqueous phase form binary complexes with chiral ligands in organic phase, HCHMa enantiomers form two ternary complexes with the binary complexes in organic phase.

$$HCHMa_j + \overline{CuN_2} \Leftrightarrow \overline{CuNCHMa_j} + \overline{HN} \quad (3)$$

The subscript "j" is used to denote the enantiomer concerned (S- or R-). The partition coefficients for S, R-enantiomer is expressed as follows:

$$K_j = \frac{\overline{[CuNCHMa_j]}}{[HCHMa_j]} \quad (4)$$

Stabilities of $CuNCHMa_j$ diastereomeric complexes in lipophilic organic phase is different. The difference in free energy of enantiomer complexes-$\Delta(\Delta G)$, stands as the difference of stability:

$$-\Delta(\Delta G) = -\Delta G_S - (-\Delta G_R) = RT\ln K_S - RT\ln K_R = RT\ln(K_S / K_R) = RT\ln\alpha \quad (5)$$

$\alpha$ (separation factor) is a very important parameter in chiral extraction system. Separation of S- and R-enantiomers is not only influenced by $K_S$ and $K_R$, but also depends on $-\Delta(\Delta G)$. The value of $-\Delta(\Delta G)$ shows separation ability of S- and R-enantiomers. In theory, only if $\alpha>1$, with certain mass transfer number, some degree of separation of S- and R-enantiomers can be achieved.

## MATERIALS AND METHODS

**Materials:** The present research were started two years ago and were completed and concluded in School of Chemical engineering and Chemistry of Central South University in 2005. Racemic HCHMa was purchased from Synergetica Changzhou, Ltd. in China. L-hydroxyproline was obtained from Xinghu Biology of Science and Technology Zhaoqing Ltd. in China, $[\alpha]_D^{20}$ = -85.2°, the purity was above 98%. Aldehyde C-12 lauric was purchased from Fluka chemika company, the purity was above 97%. Palladiumon on carbon catalyst was from Shanghai chemical reagent company in China. All other reagents are of analytical grade and purchased from different suppliers.

**Analytical method:** HPLC was performed with a LC-2010A SHIMADAZU system controller (Kyoto Japan), a sample loop injector of 20 μL, a Shimadzu C-R3A chromatopac, a Kromasil RP-18, 5 μm, 4.6 mm×150 mm column was used for the analysis. Copper ion concentrations in aqueous phase were determined by volumetric titration with EDTA using 1-(2-pyriclylaze)-2-naphthol (PAN) indicator dye. The copper concentration in the organic phase were determined by first diluting the sample with ethanol and then titrating with EDTA using PAN. HN was synthesized in our lab (Ding *et al*., 1992). The concentrations of enantiomers in the aqueous were measured by chiral mobile phase HPLC. Chromatographic conditions (Hu *et al*., 2004): concentration of β-cyclodextrin 9.5 mmol $L^{-1}$, V [aqueous $KH_2PO_4$ solution (0.075 mol $L^{-1}$)]: V (ethanol): V (acetonitrile)= 65:20:15, pH 4.8, flow rate 1 mL $min^{-1}$ and room temperature.

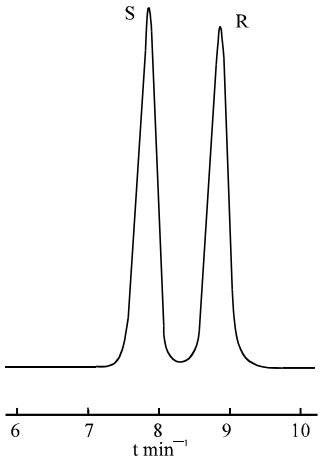


Fig. 1: Chiral HPLC chromatogram of HCHMa

**Partition experiments:** All partition experiments were performed at room temperature and an aqueous-phase ionic concentration (NaAc) of 0.1 mol $L^{-1}$. Equal volumes (3 mL) of 0.1 mol $L^{-1}$ acetate buffer containing copper ion and organic phase containing HN were placed in a centrifugal tube and stirred for 6 min. Then HCHMa enantiomers was added and strirred for 10 min. After centrifugation for 10 min at 2000 rpm, the concentration of the enantiomers in the aqueous phase was determined by HPLC (Fig. 1).

## RESULTS AND DISCUSSION

**Distribution of copper ion and binary equilibrium formation constants:** Figure 2 shows that copper ions are distributed in organic phase and aqueous phase at different pH. From Fig. 2, the concentration of copper ion in aqueous phase begins to decrease sharply at pH value of 3.0. At pH > 5.7, copper is partitioned quantitatively into organic phase, indicating that aqueous solution pH can be used to tune copper ion partitionly and thus separation efficiency in a chiral separation system.

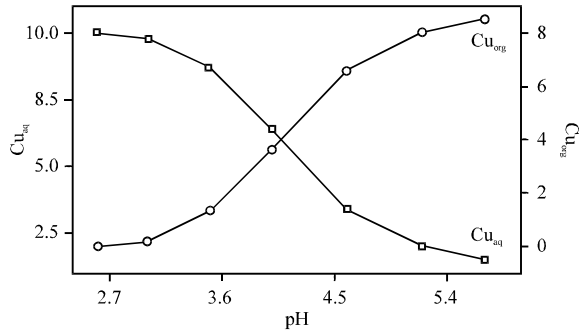


Fig. 2: Copper concentrations as a function of pH in an water/n-octanol. $[HN]_{ini}$ = 20.0 m mol $L^{-1}$; $[Cu]_{ini}$ = 10.0 m mol $L^{-1}$

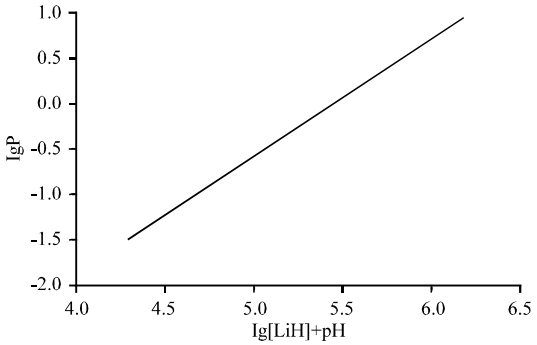


Fig. 3: Plot of lgP with pH+lg[HN]. $[HN]_{ini}$ = 20.0 mmol $L^{-1}$; $[Cu]_{ini}$=10.0 mmol $L^{-1}$; the rang of pH: 2.5~5.7

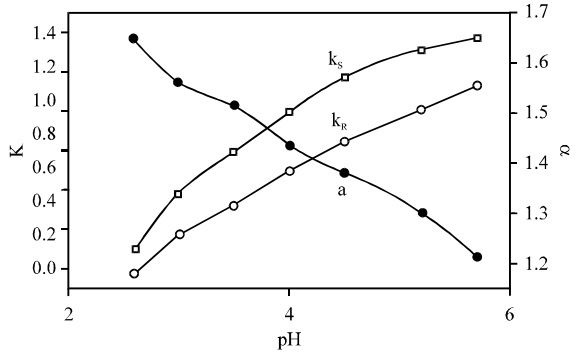


Fig. 4: Influence of pH on K and α in n-octanol/water. $[HN]_{ini}$ = 20.0 mmol $L^{-1}$; $[Cu]_{ini}$ = 10.0 mmol $L^{-1}$; [HCHMa] = 1 mmol $L^{-1}$; [NaAc] = 0.1 mol $L^{-1}$; pH = 4.0

An equilibrium formation constant for the binary bis HN: copper complex in the n-octanol phase was found from a global fit Eq. LgP = lg[HN]+pH (Fig. 3). Experimental measurements at pH values higher than 6.0 were omitted since precipitation of copper complexes can be observed. In aqueous phase, both the mono and bis binary copper/analyte complexes can form; whereas in the organic phase, only the bis Cu: ligand complex exists. Mass action predicts that the bis-binary equilibrium formation constant in the organic phase will be higher than that in aqueous phase.

**Influence of pH on K and α:** The partition of HCHMa enantiomer into organic phase is the reason that enantiomer and binary complexes of copper form ternary complexes of copper (CuNCHMa). From the discussion above, in extraction separation system of chiral ligand, pH influence the partition of copper ion in aqueous phase and organic phase. So, the pH of the aqueous phase inevitably influences the partition of HCHMa enantiomers in the two-phase system. In order to investigate the influence of pH on K and α, the distribution behavior of HCHMa enantiomers was studied in an n-octanol/water two-phase system containing HN (20 mmol $L^{-1}$) and $Cu^{2+}$ (10 mmol $L^{-1}$) over a range of aqueous pH values (Fig. 4). From Fig. 4, it can be found that the partition coefficients of HCHMa enantiomers increase with the rise of pH. As the pH rise, the partition of copper ion in organic phase increases and the concentration of binary complexes increases. From ligand-exchange equibrium of ternary complexes (CuNCHMa), it can be seen that the partition of HCHMa enantiomers in the organic phase increase with the rise of the concentration of ternary complexes (CuNCHMa) in organic phase. So partition coefficients increases with the rise of pH. The separation factor of HCHMa enantiomer decreases as pH rises. At different pH, the difference in distribution of HCHMa enantiomers in two-phase system shows the exist of diastereomeric complexes.

**Influence of the concentration of copper ion on K and α:** The concentration of copper ion influences formation of ternary complexes ($CuN_2$), so it must influence the ligand-exchange reaction of HCHMa and binary complexes ($CuN_2$) which form ternary complexes (CuNCHMa). Therefore, there is a large influence of the concentration of copper ion on partition coefficients and separation factor. It is found from Fig. 5 that the partition coefficients are biggest at the mole ratio (2:1) of chiral ligand to copper ion and then decrease with the increase of the concentration of copper ion. This reason may be that the concentrations of binary complexes increases with the rise of the concentration of copper ion below 10 mmol $L^{-1}$, reaches maximum at 10 mmol $L^{-1}$ and then changes hardly. However, the excel copper ions reacts with HCHMa enantiomers to form mono and binary complexes with rise of the concentration of copper ions beyond 10 mmol $L^{-1}$. So the concentrations of free

Table 1: Influence of organic solvents on K and α

| Solvent | $K_S$ | $K_R$ | α |
|---|---|---|---|
| 1, 2-dichloroethane | 0.06 | 0.04 | 1.500 |
| dichloromethane | 0.04 | 0.02 | 2.000 |
| chloroform | 0.02 | 0.01 | 2.000 |
| n-nonanol | 0.897 | 0.677 | 1.324 |
| n-octanol | 0.995 | 0.693 | 1.436 |
| n-heptanol | 1.125 | 0.768 | 1.464 |
| n-hexanol | 1.214 | 0.813 | 1.493 |
| n-pentanol | 1.341 | 0.879 | 1.526 |
| n-butanol | 1.467 | 0.952 | 1.541 |
| ethyl ether | 0.456 | 0.423 | 1.080 |
| ethyl benzen | 0.398 | 0.364 | 1.090 |

$[HCHMa]_{ini}$ = 1 mmol $L^{-1}$; $[Cu^{2+}]$ = 10 mmol $L^{-1}$; [HN] = 20 mmol $L^{-1}$; [NaAc] = 0.1 mol $L^{-1}$; pH = 4.0

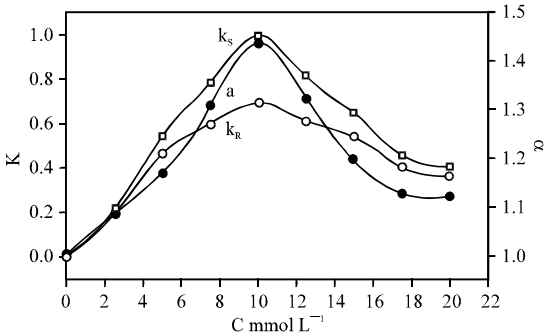


Fig. 5: Influence of copper (II) ions on the K and α in n-octanol/water. $[HCHMa]_{ini}$ = 1 mmol $L^{-1}$; $[Cu^{2+}]$ = 0~20 mmol $L^{-1}$; [NaAc] = 0.1 mol $L^{-1}$; pH = 4.0

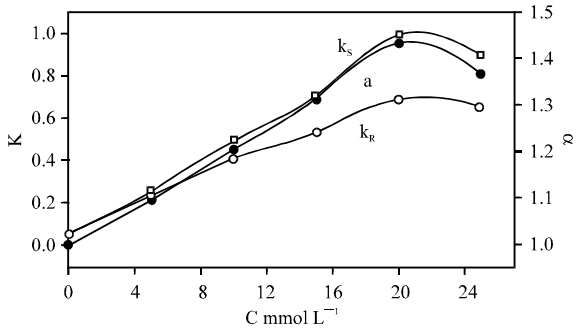


Fig. 6: Influence of the concentrations of chiral Ligand on K and α in n-octanol/water. $[HCHMa]_{ini}$ = 1 mmol $L^{-1}$; $[Cu^{2+}]$ = 10 mmol $L^{-1}$; [NaAc] = 0.1 mol $L^{-1}$; pH = 4.0

HCHMa enantiomers in aqueous phase decrease, which react with binary complexes ($CuN_2$) to form ternary complexes (CuNCHMa) and the partition coefficients of HCHMa enantiomer decrease. It is seen from Fig. 5 that at first, separation factor increases as the concentration of copper ions increases, reaches maximum at 10 mmol $L^{-1}$ and then decreases. In a word, when the mole ratio of chiral ligand to copper ion is 2:1, partition coefficients and separation factors reach the maximum.

**Influence of organic solvents on K and α:** In chiral ligand extraction system, separation of HCHMa enantiomers is dependent on the difference in the stability of the two disatereomers formed by the enantiomer with $CuN_2$ in organic phase and the nature of organic solvents might have a large influence on the difference. At pH 4.0, K and α of 1 mmol $L^{-1}$ HCHMa enantiomers in organic solvent/water two phase containing 20 mmol $L^{-1}$ HN and 10 mmol $L^{-1}$ copper ions are listed in Table 1. When alcohols were used as extraction solvents, enantioselective distribution of HCHMa enantiomers were obtained. With the alcohols studied, both of K and α decrease as the number of alkyl carbons of alcohol increases. When halohydrocarbon was used as extraction solvents, enantiomers were hardly extracted into the organic phase. K and α were low relatively when ethyl ether and ethyl benzen were used as extraction solvents.

**Influence of the concentration of chiral ligand on K and α:** The concentration of binary complexes influences formation of ternary complexes and the concentration of the chiral ligand influences formation of binary complexes. So the concentration of chiral ligand will inevitably influence the formation of ternary complexes. Therefore, there is a large influence of the concentration of chiral ligand on the partition coefficient and separation factor of HCHMa enantiomers. At first, Keeping the concentration of copper ion at 10 mmol $L^{-1}$, the distribution behavior of HCHMa enantiomers was studied at different concentrations of HN from 0 mmol $L^{-1}$ to 25 mmol $L^{-1}$ (Fig. 6). It is found from Fig. 6 that the partition coefficients and separation factors of HCHMa enantiomer increase with the rise of the concentration of the chiral ligand, reach maximum at the mole ratio 2:1 of chiral ligand to copper ion and then decrease. It is proved that the distribution of HCHMa enantiomers in two-phase is influenced directly by the concentration of binary complexes ($CuN_2$). When the concentration of chiral ligand reaches 20 mmol $L^{-1}$, the concentration of binary complexes nearly reaches maximum. Then as the concentration of chiral ligand increase, the concentration of $CuN_2$ hardly changes, but the concentration of free chiral ligand increases in the organic phase.

In addition, on the condition that the mol ratio of chiral ligand to copper ion is 2:1, the distribution behavior of HCHMa enantiomers was studied at different concentrations of HN. It is found that both partition coefficient and separation factor increase with the rise of the chiral ligand. In practical operation, large partition coefficient and separation factor are expected to reduce the ratio of phase and number of transfer units for extraction. But it is not practical to obtain relatively large

K and α only depending on the increase of the concentration of chiral ligand because of the limited solubility of chiral ligand in organic phase.

HN is suitable for separation of HCHMa enantiomers by enantioselective extraction at room temperature, which is of big K and α. N forms more stable ternary complex with S-HCHMa enantiomer than with R-enantiomer. When the mole ratio of chiral ligand to copper ion is 2:1, partition coefficients and separation factor reach the maximum. pH, solvents and concentrations of $Cu^{2+}$ and chiral ligand, have larger influence on K and α.

## ACKNOWLEDGMENT

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