Mechanisms of L-lysine extraction with sec-octylphenoxy acetic acid in sulfonated kerosene

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Abstract

BACKGROUND: Currently, the dominant theories on extraction mechanisms of amino acids are mainly deduced from mathematical models or reaction equations, it is thus necessary to obtain direct evidence for the extraction mechanisms by experimental methods. This work aims to reveal the mechanisms of L-lysine (L-Lys) extraction with sec-octylphenoxy acetic acid (CA-12) in sulfonated kerosene by atomic force microscopy (AFM), half-saturated fluorometric experiment and competition experiment.

RESULTS: The ionic bond based on the electrostatic attraction between the amino group of L-Lys and the carboxyl group of CA-12 was verified visually by high-resolution AFM. Half of the amino groups of L-Lys were labeled by o-phthalaldehyde (OPA) to form half-saturated fluorescent derivatives which were then extracted by means of CA-12 in sulfonated kerosene. The electrostatic attraction between the free amino group of L-Lys and the carboxyl group of CA-12 was illustrated by the relationships between OPA dosage and fluorescence intensities of stock solution and extracted liquid. This electrostatic interaction was also demonstrated by the competition experiment using three other amino acids including L-arginine, L-aspartic acid and L-alanine as competitive reagents.

CONCLUSION: The chemical force of L-Lys extraction with CA-12 in sulfonated kerosene was comprehensively demonstrated to be the electrostatic interaction between the amino group and the carboxyl group.

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Keywords: L-lysine; sec-octylphenoxy acetic acid; extraction mechanism; electrostatic interaction; AFM

INTRODUCTION

Liquid–liquid extraction (LLE) is a process of transferring a chemical compound from one liquid phase to the other liquid phase.^{1–3} In recent decades, LLE has been becoming more attractive for separation and purification of amino acids due to the high extraction efficiency, saving in solvent and energy, possibility of room temperature operation, continuous production and low cost.^{4–7} Amino acids dissociate in aqueous solutions, forming characteristic ionic species (i.e. the cation A^+ , zwitterion $A^\pm$ and anion A^-) as a function of the solution pH value. As a result, the solubility in non-polar solvents is very low. Therefore, LLE of amino acids is generally achieved by adding the organic phase extractants such as phosphoric acid derivatives,⁸ quaternary aliphatic amines⁹ or crown ethers,¹⁰ etc. mainly including the method of ion-exchange reaction extraction, liquid membrane extraction or reversed micelle extraction.

There are four theories on the extraction mechanisms of amino acids including electrostatic interaction,^{11–13} Lewis acid–base interaction,^{14–17} proton transfer reaction^{18–22} and complexation extraction.^{23–27} The electrostatic interaction was involved in many amino acids extraction either using acidic extractant (e.g. di(2-ethylhexyl) phosphoric acid (D2EHPA))⁸ or basic extractant (e.g. trioctylamine (TOA))¹⁴ or using reversed micelles formed by anionic surfactant (e.g. bis(2-ethylhexyl) sulfosuccinate sodium

salt (AOT))^{28,29} or cationic surfactant (e.g. trioctylmethylammonium chloride (TOMAC))⁹. The Lewis acid–base interaction was a more extensive theory which might refer to ion exchange and proton transfer in amino acids extraction using Lewis acid extractant (e.g. D2EHPA) or Lewis basic extractant (e.g. TOA).^{14,30} The proton transfer reaction theory mainly concerned the extraction and separation of electrically neutral amino acids (i.e. amino zwitterionic molecules). Liu *et al.* demonstrated that the proton-transfer reaction occurred in the extraction of α -amino acid and explained the complicated phenomenon present in the extraction of L-tryptophan with D2EHPA dissolved in n-octane and n-octanol.¹⁸ They found Lewis acid D2EHPA in the organic phase

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was likely to transfer its proton to amino zwitterionic molecule $A^{\pm}$ to form an ion-pair complex in the $3.5 < \text{pH} < 5.0$ range. It was also concluded that proton-transfer and cation-exchange reactions coexisted in the extraction of amino acid with D2EHPA. The complexation extraction was based on interaction between an electron pair donor and an electron pair acceptor or interaction between a Lewis base and a Lewis acid (the so-called complexing force). Due to the advantages of high capacity and high selectivity, reversible chemical complexation extraction has been the main method used to separate the polar, hydrophilic organic compounds in dilute solution by chelating extractant.^{22,26,27,31} Ihara *et al.* reported that six mixed-ligand nickel(II) and copper(II) chelates acted as receptors of amino acids in liquid–liquid extraction experiments between the 1,2-dichloroethane phase containing the metal chelates and the aqueous phase containing amino acids (L-phenylglycine, L-phenylalanine or L-tryptophan). The nickel(II) chelates effectively extracted amino acids from the aqueous phases under neutral conditions, forming octahedral ternary chelates.³¹ In the study of distribution behavior of L-tryptophan by extraction with D2EHPA, experimental data indicated the formation of both (1:1) and (1:2) L-tryptophan–D2EHPA (dimeric) complexes, and the complexes tended to cluster together, away from the low-polarity bulk solvent, but they did not cluster together when the polarity of the bulk solvent was improved.¹⁸ The achievements in mechanism exploration have made theoretical foundation for separation and purification of amino acids. However, the above four extraction mechanisms are mainly inferred by mathematical models or reaction equations, which are not conducive to promote the popularization and application of research ideas and achievements. Therefore, it is helpful to obtain the direct evidence by some appropriate experimental methods to further confirm the extraction mechanism of amino acids with typical extractants.

The essential amino acid L-Lys is an important amino acid applied as a special chemical in medicaments, chemical agents, food materials and feed additives.^{32–34} The separation and purification of L-Lys using an LLE method is prospective.^{7,35,36} Several authors have studied the separation and purification of L-Lys. Wu *et al.* studied the separation of low concentration L-Lys from aqueous solution by solvent sublation with dodecylbenzenesulfonic acid (DBSA) as the surfactant and D2EHPA as the extractant.³⁷ The results showed that the appropriate conditions of initial pH, D2EHPA concentration in the organic phase and DBSA concentration in the aqueous phase were 7.0, 30% and 0.2 g L^{-1} , respectively. The enrichment ratio and recovery ratio reached 13.26% and 79.57%, respectively, at room temperature. In addition, Li *et al.* studied L-Lys extraction with the polyethylene glycol (PEG)/sodium sulfate aqueous two-phase system.³⁸ The mass fraction of sodium sulfate and polyethylene glycol (PEG 1000) were controlled to be 14% and 20%, respectively. The extraction efficiency and distribution coefficient of L-Lys standard liquid separately reached 96.5% and 0.033 at 30°C and $\text{pH} = 5.5$.

Nevertheless, few studies have directly investigated the extraction mechanism of L-Lys for better understanding of the extraction process and further increasing the extraction efficiency. Zhang *et al.* studied the transfer mechanism of L-Lys in liquid emulsion membrane (LEM).³⁹ L-Lys transferred from feed solution to membrane phase when cations exchanged with carrier D2EHPA. Then free L-Lys diffused into the internal liquid membrane, followed by D2EHPA, and transferred into the internal phase when cation exchanged with strippant H^{+} . In addition, Kaghazchi *et al.* reported the extraction of L-Lys from dilute aqueous solutions by emulsion liquid membrane.¹² The forward extraction was based

on the proton-exchange mechanism. However, the mechanism of L-Lys extraction by the method of LLE has never been reported.

A half-saturated fluorometric extraction experiment was performed to analyze L-Lys and CA-12-L-Lys extracted complex. The basic principle of the fluorescence method is described as follows. The amino groups of L-Lys react with o-phthalaldehyde (OPA) in the presence of β -mercaptoethanol (β -ME) to form fluorescent derivatives, which can be monitored at excitation and emission wavelengths of 340 nm and 455 nm, respectively. L-Lys is a basic amino acid with α -amino group and ϵ -amino group.⁴⁰ Both amino groups can react with o-phthalaldehyde (OPA) to form the fluorescent derivatives L-Lys-OPA and L-Lys-(OPA)₂ in weakly alkaline conditions.⁴¹ Theoretically, the α -amino group and ϵ -amino group of L-Lys have the same reactivity with OPA, thus the half-saturated fluorescent derivative L-Lys-OPA can be obtained by making the molar ratio of OPA to L-Lys equal to 1:1. Furthermore, the fluorescent extracted complex can be formed via the reaction between a suitable anionic surfactant (as the extractant) and the free amino groups from L-Lys or L-Lys-OPA.

In this work, the common extractants sec-octylphenoxy acetic acid (CA-12), D2EHPA, trialkylamine (N235) and tributyl phosphate (TBP) were selected to extract and separate L-Lys. CA-12 is a novel carboxylic acid extractant⁴² with several advantages including easy preparation, stable composition, slow aqueous solubility, little emulsification during extraction and high extraction efficiency.⁴³ CA-12 can ionize H^{+} , and the carboxyl group of CA-12 and the amino group of L-Lys can form an ionic bond to achieve L-Lys extraction.⁴⁴ Moreover, CA-12 had the best extraction efficiency (62.8%) in preliminary experiments. D2EHPA is a cheap and stable phosphoric acid extractant, which can ionize a negatively charged ion.⁴⁵ D2EHPA[−] and the amino group of L-Lys can form an ionic bond to achieve L-Lys extraction. The extraction efficiency of D2EHPA reached 49.7% in preliminary experiments. N235 (a mixture of tertiary amine R_3N , where $\text{R} = \text{C}_8\text{--C}_{10}$, molecular weight = 350–390) is easily combined with protons and results in a quaternary ammonium cation.⁴⁶ This cation reacts with the carboxyl group of L-Lys to form an ionic bond. The extraction efficiency of N235 was 11.9% in preliminary experiments. TBP is a neutral phosphorus-containing extractant having the advantages of stability at room temperature and long-term storage.⁴⁷ Unfortunately, it was difficult to form stable ionic and hydrogen bonds with L-Lys so that the extraction efficiency of TBP was only 1.3% in preliminary experiments.

Sulfonated kerosene, also known as solvent naphtha #260, was used as diluent, with the advantages of less aromatics, low toxicity, high safety, no odor, pure quality, low sulfur, environmental protection and strong solubility.⁴⁸ Sulfonated kerosene was immiscible with L-Lys aqueous solution, and it showed high selectivity and stability in L-Lys extraction.⁷ Therefore, sulfonated kerosene was selected to be the diluent for extractant CA-12 in L-Lys extraction.

In this work, the mechanisms of L-Lys extraction with CA-12 in sulfonated kerosene were explored by atomic force microscopy (AFM), half-saturated fluorometric experiments and competition experiments. The local surface molecular structures of L-Lys, CA-12 and the extracted complex CA-12-L-Lys were directly observed by high-resolution AFM. In half-saturated fluorometric experiments, half of the amino groups in L-Lys were labeled by OPA to form the half-saturated fluorescent derivative L-Lys-OPA, which was then extracted by CA-12 in sulfonated kerosene; the fluorescence intensity before and after the extraction process was analyzed. Competition experiments between L-Lys and other amino acids,

Figure 1. Equations for the chemical reaction between L-Lys and OPA in the presence of β -ME in weak alkaline medium (pH = 9.0) at room temperature.

namely L-arginine (L-Arg), L-aspartic acid (L-Asp) and L-alanine (L-Ala) were performed to further demonstrate the interaction between L-Lys and CA-12. In addition, the theoretical and experimental influences of pH on the extraction efficiency of L-Lys with CA-12 in sulfonated kerosene were compared and discussed. This work aims to provide solid strong experimental data to demonstrate that the chemical force of L-Lys extraction with CA-12 is an electrostatic interaction, namely the ionic bond between the amino group of L-Lys and the carboxyl group of CA-12.

MATERIALS AND METHODS

Reagents and chemicals

Biochemical grade L-Lys, L-Arg, L-Asp and L-Ala were purchased from Shanghai Kangda Amino Acid Factory. Ninhydrin and 3, 5-dinitrosalicylic acid of analytical grade were provided by Eastern Sichuan Chemical Co. Ltd (Chongqing, China). Industrial grade N235, CA-12, D2EHPA and sulfonated kerosene were obtained from Laimei Yashi Chemical Co. Ltd (Shanghai, China). TBP of analytical-reagent grade was purchased from Chongqing Chemical Reagent Factory.

Refining of extractants and sulfonated kerosene

The extractants and sulfonated kerosene were refined by removing trace water-soluble impurities. CA-12, D2EHPA and N235 were insoluble in water, and the water content of industrial grade TBP was no more than 0.35%. First, D2EHPA, TBP, N235, CA-12 and sulfonated kerosene were washed repeatedly with ultrapure water until the pH reached 7.0. Second, they were centrifuged for 10 min at 3000 rpm and transferred to a separating funnel for one night. Finally, the aqueous phase was removed, while the purified extractants and sulfonated kerosene were collected and placed in brown mill mouth bottles.

Preparation of extractive solutions

Extractive solutions were prepared by slowly dissolving D2EHPA, TBP, N235 or CA-12 in deacidified sulfonated kerosene under stirring to a desired concentration and placed in brown mill mouth bottles.

Quantitative measurement of L-Lys

L-Lys concentration was determined by a ninhydrin-colorimetry method in this work. L-Lys reacted with ninhydrin to form a blue-violet compound at pH = 3.0 and 100 °C. Then the compound was detected by 722S visible spectrophotometer at 475 nm. A perfect quadratic linear relationship between the concentration of L-Lys (0–2.0 mg mL⁻¹) and the UV absorbance was achieved: $y = 5.701x^2 + 0.733x + 0.003$ ($R^2 = 0.9997$, $SD = 0.0043$), where y is the UV absorbance at 475 nm, x the concentration of L-Lys (mg mL⁻¹), and R the regression coefficient.

Extraction experiments

The extraction experiments were performed by making L-Lys solution (containing 0.5–1.5 mol L⁻¹ L-Lys) contact the extractive solutions in jacketed extractors for 15 min at 25 °C under controlled pH. The molar ratio of L-Lys solution to extractive solution was controlled to be 1:1. After reaching extraction equilibrium, the extracted liquids were centrifuged for 20 min at 10 000 rpm and then transferred to separating funnels to stand for 30 min to separate extraction phases and raffinate phases. The extraction phases were used to investigate the stripping extraction conditions, and the raffinate phases were used to measure the quantities of residual L-Lys. Then the extraction efficiencies (E) and distribution coefficients (D) of L-Lys were calculated using the following equations:

$$\text{extraction efficiency } (E, \%) = \frac{\text{quantity of L-Lys in the extraction phase}}{\text{total quantity of L-Lys in initial L-Lys solution}} \times 100\% \quad (1)$$

$$\text{distribution coefficient } (D) = \frac{\text{concentration of L-Lys in extraction phase}}{\text{concentration of L-Lys in raffinate phase}} \quad (2)$$

In this study, batches of liquid-liquid extractions were performed to study appropriate conditions in which the variables evaluated were concentrations of L-Lys and extractants, phase ratio and extraction time. The sensitive ranges were

determined by single factor experiments, and the appropriate extraction conditions were investigated by orthogonal experiments.

AFM characterization

A thin layer of dilute formaldehyde solution was pre-coated on two conductive microscopic glass slides and dried with sterile air. Then, approximately 0.05 mL dilute L-Lys solution (0.1 wt%) and extracted liquid were dropped onto the two pre-coated microscopic glass slides and dried with sterile air again. Finally, AFM IPC-208B measurements of the above prepared samples were performed under the following conditions: tungsten probes (force constant 0.06 N m^{-1}), scan range $10.5 \text{ nm} \times 10.5 \text{ nm}$, tapping mode imaging, scanning point by point at room temperature. The original image data were transmitted to a computer and further processed by G3DR software.

Half-saturated fluorometric extraction experiments

(1) Principle of half-saturated fluorometric extraction

L-Lys is a basic amino acid with α -amino group and ϵ -amino group. Both free amino groups react with OPA to form fluorescent derivatives L-Lys-OPA and L-Lys-(OPA)₂ in the presence of β -mercaptoethanol (β -ME) in weak alkaline medium (pH = 9.0) at room temperature. The pH of mixtures was adjusted by adding 1 mol L^{-1} NaOH to obtain the desired pH = 9.0. The chemical reaction equations are shown in Fig. 1.

The fluorescent derivative L-Lys-OPA can be monitored at excitation and emission wavelengths 340 nm and 455 nm, respectively. The fluorescence intensity of L-Lys-OPA is positively correlated with L-Lys concentration over a certain range. As shown in Fig. 1, the stoichiometric ratio of OPA to L-Lys is 2:1 due to the same reactivity of α -amino group and ϵ -amino group. When the molar ratio of OPA to L-Lys is lower than 1:1, OPA reacts with half of the amino group in L-Lys at most to form the half-saturated derivative L-Lys-OPA. Then the free amino group of L-Lys-OPA can react with the negatively charged extractant CA-12 to form fluorescent extracted complex. Consequently, the fluorescence intensity of the raffinate phase decreases with increasing concentration of L-Lys-OPA in the extraction phase. The extraction efficiency and the interaction between extractant and L-Lys-OPA can be analyzed by fluorescence spectrophotometry.

(2) Quantitative measurement of half-saturated fluorometric extraction

Based on this experiment, the half-saturated fluorometric standard curve was obtained. L-Lys solution with a concentration of 0.4 mg mL^{-1} was prepared using sodium bicarbonate buffer solution (pH = 9.0) and β -ME ($300 \mu\text{L}$). Then 5 mL of L-Lys solution was taken into each test tube containing 20 mg mL^{-1} OPA fluorescent reagent ($25\text{--}250 \mu\text{L}$) and shaken up. Afterwards, the above test tubes were placed in a darkroom for 5 min. Finally, the fluorescence intensity of the reaction solution in each test tube was monitored at excitation and emission wavelengths of 340 nm and 455 nm, respectively. The half-saturated fluorometric standard curve was plotted with molar concentration of OPA as abscissa and fluorescence intensity (at 455 nm) as vertical coordinate.

The half-saturated fluorometric extraction experiment for L-Lys was performed based on the molar ratio of OPA to L-Lys being 1:1. First, the quantified OPA solution and L-Lys solution were mixed in

a test tube and shaken up. Second, the mixed solution was placed in a darkroom for 5 min to prepare the half-saturated fluorometric derivative L-Lys-OPA. Third, the quantified L-Lys-OPA solution and CA-12/sulfonated kerosene solution were mixed in a jacketed extractor and stirred for 20 min. Afterwards, the extraction mixture was centrifuged for 20 min at 10 000 rpm. Then, the centrifugate was transferred into a separating funnel to stand for 30 min. Finally, the fluorescence intensities of the extraction phase and raffinate phase were measured. The extraction curves were plotted with molar concentration of OPA as abscissa and fluorescence intensities of extraction phase and raffinate phase as vertical coordinate, respectively.

Competition experiment

Several typical amino acids including basic L-Arg, acidic L-Asp and neutral L-Ala were selected as possible competitive reagents to L-Lys in the competition experiment. The molar ratios of L-Lys to each competitive amino acid (L-Arg, L-Asp and L-Ala) were controlled at 0.5, 1.0, 1.5, 2.0, and 2.5, respectively. The extraction method of the competition experiment was in accordance with that detailed in the Extraction experiments section. The amino acids were analyzed by ninhydrin-colorimetry method.⁴⁹ A curve reflecting the influences of the three typical amino acids on L-Lys extraction was plotted with the molar ratios of the other amino acids (L-Arg, L-Asp, L-Ala) to L-Lys as abscissa and the extraction efficiency as vertical coordinate, respectively.

RESULTS AND DISCUSSION

Verification of the electrostatic interaction between L-Lys and CA-12

AFM characterization

AFM is a powerful characterization tool to reveal the local surface molecular structure and chemical composition of materials with superior spatial resolution, which has already been successfully applied to study the morphologies and interaction mechanisms of ion channels,^{50,51} protein extraction,⁵² short peptides⁶ and heat shock protein.⁵³ In this study, the high-resolution AFM was utilized to clearly reveal the molecular structure of L-Lys and CA-12, as well as the interaction between L-Lys and CA-12 in the extraction phase.

The AFM morphologies of L-Lys, CA-12 and the extracted complex CA-12-L-Lys are displayed in Figs 2, 3 and 4, respectively. The red and blue areas of AFM images with high probe density indicate the existence of atoms or groups of the observed samples, while no sample was found on the green areas. As shown in Figs 2 and 3, the marked morphologies of L-Lys (Fig. 2(A)) and CA-12 (Fig. 3(A)) in AFM images are essentially consistent with their molecular models. Thus, visualization of the molecular structure of L-Lys and CA-12, as well as the structural relationship between L-Lys and CA-12 in the extraction phase, by AFM at atomic level is reliable.

Figure 4(A) shows the morphology of the extracted complex CA-12-L-Lys. There are massive chain-ring intermolecular structures, including the structure/morphology of two cyclic compounds approaching one chain compound (cyclic-chain-cyclic structure). As shown in Fig. 4(B), the chain-like molecule in the marked morphology of the so-called 'cyclic-chain-cyclic structure' is essentially consistent with that in Fig. 2(B) (namely L-Lys), while the cyclic-like molecule is consistent with that in Fig. 3(B) (namely CA-12). Moreover, it can be seen from Fig. 4(B) that both the two amino groups in the L-Lys molecule and the O-atom of C–O

Figure 2. AFM image and molecular model of L-Lys (the full-length of X and Y directions are both 10.5 nm in the AFM image).

Figure 4. AFM image and molecular model of CA-12-L-Lys (the full-length of X and Y directions are both 10.5 nm in the AFM image).

Figure 3. AFM image and molecular model of CA-12 (the full-length of X and Y directions are both 10.5 nm in the AFM image).

in the CA-12 molecule are arranged in a line and able to form an ionic bond. These observations mean that the mechanism of L-Lys extraction with CA-12 is based on the electrostatic attraction between the carboxyl group and the amino group. Therefore, it can be concluded that direct evidence of the electrostatic interaction between L-Lys and CA-12 in the extraction phase is successfully obtained by high-resolution AFM.

Half-saturated fluorometric analysis

As shown in Fig. 1, both amino groups of L-Lys can react with OPA to form the fluorescent derivatives L-Lys-OPA and L-Lys-(OPA)₂ in the presence of β -ME in weak alkaline medium, where the stoichiometric ratio of OPA to L-Lys is 2:1. Thus, when the molar

ratio of OPA to L-Lys is lower than 1:1, all of the OPA molecules will react with L-Lys while less than half of the amino group can be consumed to form the half-saturated fluorescent derivative L-Lys-OPA. The free amino group of L-Lys-OPA can react with the negatively charged extractant CA-12 to form a kind of fluorescent extracted complex. Therefore, it is feasible to use the half-saturated fluorescent derivative L-Lys-OPA to explore the electrostatic interaction in the extraction process.

Figure 5 shows the relationship between OPA dosage and the fluorescence intensity of stock solution and extracted liquid, respectively. There was 30 μ mol L-Lys in the stock solution, namely 60 μ mol amino groups. Thus, when the OPA dosage was less than 60 μ mol, the fluorescence intensity of the stock solution was positively correlated with OPA amount. If the OPA dosage was 60 μ mol, the molar ratio of amino group to OPA was 1:1, which indicated that the generated derivative L-Lys-(OPA)₂ in the stock solution had the strongest fluorescence intensity. If the OPA dosage was more than 60 μ mol, the fluorescence intensity of the stock solution was no longer changed. This result shows that the fluorescence intensity of the stock solution depends on the generated derivatives L-Lys-OPA and L-Lys-(OPA)₂. The fluorescence intensity of L-Lys-(OPA)₂ is two times of that of L-Lys-OPA. It also reveals that all OPA molecules can participate in this reaction, with a reaction efficiency of 100%.

In the extracted liquid, the fluorescence intensity first increased with increasing OPA amount until the OPA dosage was 30 μ mol, and then decreased to the minimum value when the OPA dosage was 60 μ mol. The fluorescence of extracted liquid came from the fluorescent extracted complex generated by the reaction between CA-12 and the half-saturated fluorescent L-Lys-OPA. In this extraction reaction, the carboxyl group of CA-12 reacts with the free amino group of the half-saturated fluorescent derivative L-Lys-OPA via electrostatic attraction. In other words, the fluorescence intensity of the extracted complex depends on the amount of L-Lys-OPA generated in the stock solution. As described above, when the OPA dosage was not more than 30 μ mol, the generated amount of L-Lys-OPA increased with increasing dosage of OPA

Figure 5. Fluorescence curve of L-Lys extraction with CA-12. The concentration and volume of L-Lys solution were 0.4 mg mL^{-1} and 10 mL , respectively. The concentration of OPA reagent was 20 mg mL^{-1} .

and reached a maximum at $30 \mu\text{mol}$ OPA dosage. When the OPA dosage was more than $30 \mu\text{mol}$, OPA reacted with L-Lys-OPA to form L-Lys-(OPA)₂ which could not react with extractant CA-12. As a result, the amount of L-Lys-OPA in the stock solution began to decrease and reached a minimum at $60 \mu\text{mol}$ OPA dosage. A similar tendency was observed in fluorescence intensity of the extracted liquid. This result also shows that the chemical force of L-Lys extraction with CA-12 is an electrostatic interaction between the carboxyl group and the amino group.

Ion competition from other amino acids

The influence of ion competition from other amino acids on L-Lys extraction with CA-12 in sulfonated kerosene is depicted in Fig. 6. It is obvious that the extraction efficiency of L-Lys is influenced to different degrees by the three typical amino acids. However, the influences of the three amino acids are similar, as the extraction efficiencies of L-Lys in the three curves all decrease with increasing molar ratios of the three amino acids to L-Lys. The three amino acids are all biological amino acids with α -amino group and α -carboxyl group, including the basic amino acid L-Arg, the neutral amino acid L-Ala and the acidic amino acid L-Asp. The different influences of the three amino acids on the extraction efficiency of L-Lys are mainly caused by their different side chain groups, since the contributions from their α -amino groups and α -carboxyl groups are similar.

The side chain group of L-Ala is methyl which does not react with the carboxyl group of CA-12, thus the influence of L-Ala on the extraction efficiency of L-Lys only reflects the effects of the α -amino group and the α -carboxyl group. The side chain group of L-Arg is guanidyl whose amino group has similar properties to the ϵ -amino group of L-Lys, resulting in competition between the two amino groups for the carboxyl group of CA-12. Consequently, the influence of L-Arg on the extraction efficiency of L-Lys contains the effects of not only the α -amino group and α -carboxyl group but also the guanidyl group. For the L-Asp, its side chain group β -carboxyl has the same charge as the carboxyl of CA-12, hence a mutual repulsion between the two carboxyl groups subtracts the influence of L-Asp on the extraction efficiency of L-Lys. Therefore, among the three typical amino acids, the basic amino acid L-Arg and the acidic amino acid L-Asp are determined to be the most and the least competitive amino acid, respectively. Based on the comparisons, the contribution of L-Arg to the extraction is

Figure 6. Influences of three typical amino acids on L-Lys extraction with CA-12 in sulfonated kerosene (L-Lys concentration = 0.4 mg mL^{-1} , pH of the initial solution = 10.0 , volume phase ratio = $1:1$, extraction time = 15 min).

Table 1. Extraction efficiencies of several extractants for L-Lys^a

Extractant	TBP	N235	D2EHPA	CA-12
Extraction efficiency (%)	1.3	11.9	49.7	62.8

^a L-Lys concentration = 0.2 mg mL^{-1} , molar ratio of extractant to L-Lys = $1:1$, pH of the initial solution = 7.0 , volume phase ratio = $1:1$, extraction time = 15 min .

Figure 7. Structural formulas of the four extractants (N235 is a mixture of tertiary amine R_3N , where $\text{R} = \text{C}_8\text{--C}_{10}$, molecular weight = $350\text{--}390$).

equivalent to [L-Ala] and the positive effect of [guanidine- NH_3^+], while the contribution of L-Asp to the extraction is equivalent to [L-Ala] and the negative effect of [$\beta\text{-COO}^-$]. This result is also consistent with the research conducted by Blaga *et al.*⁵⁴

Mechanisms of L-Lys extraction with several extractants in sulfonated kerosene

It is widely known that the formation of a stable extracted complex is the key step in an extraction process. The chemical forces used to form the extracted complex include electrostatic interaction, hydrophobic interaction, hydrogen bond and coordination bond.⁵⁵ Moreover, higher extraction efficiency can be obtained by

Figure 8. Reaction equation of L-Lys extraction with CA-12 ($\text{H}_3\text{N}^+ = \alpha$ -amino and ϵ -amino groups, $n = 1$ or 2).

Figure 9. Reaction equation of L-Lys extraction with D2EHPA.

electrostatic interaction because the bond energy of ion–ion interaction is much higher than that of the other chemical bonds. This is also a reason why CA-12 is selected to extract L-Lys in sulfonated kerosene in this study. Table 1 lists extraction efficiencies of several extractants for L-Lys. Figure 7 displays the structural formulas of those extractants (CA-12, D2EHPA, N235 and TBP). Obviously, the CA-12/sulfonated kerosene system has the best extraction efficiency of 62.8% at $\text{pH} = 7.0$, which is increased by 26.4% and 427.7% compared with that of D2EHPA and N235, respectively.

TBP is a neutral phosphorus-containing extractant with an extraction functional group of phosphoryl ($\text{P}=\text{O}$) oxygen atom, hence it is difficult to form stable ionic and hydrogen bonds with the amino groups of L-Lys. As for the amine extractant N235, the electron density of its nitrogen atom will increase due to the electron denoting effects of its three R-substituents, making the nitrogen atom easily combined with a proton and resulting in a quaternary ammonium cation.⁵⁶ This cation can combine with the carboxyl group of L-Lys to form an ionic bond in extraction process. However, quaternary ammonium compounds are stable only in acidic media,⁵⁶ because the proton dissociation can be enhanced by the increasing pH and accordingly the extraction ability of quaternary ammonium cations decreased continuously. In neutral medium at $\text{pH} = 7.0$ in this study, there were only a few positive charges of N235, resulting in lower L-Lys extraction efficiency.

Both CA-12 and D2EHPA are acidic extractants, with the functional group of acetate carboxyl and oxyphosphate anion, respectively. Acetic acid is an organic weak acid, whose pK_a is 4.76; while phosphate is a medium strong acid, whose pK_a is 2.12. Theoretically, both CA-12 and D2EHPA can react with the α -amino and ϵ -amino groups of L-Lys to form ionic bonds, as shown in Fig. 8 and Fig. 9, respectively. In this study, D2EHPA reacts with L-Lys to form a medium strong acid–weak base salt $\text{D2EHPA-O}^+\text{H}_2\text{N-Lys}$, while CA-12 reacts with L-Lys to form a weak acid–weak base salt $\text{CA-12-COO}^+\text{H}_2\text{N-Lys}$ which has a much lower ionization degree than $\text{D2EHPA-O}^+\text{H}_2\text{N-Lys}$. As a result, the extraction efficiency of CA-12 is higher than that of D2EHPA, because the stability of $\text{CA-12-COO}^+\text{H}_2\text{N-Lys}$ is significantly higher than that of $\text{D2EHPA-O}^+\text{H}_2\text{N-Lys}$.⁵⁷

As mentioned above, CA-12 is an organic weak acid, whose carboxyl dissociation is influenced by acidity; while L-Lys is a basic amino acid, and the charge properties of its amino groups are also influenced by acidity. Accordingly, in this study, the electrostatic interaction between CA-12 and L-Lys is controlled by acidity, so is the extraction efficiency. The pK_a of CA-12 carboxyl and the

pH at the carboxyl's total dissociation are calculated to be 3.52 and 8.53, respectively.⁵⁸ Theoretically, when pH is less than 8.53, the concentration of CA-12 anion is positively correlated with pH, resulting in an increased extraction efficiency of L-Lys until reaching the maximum at $\text{pH} = 8.53$. The pK_a values of α -amino and ϵ -amino groups of L-Lys are calculated to be 8.95 and 10.53, respectively, hence the two amino groups are positively charged when pH is less than 7.0. However, the positively charged amino groups decrease gradually with increasing pH when pH is more than 7.0. Consequently, the concentration of CA-12-Lys and the extraction efficiency of L-Lys would be reduced. Therefore, the theoretical curve of the extraction efficiency versus the pH would be a polygonal line with $\text{pH} = 7.0$ as the vertex.

As shown in Fig. 10, with increasing pH, the experimental extraction efficiency increased significantly first ($\text{pH} < 4.0$, curve slope of 7.91), then increased slowly ($\text{pH} = 4.0$ to 8.0 , curve slope of 3.42), and later increased significantly again ($\text{pH} > 8.0$, curve slope of 13.95). This result is obviously different from that of the theoretical curve mentioned above, and the possible reasons are described as follows. (1) When pH is below 4.0, there are only a few dissociated CA-12 carboxyls due to its low pK_a value (3.52); when the pH is raised, the dissociation of CA-12 carboxyl and the extracted complex can be increased significantly. (2) When pH exceeds 4.0, most of the CA-12 carboxyls are dissociated; although the pH is raised to 8.0 continually, the dissociation of CA-12 carboxyl and extracted complex increase slowly. (3) When pH is higher than 8.0, CA-12 carboxyl is completely dissociated, achieving the maximum extraction ability; however, much of the sulfonated kerosene sulfonic groups (SO_3H^-) ($\text{pK}_a = 7.0$) are dissociated, thus the extraction efficiency can be increased significantly again.

CONCLUSIONS

The conclusions drawn from this study of the extraction mechanisms of L-Lys with CA-12 in sulfonated kerosene are summarized below.

- (1) The chemical force of L-Lys extraction with CA-12 is based on the electrostatic interaction between the carboxyl group and the amino group. Direct evidence of the electrostatic interaction between L-Lys and CA-12 in the extraction phase was successfully obtained by high-resolution AFM.
- (2) It is feasible to use the half-saturated fluorometric extraction experiment to study the electrostatic interaction during the extraction process: CA-12 reacts with half-saturated derivative

Figure 10. Influence of pH on the extraction efficiency of L-Lys with CA-12 in sulfonated kerosene (L-Lys concentration = 0.2 mg mL^{-1} , volume phase ratio = 1:1, extraction time = 15 min).

L-Lys-OPA to form the fluorescent extracted complex via electrostatic interaction.

- (3) The basic amino acid L-Arg and the acidic amino acid L-Asp were determined to be the most and the least competitive amino acid, respectively; the influence of ion competition from L-Arg and L-Asp on the extraction efficiency of L-Lys could be described as $[\text{L-Ala}] + [\text{guanidine-NH}_3^+]$ and $[\text{L-Ala}] - [\beta\text{-COO}^-]$, respectively.
- (4) The analysis of pH influence on the extraction efficiency further indicates that the chemical force of L-Lys extraction with CA-12 in sulfonated kerosene is by electrostatic interaction.

In conclusion, the mechanisms of L-Lys extraction with CA-12 in sulfonated kerosene were comprehensively studied in this work. The chemical force between L-Lys and CA-12 was successfully demonstrated to be via an ionic bond based on the electrostatic interaction.

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