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Extraction and separation of D/L-lactic acid in simulated fermentation broth

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Abstract−The recovery of lactic acid from fermentation broth plays an important part in production of lactic acid. In this case, the extraction of lactic acid from simulated fermentation broth was processed by tri-n-octylamine dissolved in oleyl alcohol. The extraction efficiency was investigated with several variables, and the optimal condition of extraction of lactic acid (10 mg mL^{-1}) from aqueous solution was tri-n-octylamine/oleyl alcohol (30/70, v/v) and solvent phase/fermentation (1/2, v/v) stirred for 60 min under room temperature. The optimal back extraction of lactic acid was obtained by hot water (~90 ºC) with solvent phase/water (1/4, v/v). The back extracted racemic lactic acid was direct enantiomeric analyzed and separated by chiral ligand chromatography due to strict requirement of absolute configuration in pharmaceutical field and food science. The effect of various parameters on enantioselectivity was discussed and the (L)-phenylalaninamide·HCl (6.0 mmol L^{-1}) and $CuSO_4 \cdot 5H_2O$ (3.0 mmol L^{-1}) dissolved in methanol/water (5/95, v/v) at pH=6.0 was the suitable mobile phase for chiral ligand exchange separation of (D, L)-lactic acids. By the investigations, a convenient systemic method was established for extraction and separation of (D, L)-lactic acid.

Key words: Lactic Acid, Fermentation, Extraction, Separation, Ligand Exchange

INTRODUCTION

Lactic acid is an important chemical that can be used in food, pharmaceutical, leather and textile industries [1]. It can be produced either by synthesis or by fermentation [2,3]. The conventional fermentation was considered to be prevailing method. To recover the lactic acid from fermentation broth, several kinds of methods have been employed, such as solvent extraction [1,4,5], reverse osmosis [6], adsorption [7,8], distillation [9], electrodialysis [10] and nanoflitration [11].

Solvent extraction has been proposed to be a convenient method for lactic acid recovery with low cost. Von Frieling et al. [4] reported that the recovery of lactic acid from fermentation broths can be obtained by a carrier/modifier/solvent combination. A secondary amine (Hoe F 2562), a tertiary amine (Hostarex A 327) as well as a phosphine oxide as carriers and butylacetate and kerosene as solvents were adopted in the experiments. Yankov et al. [5] carried out an extraction of lactic acid from aqueous solutions and simulated fermentation broth by the selected system (30% tri-n-octylamine, 20% decanol and 50% dodecane) with high efficiency. Recently, a high performance extractive fermentation process with high extractive efficiency was achieved by the use of tri-*n*-decylamine (TDA) as the extractant was developed [1].

The D or L lactic acid can be directly produced by the bacterial fermentation and extracted by solvent extraction. However, enantiomeric analysis and separation should be adopted due to the strict requirement of optical purity in pharmaceutical and food industries. Previous studies indicated that the analysis of enantiomeric purity of lactic acid can be obtained by nuclear magnetic resonance (NMR) [12] or chromatographic methods with chiral stationary phase [13]. Later, Galaverna et al. [14] developed a ligand exchange HPLC for chiral separation of (D, L)-lactic acid. This method provides a convenient approach for analysis and separation of (D, L)-lactic acid.

We developed a method to extract lactic acid from lactic acid aqueous solution and simulated fermentation broth by a solvent composed of tri-*n*-octylamine (TOA) and oleyl alcohol (OAL). The influences on the extraction were studied. Subsequently, the lactic acid was enantiomeric analyzed and separated by chiral ligand exchange HPLC after the removal of interference in fermentation broth by solvent extraction. The extraction and enantiomeric separation were combined to establish a convenient system method for the procedure of recovery of (D, L)-lactic acid from fermentation broth.

MATERIALS AND METHODS

1. Materials

(D, L)-Lactic acid (90%) was purchased from Fluka (Milwaukee, USA) Tri-*n*-octylamine (TOA), (L)-phenylalanine, and (L)-phenylalaninol (L)-phenylalaninamide·HCl were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Sodium hydroxide (NaOH), methanol and oleyl alcohol (OAL) were purchased from DAE JUNG Chemicals and Metals Co., LTD. (Ansan, Korea). Sodium Phosphate, Dibasic 12-water ($Na_2HPO_4 \cdot 12H_2O$), Copper (II) Sulfate pent hydrate ($CuSO_4 \cdot 5H_2O$) and Sodium Acetate (CH_3COONa) were from DUKSAN Pure Chemical CO., LTD. (Ansan, Korea), Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) was from SAMCHUN Pure Chemical CO., LTD. (Pyongtack, Korea). Distilled water was filtered using a vacuum pump (Division of Millipore, Waters, USA) and a filter (HA-0.45, Division of Millipore, Waters, USA). All solvents used in the experiment were HPLC or analytical grade. All the samples were filtered by a filter (MFS-25, 0.2 μm TF, WHATMAN, U.S.A.) before being injected into the HPLC system.

2. Extraction of Lactic Acid

The aqueous solution, containing 5-100 g L^{-1} (A_0) two enanti-

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omers of lactic acid, was used for the extraction studies. TOA was used as a carrier. The solvent phase was composed from TOA dissolved in OAL. The experiment was performed in a 100 mL flask. Aqueous phase (20 mL) containing lactic acid and solvent phase was stirred under different temperatures. After complete separation of the phases, the concentration of lactic acid (A_1) in aqueous phase was measured by HPLC. The extracted amount of lactic acid was obtained by A_1 subtracting from A_0 according to the mass balance.

To simulate the real fermentation broth, salts (CH_3COONa, $Na_2HPO_4 \cdot 12H_2O$ and $MgSO_4 \cdot 7H_2O$) were added in the lactic acid aqueous solution with different pH values adjusted by 1.0 mol L^{-1} NaOH aqueous solution.

The back extraction was obtained by extracting lactic acid from the solvent phase containing lactic acid (A_1) with hot water (~90 °C). After 60 min stirring, the concentration of lactic acid in hot water (A_2) was determined by HPLC.

3. HPLC Analysis

The HPLC system was comprised of an M930 solvent delivery pump (Young Lin Co. Korea), a UV detector (M 720 Absorbance Detector, Young-In Scientific Co., Korea) and an integrated data system (Autochrowin. Ver. 1.42, Young Lin Co., Korea). Injection valves with 20 μL sample loops were used. The HPLC analysis was performed with a commercial C_{18} column (4.6 * 150 mm, 5 μm) purchased from RStech Co. (Daejeon, Korea). In the extraction of lactic acid process, the mobile phase was methanol/water (20/80, v/v), the flow-rate was set at 0.5 mL min^{-1}, the UV wavelength was set at 210 nm, and the injection volume was 10 μL. While in the analysis of (D, L)-lactic acid, the (L)-phenylalaninamide and $CuSO_4 \cdot 5H_2O$ (3 mmol L^{-1}) were dissolved in methanol/water solution with different pH values. The flow rate was 0.5 mL min^{-1} and the UV wavelength was at 254 nm.

RESULTS AND DISCUSSION

1. Extraction and Back Extraction of Lactic Acid from Aqueous Solution and Simulated Fermentation Broth

The principle of extraction was due to the interactions between lactic acid and tertiary amine through hydrogen bonding (Eq. (1)) or ion-pair formation (Eq. (2)) [5].

$$R_3N + HA \leftrightarrow R_3N \cdots HA \quad (1)$$

$$R_3N + HA \leftrightarrow R_3NH^+A^- \quad (2)$$

Where, R_3N represents the tertiary amine and HA, H^+ and A^- represent lactic acid and its ions, respectively.

The factors that might influence the extraction were investigated, such as time, temperature, the ratio of tri-*n*-oxtylamine (carrier) to oleyl alcohol (solvent) on the base of volume (RCS), the ratio of solvent phase to fermentation on the base of volume (RSF), the concentration of lactic acid in aqueous solution and pH values (Table 1). The ratio of solvent phase (with lactic acid) to water (~90 °C) on the base of volume (RSW) was investigated for the back extraction.

The extraction efficiency and back extraction efficiency of lactic acid were calculated by the following equations:

$$\text{Extraction efficiency } (\%) = \left(1 - \frac{A1}{A0}\right) \times 100 \quad (3)$$

Table 1. The factors that influence the extraction of lactic acid from aqueous solution

Variables	Range
Extraction of lactic acid from aqueous solution	
Time/min	30, 60, 90, 120
Temperature/°C	20, 30, 40, 50, 60
pH	2, 3, 4, 5, 6
RCS	0 : 100, 10 : 90, 20 : 80, 30 : 70, 40 : 60, 50 : 50
RSF	1 : 4, 1 : 2, 1 : 1, 2 : 1
Concentration of lactic acid in aqueous solution/mg mL^{-1}	5, 10, 25, 50, 100
Back extraction of lactic acid by hot water (~90 °C)	
RSW	1 : 4, 1 : 2, 1 : 1, 2 : 1, 4 : 1

$$\text{Back extraction efficiency } (\%) = \left(\frac{A2}{A0 - A1}\right) \times 100 \quad (4)$$

Where, A_0 and A_1 represent the concentration (g L^{-1}) of lactic acid in the fermentation solutions before extraction and after extraction, A_2 represents the concentration (g L^{-1}) of lactic acid in hot water of back extraction, respectively.

1-1. Extraction of Lactic Acid from Aqueous Solution

The extraction efficiency was first investigated by extraction of lactic acid from its aqueous solution. The effect of extraction time was investigated by mixing the solvent phase (RCS=10/90) with the lactic acid aqueous solution and stirring for a period of 30-120 min under room temperature. Fig. 1 shows that with different RSF (1/4, 1/2, 1/1 and 2/1), there was no obvious increase of extraction efficiency after 60 min, so 60 min was selected in the following experiments.

The effect of RCS on extraction efficiency with different RSF was then investigated under room temperature. The results are shown in Fig. 2. With increasing the RCS and RSF, the extraction efficiency of lactic acid increased. However, the extraction efficiencies

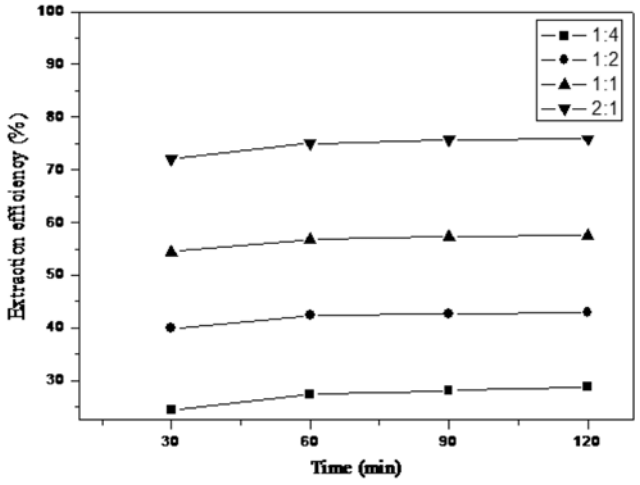

Fig. 1. Effect of extraction time on extraction efficiency with different RSF (Temperature=20 °C, RCS=10 : 90, concentration of lactic acid=10 mg mL^{-1}).

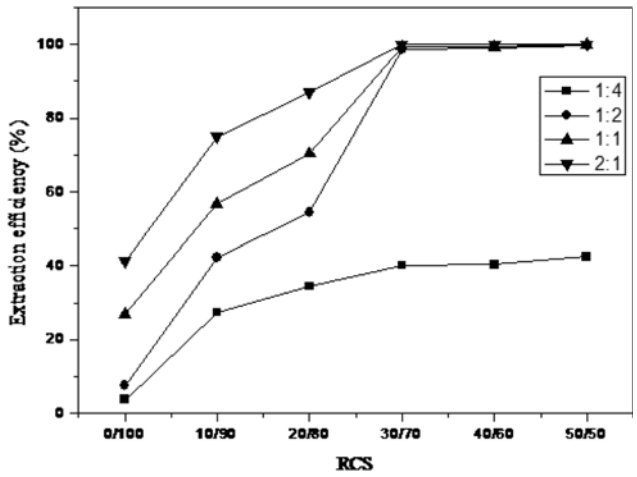

Fig. 2. Effect of RCS on extraction efficiency with different RSF (Temperature=20 °C, time=60 min, concentration of lactic acid=10 mg mL^{-1}).

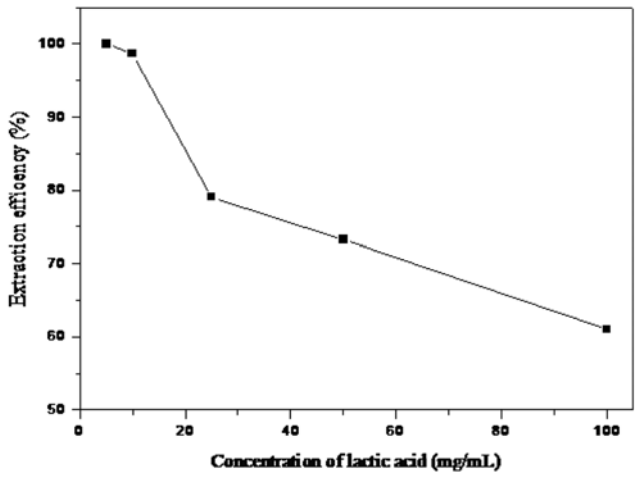

Fig. 4. Effect of concentration of lactic acid on extraction efficiency (RCS=30/70, RSF=1/2, temperature=20 °C, extraction time =60 min).

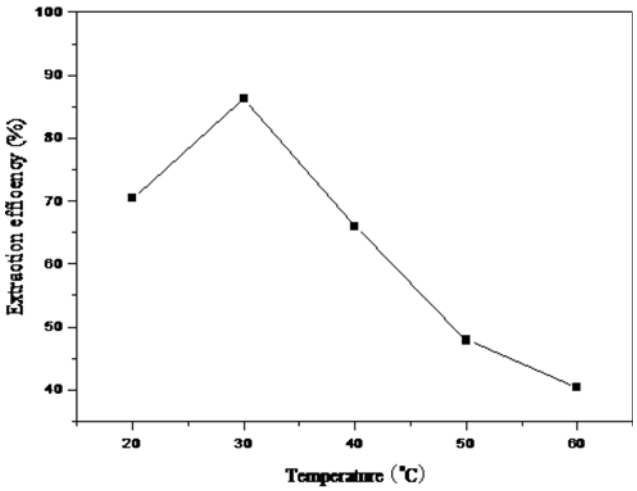

Fig. 3. Effect of temperature on extraction efficiency (RCS=20/80, RSF=1/1, extraction time=60 min, concentration of lactic acid=10 mg mL^{-1}).

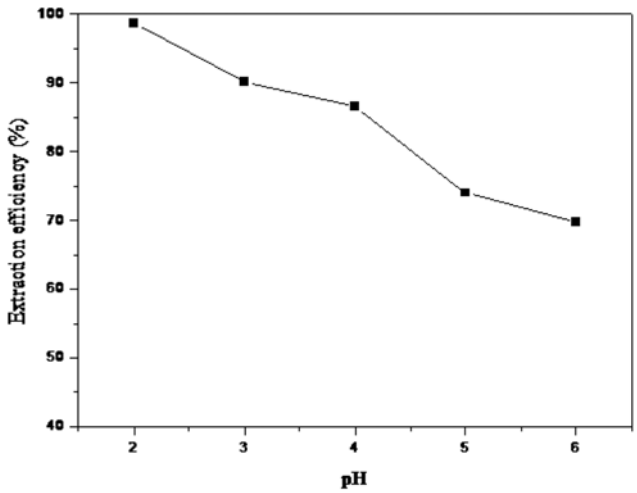

Fig. 5. Effect of pH on extraction efficiency (Na_2HPO_4=2 mg mL^{-1}, CH_3COONa=5 mg mL^{-1}, $MgSO_4$=0.6 mg mL^{-1}, RCS=30/70, RSF=1/2, temperature=20 °C, extraction time=60 min, concentration of lactic acid=10 mg mL^{-1}).

did not increase significantly when RCS increased to 30/70. To minimize the use of TOA and solvent phase, RCS=30/70 and RSF=1/2 were preferred.

To study the effect of temperature on extraction efficiency, solvent phase (RCS=20/80) and lactic acid aqueous solution were mixed (RSF=1/1) and stirred for 60 min at 20-50 °C. The results are in Fig. 3, which shows that the highest extraction efficiency (86.3%) was obtained at 30 °C. Further increase of temperature caused the decrease of extraction efficiency. This result was caused by the equilibrium between diffusion and interactions of extraction. The diffusion of lactic acid increased with increasing the temperature, but the interactions between lactic acid and tertiary amine decreased. Although the highest extraction efficiency was obtained at 30 °C, the 20 °C with moderate extraction efficiency was adopted, considering the energy consumption.

The effect of the concentration of lactic acid in aqueous solution on extraction efficiency was obtained by adding different concentration of lactic acid into aqueous solution and stirring with solvent phase (RCS=30/70, RSF=1/2) under room temperature for 60 min. The extraction efficiency decreased to 60% with the concentration of lactic acid increasing from 5 g L^{-1} to 100 g L^{-1} (Fig. 4).

1-2. Extraction of Lactic Acid from Simulated Solution

The previous studies have shown that glucose, peptone, yeast extract [15] and lactose [16] do not affect the lactic acid extraction. In this case, Na_2HPO_4, sodium acetate and $MgSO_4$ were contained in the lactic acid aqueous solution according to the reference [17]. Because the extraction efficiency depended on the acidity of the aqueous solution, the pH values were adjusted from 2-6 (Fig. 5). It was seen that the extraction efficiency was poor at high pH value, which also could be explained by the equilibrium of the interactions between lactic acid and tertiary amine.

1-3. Back Extraction of Lactic Acid from Solvent Phase

The back extraction of lactic acid with hot water (~90 °C), which

is plotted in Fig. 6, increased with the RSW increasing. Because of the increase of water amount and temperature, the equilibrium of interactions (Eqs. (1) and (2)) moved to the left, and the highest back extraction efficiency was obtained at the RSW=1/4.

2. Chiral Separation of (D, L)-Lactic Acid by Ligand Exchange Chromatography

The extracted (D, L)-lactic acids were enantiomeric analyzed and separated by ligand exchange chromatography using chiral ligand and copper (II) sulfate as additives to the mobile phase in connection with an achiral reversed phase column (C_{18}). Due to the copper complex, the detection was performed by monitoring the UV absorption at 254 nm. The retention factor was calculated from the equation $k=(t-t_0)/t_0$, where t and t_0 are the retention times of analyte and unretained solutes, respectively. And the enantioseparation factor was calculated from the equation $\alpha=k_L/k_D$, where k_L and k_D are the retention factors of (L)-enantiomer and (D)-enantiomer, respectively.

To investigate the effects of different ligands on the chiral separation, (L)-phenylalanine, (L)-phenylalaninamide and (L)-phenylalaninol were used as ligand agents, respectively. (L)-phenylalaninamide was the only ligand agent which was found to be suitable for chiral separation of (D, L)-lactic acid.

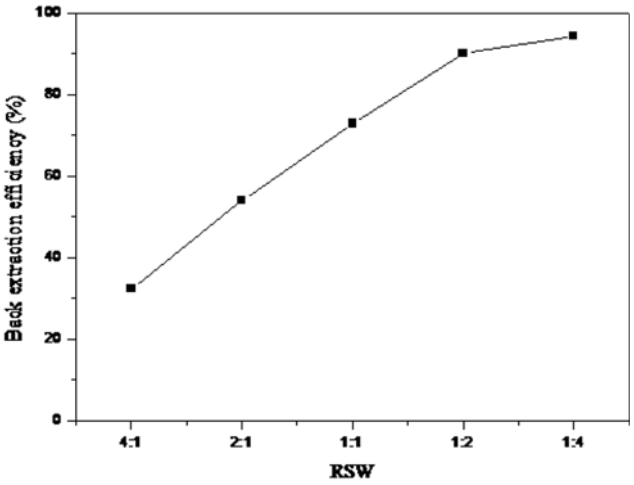

Fig. 6. Effect of RSW on back extraction efficiency (RCS=30/70, temperature=~90 °C, extraction time=60 min).

The effects of different concentration of (L)-phenylalaninamide in the mobile phase on the enantioselectivity were investigated in the range of 2.0-8.0 mmol L^{-1} and the results are shown in Fig. 7. It indicated that the concentration of ligand agent in the mobile phase could affect the k and α. The value of k and α increased when the concentration of (L)-phenylalaninamide was varied from 2.0 to 6.0 mmol L^{-1} and tended to decrease above the latter value. Increasing the concentration of ligand in the mobile phase could result in the formation of more ligand complexes, and as a result, the retention and separation of the enatiomers would be increased. When the concentration of ligand reached a certain threshold (6 mmol L^{-1}), the ligand would compete with ligand complexes to distribute on the stationary phase, so the k and α decreased.

The pH of the mobile phase was another critical factor affection enatioselectivity. To investigate the effect of pH on the enantioseparation, the pH of the mobile phase was varied from 4.0 to 7.0. Fig. 8 shows that the selectivity and retention of the two enantiomers increased with the pH increasing to 6.0; further increase of the pH tended to decrease the k and α. Moreover, higher pH would cause the precipitation of exceeding Cu^{2+}. In this case, pH=6.0 was a suitable pH value.

Different concentrations of methanol were used as the organic modifier in the mobile phase to investigate the effect of organic modifier on chiral separation. Fig. 8 shows that both the retention and separation of the two enantiomers decreased when the concentration of methanol in the mobile phase increased. This was attributed to the high concentration of organic solvent, which resulted in precipitation of the electrolyte in the mobile phase. Although the k and α increased when methanol was below 5%, the mobile phase was not suitable for the protection of C_{18} column.

By previous investigations, the optimal condition was fixed. The (L)-phenylalaninamide·HCl (6.0 mmol L^{-1}) and $CuSO_4 \cdot 5H_2O$ (3.0 mmol L^{-1}) dissolved in methanol/water (5/95, v/v) at pH=6.0 was

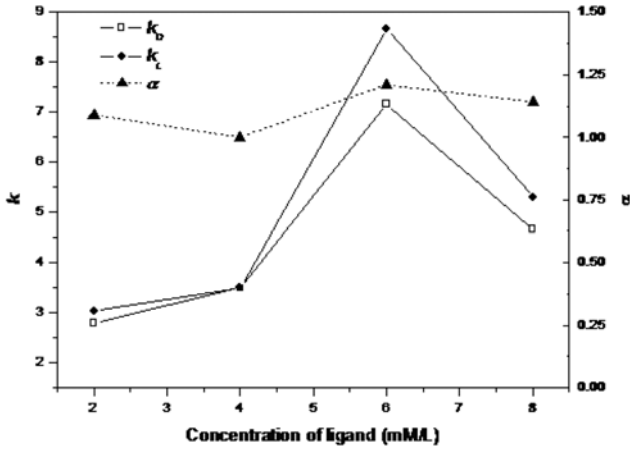

Fig. 7. Effect of the concentration of ligand (L-Phenylalaninamide, $Cu(SO_4)_2 \cdot 5H_2O$ (3 mmol L^{-1}), pH=6.0, room temperature, C_{18} column (150 * 4.6 mm), flow rate 0.5 mL min^{-1}, UV detector at 254 nm).

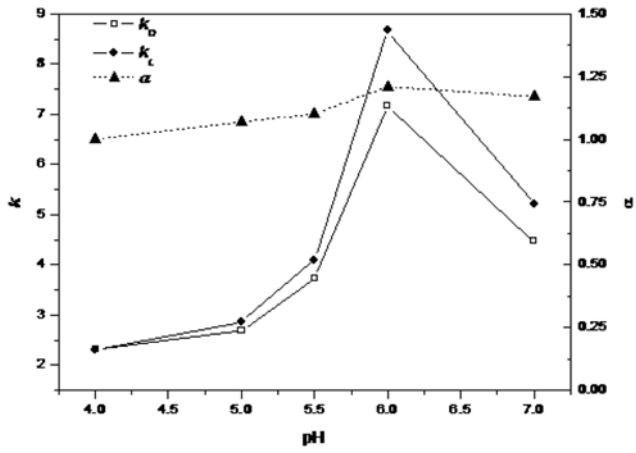

Fig. 8. Effect of pH value (L-Phenylalaninamide (6 mmol·L^{-1}), Cu $(SO_4)_2 \cdot 5H_2O$ (3 mmol L^{-1}), room temperature, C_{18} column (150 * 4.6 mm), flow rate 0.5 mL min^{-1}, UV detector at 254 nm).

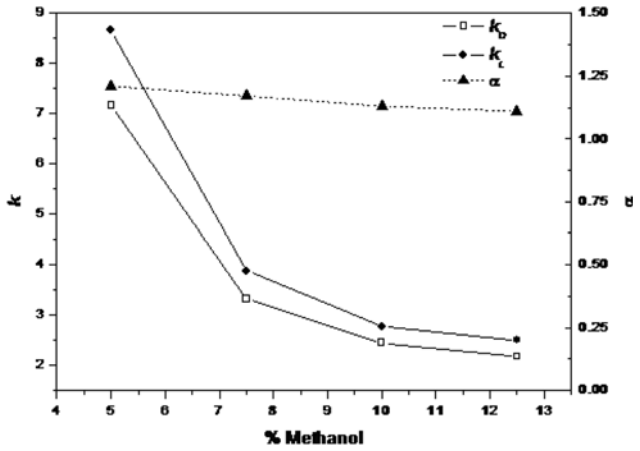

Fig. 9. Effect of concentration of methanol (L-Phenylalaninamide (6 mmol L^{-1}), $Cu(SO_4)_2 \cdot 5H_2O$ (3 mmol L^{-1}), room temperature, C_{18} column (150 * 4.6 mm), flow rate 0.5 mL min^{-1}, UV detector at 254 nm).

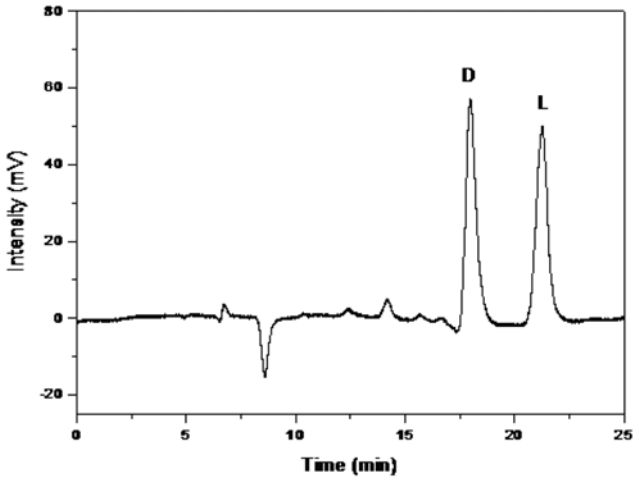

Fig. 10. Chromatogram of chiral separation of (D, L)-lactic acid (L-Phenylalaninamide (6 mmol L^{-1}), $Cu(SO_4)_2 \cdot 5H_2O$ (3 mmol L^{-1}), room temperature, C_{18} column (150 * 4.6 mm), flow rate 0.5 mL min^{-1}, UV detector at 254 nm).

the suitable mobile phase for chiral ligand exchange separation of (D, L)-lactic acids (Fig. 10).

CONCLUSION

The studies showed that the selected system (TOA and OAL) was effective for the extraction of lactic acid from aqueous solutions and simulated fermentation broth. All the variables that affected the extraction of lactic acid were investigated to get the suitable condition. After the extraction of lactic acid, the extracted two enantiomers of lactic acid were applied to investigate the enantiomeric analysis and separation by ligand exchange chromatography and a conventional method was established. The combination of extraction and enantiomeric separation was established as a convenient system for the procedure of recovery of (D, L)-lactic acid from fermentation broth.

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