Purification of Lactic Acid from Fermentation Broth by Spherical Anion Exchange Polymer

Jun Zhou, Wentao Bi, Kyung Ho Row

Department of Chemical Engineering, Inha University, 253 Yonghyun-Dong, Nam-Ku, Incheon 402-751, Korea

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ABSTRACT: Lactic acid, as a versatile chemical, was purified from fermentation broth by solid phase extraction with a spherical porous poly(4-vinylpyridine). Adsorption isotherm of lactic acid on poly(4-vinylpyridine) was first investigated with model solutions. The factors which affect the performance of separation and purification were then investigated. The obtained results indicate that the sorbent had a high adsorption capability of lactic acid, of which the maximum adsorbed lactic acid was 180.0 mg g^{-1}. Under the optimal condition the lactic acid was purified from fermentation broth by solid phase extraction with the purity of 88% and recovery yield of 95%. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 120: 2673–2677, 2011

Key words: lactic acid; porous polymers; adsorption; separation techniques; ion exchangers

INTRODUCTION

Lactic acid is a versatile chemical which plays an important role in food, pharmaceutical, leather, and textile industries.[1] It can currently be produced either by chemical synthesis or by fermentation from biomass,[2,3] and the latter one is conventional and prevailing. To purify the lactic acid from fermentation broth, several kinds of separation methods have been adopted, such as solvent extraction,[1,4,5] reverse osmosis,[6] adsorption,[7,8] distillation,[9] electro dialysis,[10] and nanoflitration.[11] With the development of industry and demand of large amount and high purity of lactic acid, purification processes which are inexpensive and efficient are imminently needed.

Recently, ion exchange technique is widely used in biological separation of amino acid or protein.[12,13] In the past few years, several ion exchange resins have been studied on the recovery and purification of lactic acid from fermentation broth, such as neutral adsorbent Amberlite XAD 1600,[14] PVP,[15] IRA-420,[16] IRA-400,[17,18] and DOWEX-50W.[19]

In this article, a spherical and porous poly(4-vinylpyridine) (P4VP) copolymer was synthesized. The basic ion exchange polymer is selected as the sorbent for the purification of lactic acid from fermentation broth because of its excellent adsorption capability of lactic acid. In this case, the adsorption isotherm of lactic acid on P4VP was studied. The lactic acid in the fermentation broth was then purified by the solid phase extraction (SPE) with the P4VP sorbent; meanwhile, the washing and elution steps were investigated to obtain the optimal condition.

Correspondence to: K. H. Row (rowkho@inha.ac.kr).

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EXPERIMENTAL

Materials

The 4-vinylpyridine (95%) was obtained from Aldrich (Milwaukee, WI). Divinylbenzene (55%) and poly(vinylpyrrolidone) (K 30) were purchased from Tokyo Chemical Industry (Tokyo, Japan). The 2, 2′-Azobisisobutyronitrile was from Junsei Chemical (Tokyo, Japan). (D,L)-lactic acid (90%) was purchased from Fluka (Milwaukee, USA). Methanol, ethanol, acetonitrile, ethyl acetate, *n*-heptane, acetic acid (99.0%), hydrochloric acid (35.0–37.9%), phosphoric acid (85.0%), and sodium phosphate monobasic ($NaH_2PO_4 \cdot 2H_2O$) were from DUKSAN Pure Chemical (Ansan, 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, USA) before injected into the HPLC system.

HPLC analysis

The HPLC system was comprised of a M930 solvent delivery pump (Young Lin, Korea), a UV detector (M 720 Absorbance Detector, Young-In Scientific,

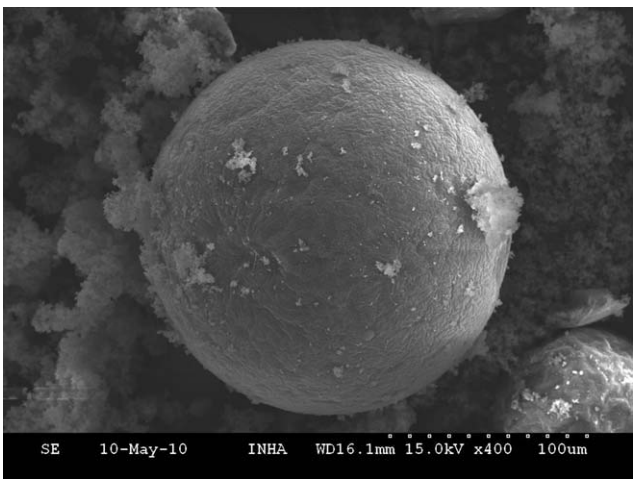

Figure 1 FE-SEM micrograph of P4VPB at magnifications of ×400. Surface area: 39.67 m^2 g^{-1}; pore volume: 0.14 cm^3 g^{-1}; pore size: 126.3 Å.

Korea), and an integrated data system (Autochrowin, Ver. 1.42, Young Lin, Korea). Injection valves with 20-μL sample loops were used. The HPLC analysis was performed with a commercial C_{18} column (4.6 × 250 mm^2, 5 μm) purchased from RStech (Daejeon, Korea). The mobile phase was 0.01 mol L^{-1} NaH_2PO_4, pH = 2.7 (adjusted by 1.0 mol L^{-1} H_3PO_4), 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.

Preparation of poly(4-vinylpyridine) (P4VP)

The copolymerization of 4-vinylpyridine with divinylbenzene was performed according to the literature.[20] Poly(vinylpyrrolidone) (0.7 g), NaCl (2.0 g) and 100 mL water were mixed in a 250.0 mL two necked flask. An organic phase consisting of a mixture of 4-vinylpyridine (3.42 g), divinylbenzene (5.1 g), *n*-heptanes (6.25 g), and AIBN (0.0625 g) was added drop wise into the aqueous solution. The solution was rapid stirred and heated to 60–65°C for 14 h under a nitrogen atmosphere. The obtained dispersion was filtered and washed with ethanol three times to remove any coagulated and soluble impurities. The poly(4-vinylpyridine) (P4VP) product was dried at 60°C for 10 h.

Adsorption isotherm

The stock standard solutions with different of concentration of lactic acid (2.5, 5.0, 10.0, 15.0, 20.0, 25.0, and 30.0 mg mL^{-1}) were prepared in vials and stored at 4°C in a refrigerator.

About 0.4g P4VP and 20 mL lactic acid standard solutions with different concentration were added into vials with stirring at room temperature. The concentration of unabsorbed lactic acid in the solution was measured every 10 min by HPLC until the equilibrium adsorption was obtained. Adsorbed lactic acid on P4VP was calculated by subtracting the concentrations of unabsorbed lactic acid.

Pretreatment of lactic acid fermentation broth

Lactic acid fermentation broth was supported by Biological Reaction Engineering Lab in Inha University. Lactobacillus was applied to ferment monosaccharide for 72 h under 37°C with pH = 5.5. The fermentation broth was filtered and clarified by centrifugation at 10,000 rpm for 10 min at 4°C to remove the insoluble proteins. Then the soluble proteins were precipitated by acetonitrile. After mixture and centrifugation, the supernatant was removed, diluted with water, and kept at 4°C in a refrigerator in darkness for further purification procedure.

Procedure of SPE

The P4VP (0.1 g) was packed into an empty cartridge and preconditioned with 4.0 mL of distilled water, then loaded by 3.0 mL 1.0 mol L^{-1} HCl for 3 h of equilibrium, and washed by distilled water until the pH of water at outlet of cartridge had no obvious change comparing with distilled water. At last, certain volume (determined by adsorption isotherm) of pretreated fermentation broth containing lactic acid was continuously loaded on the SPE cartridge until 1.0 mL for 1 h adsorption and then washed with 1.0 mL of distilled water for four times. Then, lactic acid was eluted with HCl solution. The filtrate each time was evaporated to dryness and reconstituted in 2.0 mL of mobile phase for further HPLC analysis. The recovery was investigated by the total eluted amount of lactic acid divided by the loading amount of it.

RESULTS AND DISCUSSION

Performance evaluation

As shown in Figure 1, the P4VP particles prepared had a diameter around 150 μm, and the porous structure is observed through the broken sphere around the main particle in this image. Brunauer-Emmett-Teller (BET) data was measured by ASAP2010 (Micromeritics, USA) to investigate the porous characteristics. The surface area, pore volume, and pore diameter were 39.67 m^2 g^{-1}, 0.14 cm^3 g^{-1}, and 126.3 Å, respectively. The appearance of the porous structure can increase the efficiency of this polymer.

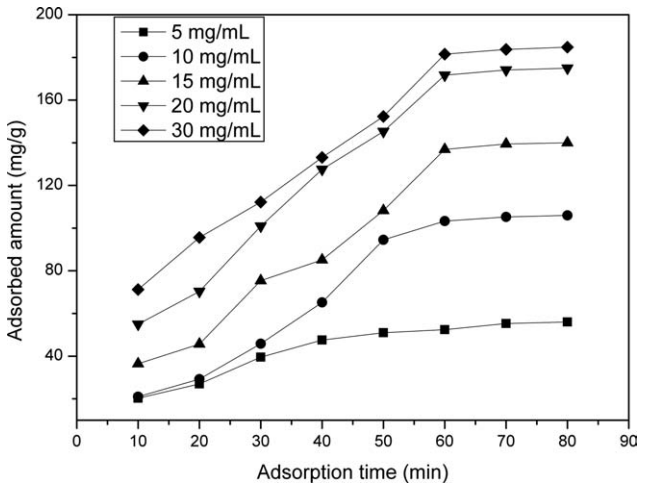

Figure 2 Effect of adsorbed time.

Adsorption isotherms

The effect of adsorption time was studied. In Figure 2, the adsorbed amounts of lactic acid (5.0–30.0 mg mL^{-1}) on P4VP increased as the adsorption time increased from 10 to 60 min. After 60 min, no obvious increase in the adsorbed amount of lactic acid was observed (Fig. 2).

The adsorption of lactic acid on P4VP was investigated by plotting the adsorption isotherms of standard lactic acid (Fig. 3). And this experiment data were fitted to the following adsorption isotherm models:

$$Q = aC + b \tag{1}$$

$$Q = \frac{aC}{1 + bC} \tag{2}$$

$$Q = aC^{1/c} \tag{3}$$

where, C (mg mL^{-1}) in the three equations is the equilibrium concentration of the solute in the liquid-phase, while Q (mg g^{-1}) the adsorbed amount of the solute in the solid-phase, a, b, and c are the parameters. These adsorption isotherm models are the linear (1), Langmuir (2), and Freundlich (3) equations, respectively.

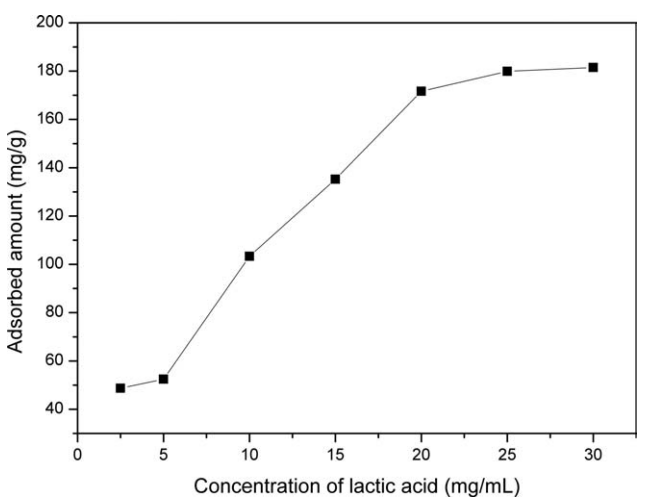

Figure 3 Adsorption isotherm of lactic acid on P4VP. Adsorption time = 60 min.

TABLE I
Parameters in Adsorption Isotherm Equations of Lactic Acid on the P4VP

Compound	Adsorption isotherm equation no.	Parameters			
		a	b	c	r^2
Lactic acid	(1)	50.64	5.04	–	0.92
	(2)	18.57	0.066	–	0.97
	(3)	32.44	–	1.90	0.96

According to the regression coefficient (r^2) in Table I, the Langmuir equation can be fitted with the compounds better than the other two equations in this experiment.

Optimum SPE conditions

The loading amount of fermentation broth was evaluated by the Langmuir equation. The concentration of lactic acid in the fermentation broth was 10.0 mg mL^{-1}. And the theoretical loading volume was determined by eq. (2) and the following equations:

$$Q_{max} = mQ \tag{4}$$

In this experiment, $m = 0.1$ g, thus, the maximum theoretical loading volume is:

$$V_{max} = \frac{0.1a}{1 + bC} \tag{5}$$

where, Q (mg g^{-1}) is the adsorbed amount of the solute in P4VP, C (mg mL^{-1}) is the equilibrium concentration of the solute in the mobile phase, m (g) is the mass of P4VP packed into the SPE cartridges, Q_{max} (g) is the mass of the total adsorbed amount of target compounds, V_{max} (mL) is the maximum theoretical loading volume of extraction solution loaded onto the SPE cartridges, and a and b the parameters.

According to the eq. (5) and Table I, the theoretical loading volume was obtained as 1.13 mL. For some interference in the fermentation broth, the experimental loading volume was fixed to 1.0 mL, in this case, lactic acid in the fermentation broth could be fully absorbed by P4VP, and the loading amount of lactic acid was 1.0 mg.

Then washing and eluting steps were investigated to optimize the process of selective extraction. Initially, different washing solvents with different polarity (water, ethanol, methanol, acetonitrile, and ethyl acetate) were investigated. The water was

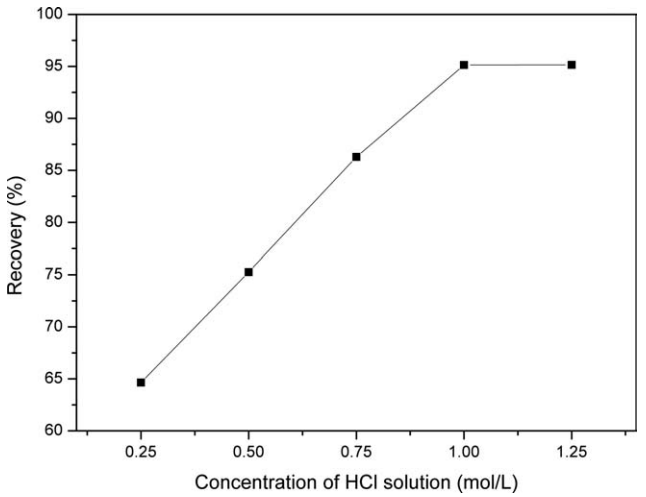

Figure 4 Recovery yield of lactic acid from fermentation broth with different concentration of HCl solutions.

found to be the most suitable washing solvent in the washing step. The HCl solution was followed to elude lactic acid. Different concentrations of HCl solutions were used as elution solvent (Fig. 4), which showed that 1.0 mol L^{-1} HCl had the highest efficiency to elude lactic acid and the recovery yield of lactic acid was 95%. Further increase of the concentration of HCl solution cannot increase the recovery yield of lactic acid from fermentation broth. Elution of lactic acid with different volumes of 1.0 mol L^{-1} HCl solutions ranging from 1.0 to 6.0 mL was used to obtain the optimum volume. The eluted lactic acid increased with increasing the elution volume below 5.0 mL. However, the extraction amounts of lactic acid was fixed, when a volume larger than 5.0 mL was used. Therefore, 5.0 mL was found to be the optimum volume.

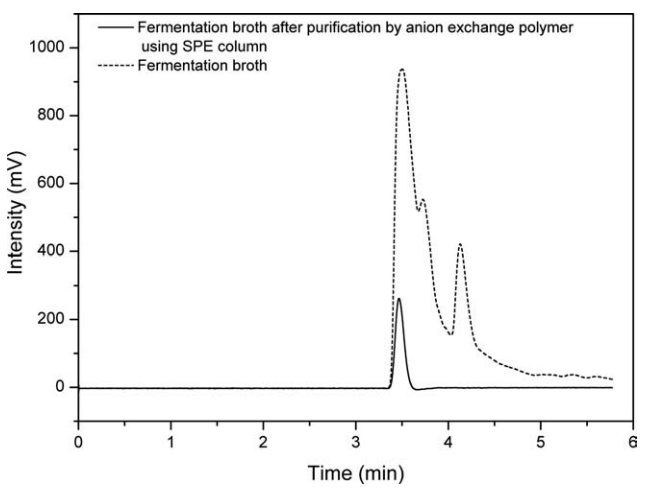

Figure 5 Chromatogram of lactic acid from fermentation broth (mobile phase 0.01 mol L^{-1} NaH_2PO_4 , pH = 2.7; flow rate 0.5 mL min^{-1}; injection volume 10 μL; UV wavelength 210 nm.

TABLE II
RSDs and LOD of Lactic Acid

	RSD (%)		
Compound	Intra-day	Inter-day	LOD (μg mL^{-1})
Lactic acid	0.015	0.034	6.01

The previous optimization indicated the following steps to separate the target compounds and interferences. First, the pretreated fermentation broth was loaded on SPE cartridges, and the interferences were washed out by distilled water. Then, the lactic acid was eluted by 5 mL 1.0 mol L^{-1} HCl solution (Fig. 5). Finally, the P4VP polymer was regenerated by washing with HCl solution and water.

Validation of proposed analytical method

The calibration curves were constructed using the chromatographic peak areas that were measured at seven increasing concentrations, ranging from 0.5 to 30 mg mL^{-1}. Each measurement was repeated three times, and a good linear correlation equation was $Y = 0.00362X - 3.4677$ ($r^2 = 0.99918$). The assays of the repeatability were calculated from the RSDs, which were determined by injecting standard solutions of lactic acid five times over a 5-day period. The standard solutions of LQ and glycyrrhizin were diluted and injected until the limit of detection (LOD) was obtained at a signal-to-noise ratio of 3. Table II lists the RSD of the precision tests and the LOD of the standard solutions. It confirmed that these values had acceptable precision and accuracy.

Recycling of P4VP in SPE

The recycling of P4VP in the SPE cartridge was investigated. The recovery yields of the LA from

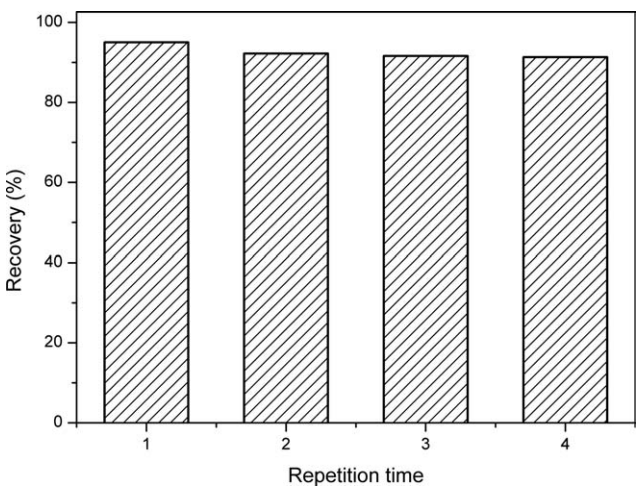

Figure 6 Recovery (%) of LA from fermentation broth within four repetitions of P4VP.

fermentation broth within four recycles of P4VP were summarized in Figure 6. The recovery yields of LA exhibited little decrease, indicating that this sorbent has a stable characteristic.

CONCLUSIONS

In this study, the equilibrium adsorption time of lactic acid on P4VP was found to be 60 min, and the Langmuir equation was selected as a suitable model for the adsorption isotherm of lactic acid on P4VP. By optimizing the loading volume of fermentation broth, washing and elution solvents for SPE, the lactic acid was successfully purified from fermentation broth. The recovery yield and purity were found to be 95 and 88%, respectively.

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