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Towards the Recovery of L-Aspartic Acid from Aqueous Solution: Exploiting pH-Dependent Solubility via Carbon Dioxide Addition

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ABSTRACT

While the use of gas antisolvents has been described widely in the literature for precipitation of solutes from organic solutions, fewer studies describe their use on aqueous solutions. The primary objective of this initial investigation was to explore the use of carbon dioxide gas to exploit the pH-dependent solubility of L-aspartic acid for its separation from aqueous solution. Experimental data are presented and discussed for the aqueous solubilities of L-aspartic acid at 25°C over the pH range 2 to 6. A minimum solubility of 0.039 M was found near the isoelectric point at pH = 2.79; solubilities increased moving away from the isoelectric pH to higher and lower pH values. Preliminary data that demonstrate using carbon dioxide as an acidulent to precipitate L-aspartic acid from aqueous solution are also presented. These preliminary data are used to draw

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inferences regarding the characteristics of compounds that might exploit this separation technique.

Key Words: Amino Acids; Antisolvent; Precipitation.

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INTRODUCTION

Numerous techniques have been developed to separate amino acids from dilute aqueous solutions. The separation techniques studied most commonly fall into two broad categories: adsorption/extraction and precipitation/crystallization. The former comprises ion exchange,[1–3] membrane extraction,[4–6] micellar extraction,[7–10] electrophoresis,[11] chromatography,[12,13] and (reactive) solvent extraction.[14–17] For a discussion of these techniques, see Cameron.[18] Separation techniques that fall into the latter category typically exploit one of three facts: (1) amino acids have aqueous solubilities that depend on pH strongly[19–21]; (2) their aqueous solubilities depend on the concentration(s) of other electrolytes in solution[22]; and (3) amino acids typically have lower solubilities in conventional organic solvents than in water.[20,23] Adjusting pH via addition of conventional acids or bases[21] or/and addition of an organic antisolvent[24] provides a means to alter aqueous solubilities and thereby to induce amino acid precipitation. Addition of an organic antisolvent requires an additional separation process to recover the antisolvent for reuse. In effect, a difficult separation problem is traded for one that is easier.

The separation method under consideration exploits the fact that amino acid solubilities depend strongly on pH; solubilities increase as the pH increases or decreases away from the amino acid isoelectric point. If the solubility limit is reached, controlling-solution pH affords the ability to change amino acid solubility, possibly inducing precipitation. An objective of this preliminary research was to provide information and insight concerning the recovery of amino acids from dilute aqueous solution by addition of carbon dioxide to alter solution pH. Carbon dioxide can be used as an acidulent by the following aqueous solution reactions:

$$CO_2 + H_2O \leftrightarrow H^+ + HCO_3^- \quad (1)$$

$$HCO_3^- \leftrightarrow H^+ + CO_3^{2-} \quad (2)$$

In addition to affecting amino acid solubilities by altering pH, carbon dioxide might be expected to affect solubilities by acting as an antisolvent. It was not

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the focus of this work to decouple these effects; rather, the aim was to test whether pressurization with carbon dioxide *at low pressures* could be used to precipitate L-aspartic acid from water. If successful, this method would have advantage over traditional antisolvent methods that use organic antisolvents; it would replace the distillation step needed to recover the antisolvent with a flash/compression cycle.

L-Aspartic acid was chosen as a model compound for three reasons: (1) It is produced typically in aqueous solutions using microorganisms. (2) It is a high-volume chemical in the food and pharmaceutical industries. In medicine, it is used as an ammonia-detoxifying agent and a fatigue refresher. In foods, it has found widespread use as a raw material for Aspartame, a low-calorie sweetener. (3) Finally, as elaborated in the Product and Process section, L-aspartic acid displays pH-dependent solubilities at moderate-to-high pH values (>5).

Results of equilibrium studies are presented and discussed for the solubilities of L-aspartic acid in water at 25°C over the pH range 2 to 6. Additional results are presented that demonstrate the ability, albeit with low-percentage recoveries, to precipitate L-aspartic acid via carbon dioxide addition at low pressures (<100 psi).

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EXPERIMENTAL MATERIALS AND METHODS

Materials

Chemical Reagents

L-Aspartic acid (Sigma, St. Louis, MO, USA, 99 + %; SigmaUltra), potassium hydroxide (Sigma, 88%), and carbon dioxide (National Welders, Charlotte, NC, USA, 99.5%) were used as received. All aqueous solutions were prepared from distilled water that had been passed through a Milli-Q water purification system (Millipore, Billerica, MA, USA).

Methods

High-performance liquid chromatography (HPLC; Hewlett-Packard, Palo Alto, CA, USA, Series 1100 system) was used to measure L-aspartic acid concentrations in solution. Measurements were performed at 40°C using a Hewlett-Packard ZORBAX SB-C18 column and a 0.01-N H_2SO_4 mobile-phase with a flow rate of 0.5 mL/min. Detection was performed by ultraviolet spectroscopy using a flow-through cell operating at 210 nm. A calibration plot

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was constructed of peak area (mAu·s) vs L-aspartic acid concentration (M); aqueous L-aspartic acid solutions of known concentrations (0.01, 0.02, and 0.03 M) were used to prepare this calibration plot. Peak area measurements were done for 10-μL injection volumes; reported values represent the average of three replicates. As expected from Beer's law and shown in Fig. 1, the calibration plot was linear over the low-concentration range employed and allowed for the determination of L-aspartic acid concentration with a relative error of ±0.8%.

Dissolution Rate

Measurements were made to determine the time required for an aqueous solution of L-aspartic acid to reach equilibrium with its solid. L-Aspartic acid was contacted with 100 mL of distilled water in a 125-mL Erlenmeyer flask;

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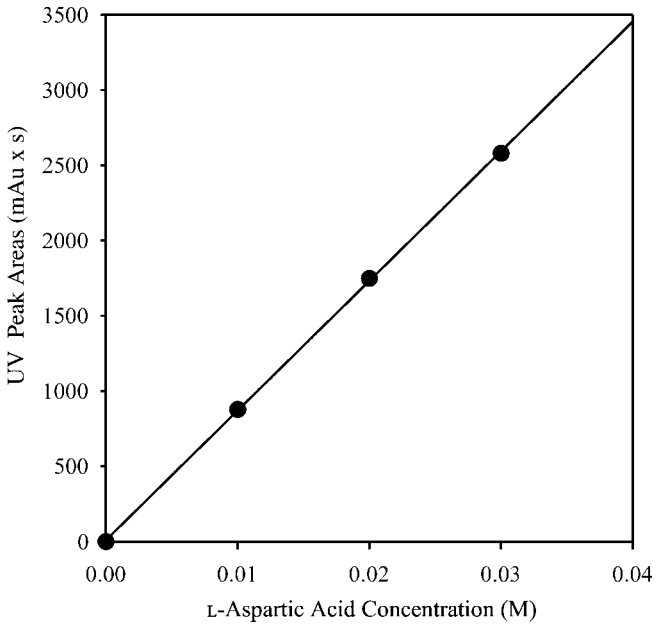

Figure 1. HPLC-UV spectroscopy calibration plot for L-aspartic acid. Measurements on 10-μL samples were performed using a ZORBAX SB-C18 column maintained at 40°C with a 0.01-N H_2SO_4 mobile phase flowing at 0.5 mL/min and UV detection at 210 nm.

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L-aspartic acid was added in excess of the mass required for saturation. The flask was agitated in a constant-temperature reciprocating shaker bath operating at 25°C and 80 rpm. Samples of the supernatant liquid phase were taken periodically and filtered through a Millex syringe filter with a PVDF/PE membrane (13-mm diameter, 0.22-μm pores). Sample L-aspartic acid concentrations were determined using measurements of HPLC peak areas and Fig. 1. This procedure was repeated until no change of concentration was observed with additional time. Figure 2 shows that about 24 hours were required to reach equilibrium within the experimental error.

Solubility-pH Isotherm

L-Aspartic acid solubilities were measured at 25°C over the pH range 2 to 6. All pH adjustments were done using aqueous solutions of hydrochloric acid or potassium hydroxide. Potassium was chosen as the strong-base cation because its salts with carbonate and bicarbonate have higher solubilities in water (8.11 M and 3.61 M at 25°C,[25], respectively) than the corresponding

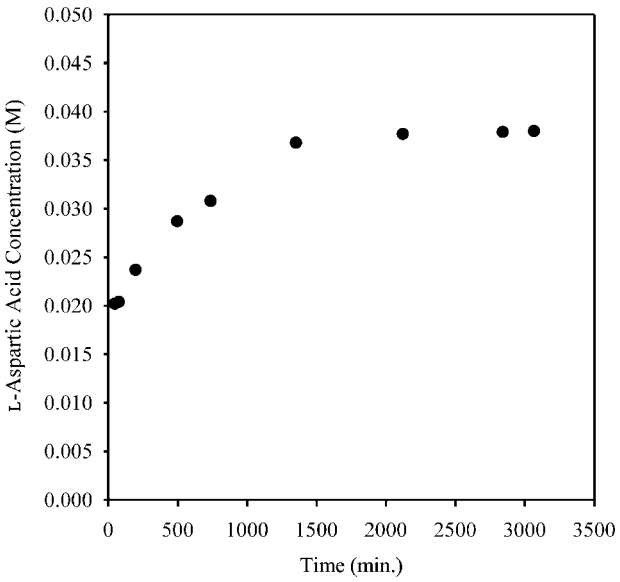

Figure 2. Rate of dissolution of solid L-aspartic acid into its aqueous solution at 25°C. Agitation was done in a constant-temperature, reciprocating-shaking bath at 80 rpm.

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sodium salts (2.84 M and 1.23 M at 25°C,[25] respectively). This fact allows higher initial L-aspartate concentrations to be used without concern for precipitation of carbonate or bicarbonate salts in the experiments with carbon dioxide. Solid L-aspartic acid was added in excess to vials containing solutions with different concentrations of HCl or KOH. The vials were agitated for at least 48 hours in the reciprocating shaker bath operating at 25°C and 80 rpm, after which, the liquid and solid phases were separated by filtration. The equilibrium pH was measured using a Fisher Scientific (Pittsburgh, PA, USA) Accumet pH meter (Model AR50) and a single junction Ag/AgCl electrode. Aqueous-phase L-aspartic acid concentrations were determined by HPLC, as described previously. High-concentration samples were diluted into the linear region of the calibration plot for peak area measurements.

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Pressurization with CO_2

The equilibrium cell used in all of the CO_2 experiments was a 15-mL glass cylinder (Waters, Milford, MA, USA) rated to 100 bar. The cell was maintained at 25°C in a constant-temperature, reciprocating-shaker bath operating at 80 rpm. Saturated aqueous L-aspartic acid solutions of known initial concentration were contacted in the cell with carbon dioxide in the presence of solid L-aspartic acid. The solid phase was maintained in contact with the solution to avoid metastable supersaturated conditions. To purge the system of air, CO_2 was introduced to one end of the cell from a pressurized CO_2 gas cylinder. Air and CO_2 exited the cell through the opposite end of the column, which was connected to a bubble flow meter that served to measure the volumetric flow rate of CO_2 into the cell. To ensure that essentially all of the air was removed from the vapor space, high purge volume-to-vapor volume ratios (>30) were employed during this step.

When the system had been purged of air, the outlet to the bubble flow meter was closed, and the system was pressurized with CO_2 to the desired working pressure Three carbon dioxide pressures were used: 53 psig, 65 psig, and 86 psig. Working pressures were measured with a Bourdon-tube type, Heise (Shelton, CT, USA) gauge (Model CM) that had been calibrated against a Budenberg dead-weight gauge (Model 380 H). Estimated uncertainty for pressure was ± 1 psig.

After 24 hours, the concentration of L-aspartic acid in the aqueous solution was measured by HPLC, as described previously. Additional measurements showed that equilibrium was reached within the experimental error in the batch CO_2-equilibration experiments within 24 hours.

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DESCRIBING AND MODELING THE SYSTEM

Amino Acid Solution Chemistry

L-Aspartic acid is a dicarboxylic amino acid; it has one amino group and two carboxyl groups. The degree of ionization of the three functional groups on L-aspartic acid is governed by the association-dissociation equilibria in solution. L-Aspartic acid participates in the following association-dissociation reactions in solution:

$$AA^{+} \overset{K_1}{\longleftrightarrow} AA^{\pm} + H^{+} \tag{3}$$

$$AA^{\pm} \overset{K_2}{\longleftrightarrow} AA^{-} + H^{+} \tag{4}$$

$$AA^{-} \overset{K_3}{\longleftrightarrow} AA^{=} + H^{+} \tag{5}$$

where AA^{+}, $AA^{\pm}$, AA^{-}, and $A^{=}$ represent the cation, zwitterion, anion, and dianion forms of L-aspartic acid. The equilibrium constants, K_1, K_2, and K_3 are the first-, second-, and third-ionization constants, respectively; apparent ionization constants are $pK_1 = 1.88$, $pK_2 = 3.65$, and $pK_3 = 9.60$ for L-aspartic acid at 25°C.[23] The term "apparent" refers to the fact that these constants were determined from concentration measurements, not activity measurements.

When L-aspartic acid is dissolved in water in the absence of other ionizing solutes, the predominant form is zwitterionic and the molecule has zero net charge. The pH where the amino acid has zero net charge is called the isoelectric point, pI; it is $pI = 2.77$ for L-aspartic acid at 25°C.[23] For solutions at $pH < pI$, the amino acid is charged positively; for solutions at $pH > pI$, the amino acid is charged negatively.

The total amino acid solubility in aqueous solution comprises the solubilities of each of the ionic forms of the amino acid. The total solubility, S, can be written as the sum of the individual solubilities:

$$[S] = [S^{\pm}] + [S^{+}] + [S^{-}] + [S^{=}] \tag{6}$$

Species concentrations are denoted by square brackets and are generally expressed in units of molarity. From the association–dissociation equilibria reactions, eqs. (3)–(5), an expression for the total solubility can be written in terms of the equilibrium solubility of the zwitterion, pH, and the apparent pK values:

$$[S] = [S^{\pm}] \cdot [1 + 10^{pK_1 - pH} + 10^{pH - pK_2} + 10^{2 \cdot pH - pK_2 - pK_3}] \tag{7}$$

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This expression assumes that the activity coefficients of all species are lumped into apparent pK values and that these apparent pK values are constant over the range of experimental conditions employed. Independent measurements[23–26] taken at 25°C for L-aspartic acid at its isoelectric point give [S] = 37.6 mM; the corresponding value for the zwitterion can be calculated to be $[S^{\pm}] = 29.8$ mM. With this independent datum point, eq. 7, can be used to predict the solubility-pH curve for L-aspartic acid over the pH range studied.

RESULTS AND DISCUSSION

While the use of gas antisolvents has been described widely in the literature for precipitation of solutes from organic solutions (e.g., Dixon and Johnston[27]), fewer studies[28,29] describe their use on aqueous solutions. Amino acids are produced typically in aqueous solutions; this fact motivates the overall research plan, which is to test the efficacy of recovering amino acids by direct addition of carbon dioxide to aqueous amino acid solutions. L-Aspartic acid was chosen as a model amino acid based on considerations outlined in the Product and Process Considerations section. Here, experimental results and model predictions are presented that demonstrate gas antisolvent precipitation of L-aspartic acid from aqueous solution at 25°C using low-pressure carbon dioxide.

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Aqueous Solubilities of L-Aspartic Acid

Figure 3 shows the equilibrium solubility-pH data for L-aspartic acid in aqueous solutions at 25°C. Experimental data are represented by symbols. The solid curve represents the mathematical model to describe solubilities for this system. Amino acid solubilities increased with increasing and decreasing values of pH away from the isoelectric point, pI = 2.77. This result is consistent with the notion that aqueous solubilities increase with increasing net charge on the amino acid molecule. These results are also consistent with previous studies[19–21] of amino acid solubilities. The solubility minimum had a value of 39.0 mM at pH = 2.79; this value compares well with the previously reported value of 37.6 mM.[23]

Equation (7) was used to describe the experimental data. The value for the solubility at the isoelectric point was obtained from the literature, so that eq. (7) could be used as a predictive tool for solubility-pH data. The predicted

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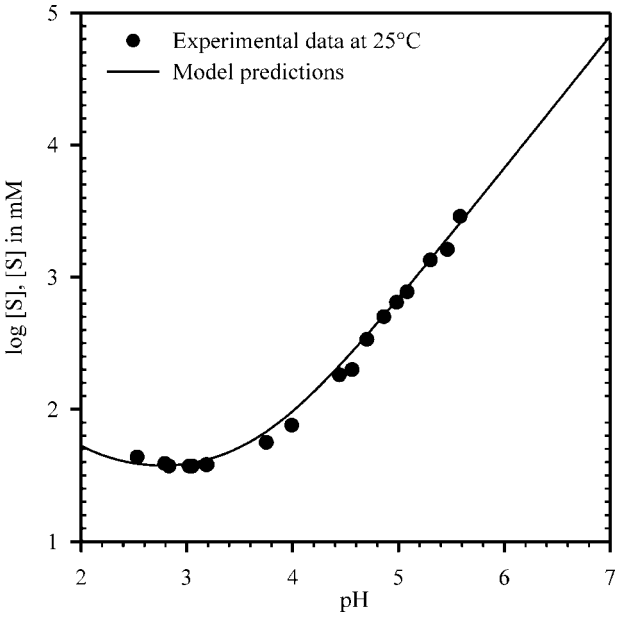

Figure 3. Comparison of predicted and measured solubility-pH isotherms for L-aspartic acid in equilibrium with its aqueous solutions at 25°.

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solubilities shown in Fig. 3 are in good agreement with experimental data over the pH range studied.

Precipitation by Carbon Dioxide Addition

The objective of this preliminary investigation was to demonstrate the ability to precipitate L-aspartic acid from aqueous solution via carbon dioxide addition at low pressures. Saturated aqueous L-aspartic acid solutions of known initial concentration were contacted with carbon dioxide in the presence of the solid phase. The solid phase was maintained in contact with the solution to avoid metastable supersaturated conditions.

A simple model was developed based on modifications from that described by Husson and King[30] to predict experimental results. The modifications were to replace the lactic acid dissociation and adsorption equilibria relations with L-aspartic acid dissociation and solubility-pH relations. The model allowed the calculation of equilibrium solubility-pH

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Table 1. Results of carbon dioxide pressurization on the solubility of L-aspartic acid in aqueous solution at 25°C. Initial solutions were saturated at pH > pI.

Pressure (psig)	Measured initial concentration (M)	Measured final concentration (M)	Calculated final concentration (M)
53 ± 1	2.98 ± 0.02	2.91 ± 0.02	2.96
65 ± 1	3.29 ± 0.03	3.21 ± 0.03	3.24
86 ± 1	3.42 ± 0.03	3.32 ± 0.03	3.38

for L-aspartic acid in the presence of carbon dioxide. Input values included the CO_2 pressure and the initial concentration of strong-base cation (i.e., K^+). Only pH effects on solubility were considered in this model; changes in physical solvation due to the introduction of carbon dioxide antisolvent into the solution were ignored, realizing that this effect might likely be important, especially for experiments at high pressure.

Since physical-solvation effects will likely be more important at higher carbon dioxide pressures, initial studies employed low carbon dioxide pressures, where our model assumptions would then apply. Table 1 presents the results of the precipitation experiments. The first column shows the CO_2 pressure. The remaining columns show the initial solution L-aspartic acid concentration prior to contact with CO_2, and the experimentally measured and model calculated solubilities. Because small solubility differences were expected and measured for these low pressures, the following experimental precautions were taken: initial- and final-solution L-aspartic acid concentrations were measured back to back during a single chromatography sequence. At least three concentration measurements were made for each solution. Uncertainties in Table 1 correspond to the propagation of uncertainties from sample and standard concentration measurements.

PRODUCT AND PROCESS CONSIDERATIONS

These preliminary low-pressure studies motivate further investigations. However, the low-percentage recoveries of L-aspartic acid under the conditions studied prompt the questions: What product compounds might be suited to such a separation process, and how might higher pressures help the separation process?

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Product Compounds

Our discussion is limited to those compounds that display pH-dependent solubility. Two facts about carbon dioxide help to define suitable compounds. Carbon dioxide acts as a weak acid in aqueous solution, and it has a relatively low solubility at low pressures (Henry's constant is 1676 bar at 25°C[31]). Therefore, to take advantage of pH-dependent solubilities using low-to-moderate CO_2 pressures (< 20 bar), the compound must display pH-dependent solubilities at moderate-to-high pH values (> 5). It must also occur in solution at these moderate-to-high pH values and at concentrations above the minimum point displayed by the pH-solubility curve (e.g., the isoelectric solubility for amino acids). Of course, these values will depend on the compound solubilities; higher solubilities at a given initial pH will result in lower pH shifts for a given charge of CO_2. Husson and King[30] show such a buffering effect for solutions of lactate anion in contact with CO_2. In addition to being a high-volume amino acid product, L-aspartic acid was chosen as a model compound that meets these requirements.

High-Pressure CO_2

Carbon dioxide is a relatively poor solvent for charged compounds. This fact proves to be beneficial for the separation process under consideration. In addition to altering solubility by changing solution pH, CO_2 acts as an antisolvent for L-aspartic acid. At low pressures, this secondary effect on L-aspartic acid solubility is likely less important than the pH effect. At higher pressures, it is expected that this effect becomes important as pressurization has a diminishing impact on changes in solution pH. Our low-pressure results indicate a need for high pressure. Obviously, the drawback to using CO_2 as an antisolvent is the need for high-pressurization equipment. It does have advantages over conventional antisolvents like methanol; notably, subsequent separation from water is easier.

CONCLUSION

This work described a separation process for recovering L-aspartic acid from dilute aqueous solution by precipitation with carbon dioxide at low pressure. Pressurization of saturated L-aspartic acid solutions resulted in precipitation of L-aspartic acid. Under the conditions studied in this preliminary research, the principal mechanism for this precipitation is

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thought to be this: Carbon dioxide alters the solution pH by acting as an acidulent. L-aspartic acid solubilities change with changing pH. If carbon dioxide shifts the pH toward the isoelectric point, then the corresponding solubility limit of L-aspartic acid will be reached, thereby causing its precipitation.

Percentage recoveries were low (2.35% to 2.92%) for the low pressures studied in this preliminary work. This result is important because it shows that carbon dioxide is too weak of an acid in aqueous solution to recover L-aspartic acid by pH swing alone. For this separation process to be viable, higher pressures are needed. Higher pressures are expected to increase percentage recoveries by decreasing solvent power as more carbon dioxide is added to solution.

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