

**Study on Succinic Acid Extraction by Liquid Membranes and  
Aqueous Two-phase Systems Containing Ionic Liquids**

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**Aulia Indah Pratiwi**

**Graduate School of Science and Engineering**

**Doshisha University**

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# **CHAPTER 1**

## **GENERAL INTRODUCTION**

# 1

## GENERAL INTRODUCTION

Succinic acid is a dicarboxylic acid with 4 atoms (Figure 1), which has wide application in industrial sector as commodity chemicals and specialty chemicals (Figure 2) [1].

So far, succinic acid was synthesized from petroleum chemicals (paraffin hydrocarbon) by hydrogenation of maleic acid, maleic anhydride or fumaric acid and by oxidation of 1,4-butanediol [2]. But it was costly production due to high petroleum cost and high CO<sub>2</sub> emission [3]. Therefore, production of succinic acid from the fermentation of renewable raw materials by microorganisms such as *Anaerobiospirillum succiniciproducens* [6], *Escherichia coli* [7], *Mannheimia succiniciproducens* [8], *Actinobacillus succinogenes* [9], *Corynebacterium glutamicum* [10], is emerged as the possible alternative of petroleum route. Succinic acid produced from fermentative processes was called as bio-succinic acid or bio-based compound in downstream processes or bio-refinery production. Fermentative production of succinic acid could produce ethanol and diethyl succinate as by-products, which are very favorable in industrial sectors.

In 2010, succinic acid as bio-based product was commercially produced from carbohydrates such as sugar [4]. However, Akhtar *et al.* [5] described that

the advances in efficient downstream processing are needed for higher earnings and profit in succinic acid production in the future.

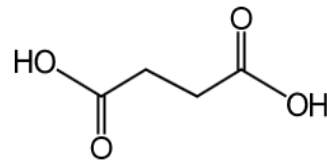


Figure 1. Succinic acid

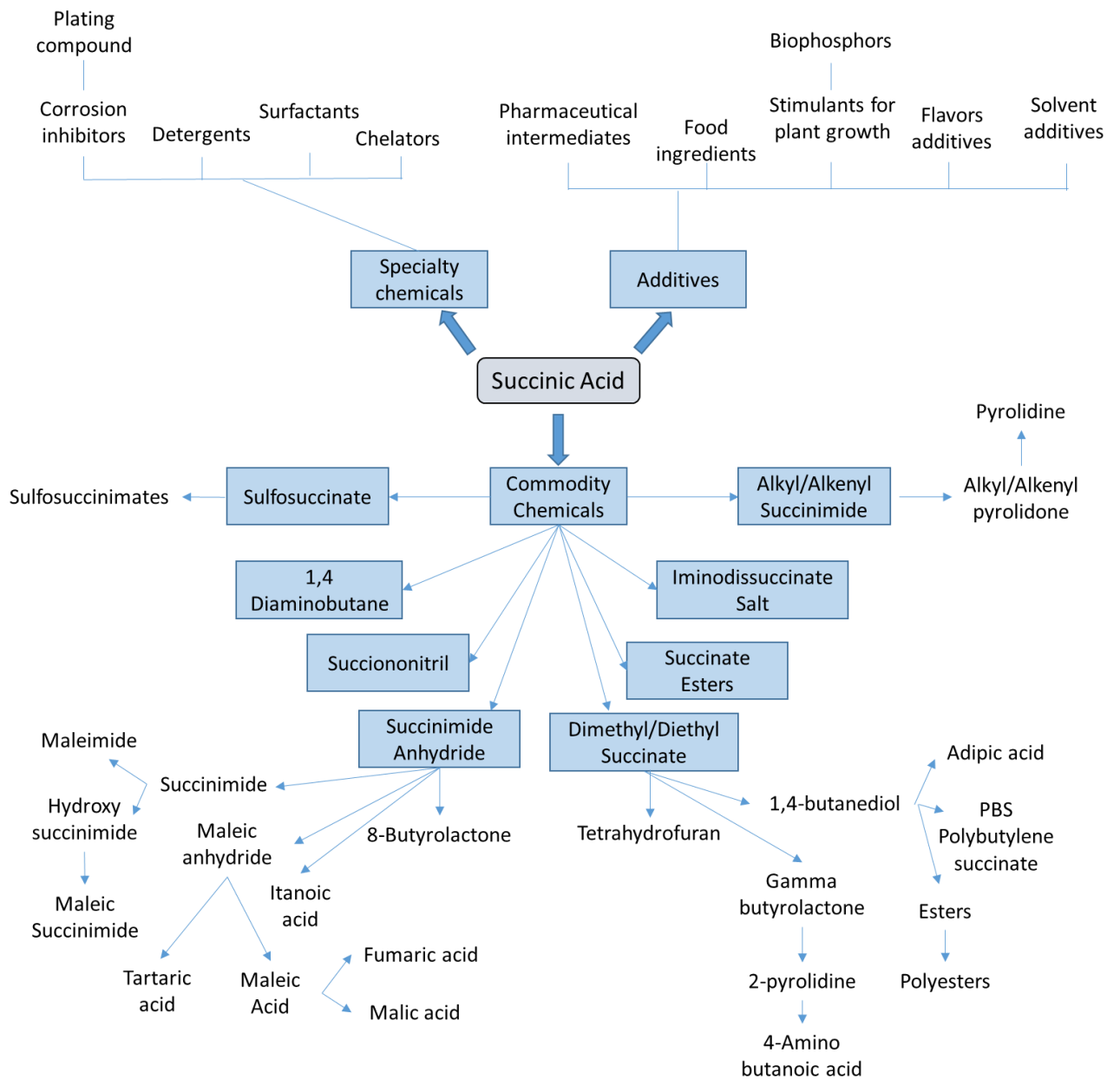


Figure 2. The application of succinic acid [1]

Some factors that affect the price of succinic acid are the nutrients price, recovery and purification processes and so on. The major contributor for the final price is recovery and purification sector [11]. Hence it is needed to look for the suitable condition and correct method to separate succinic acid from fermentation broth. Generally, proposed separation techniques to recover succinic acid from fermentation broth are precipitation, crystallization, electrodialysis, liquid membrane and liquid-liquid extraction. Garzon *et al.* [12] also explained in detail about the primary recovery from fermentation broth after removing cells such as adsorption, extraction, precipitation, nanofiltration and reverse osmosis, and conventional electrodialysis. However, it is still needed to find separation process, which is shorter procedure, cost effective, and high extractability.

In the following, two common extraction techniques, liquid membrane separation techniques and aqueous two phase extraction process, were explained in detail.

### **Extraction techniques**

There are many techniques chosen to extract succinic acid from fermentation broth. However in this thesis, liquid membrane and aqueous two phase extraction method were well studied for succinic acid separation.

#### **(a) Liquid membrane techniques**

Generally, the advantages of membrane separation techniques are the simple concept and operations, modular and easy to scale up, and low energy

demand [13]. If the liquids used as membrane, which is immiscible with the feed and receiving phases, it is referred to liquid membranes (LMs) [14]. LMs are using less organic solvents compared to solvent extraction technique, hence it was developed into several LMs configuration such as bulk liquid membranes [15;16], emulsion liquid membrane [17;18], supported liquid membranes (SLM) [19;20;21;22] and polymer inclusion membrane (PIM) [23]. Figure 3 shows each configuration between feed phase (F), receiving phase (R), and membrane (M) [14]. Configuration of PIM is similar to that of SLM. SLM and PIM will be discussed in detail in Chapters 2 and 3 respectively. In SLM and PIM systems, the membrane was put between feed and receiving phases.

Matsumoto *et al.* compared the solvent extraction and supported ionic liquid membrane for the extraction of penicillin G which is a kind of organic acid [20]. Penicillin G was extracted in both methods of separation. In LM operation, uphill transport was successfully accomplished by using ionic liquid, trioctylmethylammonium chloride (TOMAC) as a membrane solution. Therefore, in Chapter 2, supported liquid membrane impregnated with ionic liquid (SILM) as a membrane solution is used to study the permeation mechanism of succinic acid.

PIMs are formed by casting a solution containing a carrier, plasticizer, and base polymer such as cellulose triacetate (CTA) or poly(vinyl chloride) (PVC) to form a thin, flexible, and stable film [25]. PIM has been extensively used as transition of metal ions [26], removal of strontium from nuclear waste [27], cation separation ( $\text{Cd}^{2+}$ ) [28], and for separation of organic acid [23]. PIMs are gelled liquid membranes.

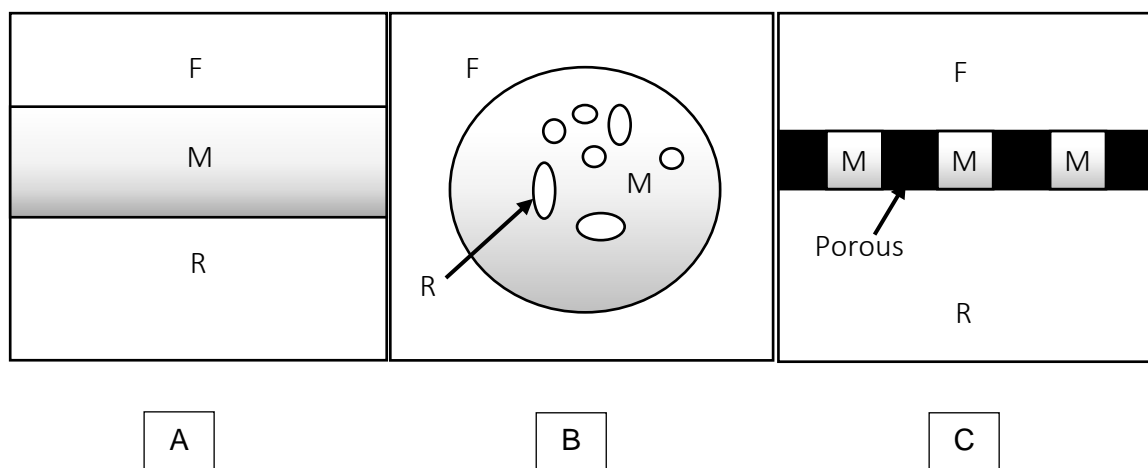


Figure 3. Liquid membranes configuration: bulk (A), emulsion (B), and supported (C). F, feed; M, membrane; R, receiving<sup>[14]</sup>



The advantage of PIM over SILM is membrane stability and many research refers to this topic. PIM has been used in chemical sensing as potentiometry [28] and ion-selective electrodes [27] and it was demonstrated that PIMs had stable membrane and high selective transport. However the stability of membrane also was affected by the feed phase used [27]. Hence in this thesis (Chapter 3), the author would discuss about the stability of membrane when using Aliquat 336 as a carrier.

(b) Aqueous two-phase system method

Aqueous two-phase system (ATPS) is composed of two-immiscible aqueous rich phases based on polymer-polymer, polymer-salt or salt-salt combinations [29]. They consist of the more hydrophobic and hydrophilic aqueous two-phases. ATPS has a unique phase diagram under a particular set of conditions such as pH, temperature and ionic strength [30].

Basically, we should understand the extraction mechanism of the fermentative product when we use ATPS as the method in purification and separation. This leads to an increase in the yield and efficiency of the process itself. Therefore, some features mentioned below are used to analyze the mechanism in ATPS containing polymer and salt [31]:

- 1) The physicochemical characteristic in solvents,
- 2) Influence of polymer composition,
- 3) Influence of the salt composition,
- 4) Influence of the polar groups of solute, and
- 5) Hydrophobic and polar hydration effect

Recently, ATPS was focused again as the greener solvent and non-volatile solvent. Moreover, water-soluble organic solvents and ionic liquids (ILs) instead of polymers as a phase separating agent have been used. Specially, ILs were much interested as the replacement of volatile organic solvent because of its capability in the formation of two-phase in the tailoring their polarities and affinities by a proper manipulation of the cation and anion design and their combination itself (29). Afterwards, in Chapters 4 and 5, the ATPS techniques will apply with ILs to gain better extractability of succinic acid.

### **Ionic Liquids as green solvent for extraction and separation system**

Nowadays, Ionic Liquids (ILs) has been well known as green solvent among chemicals in extraction techniques. As molten salt, ILs have unique properties because it has negligibly small vapor pressure, high thermal stability, high viscosity, good solvation ability, a wide liquid range, and tunable polarity [24].

Due to those advantageous of ILs, many researches lead to application of ILs into separation method, such as supported ionic liquid membrane (SILM), polymer inclusion membrane (PIM), and aqueous two-phase system (ATPS), which are going to discuss in the following chapters.

Though supported liquid membrane has many benefit among separation techniques such as simple to scale-up, very low energy requirements, low capital and operating cost and high separation factors [35], but the stability and long term performance of this method are concerned as well. Therefore ILs, which has unique characters, recently been used as stable impregnated liquids in the supported liquid membrane process [35,36,37].

ILs applied in ATPS could form two-phase due to salting-out. Gutowsky *et al.* [38], shows that imidazolium based IL were suitable for the salting-out based ATPS and since there are many applications of ILs for ATPS [20,33,34]. Author will discuss in Chapters 4 and 5, applied for extraction of succinic acid in various ILs by salting-out and sugaring-out.

## **OUTLINE OF THIS THESIS**

In this thesis, separation and extraction methods of succinic acid were studied using supported ionic liquid membrane (SILM), polymer inclusion membrane (PIM), and aqueous two-phase system (ATPS). In order to find basic information for extraction of succinic acid from fermentation broth, author is used not fermentation broth but the model solution containing succinic acid only. The pH condition of each experiment series will be considered to considerably affect the extraction behavior of succinic acid by the membrane or the ATPS. In Chapter 2, the SILM method were applied by using Aliquat 336. Author examined the effect of pH and sodium chloride concentration on the permeability and distribution ratio. The permeation mechanism was explained using the solution-diffusion model. In Chapter 3, the PIM method also using Aliquat 336 ionic liquid which were formed by casting solution to form flexible and stable film. The uphill transport was observed by using feed solution  $\text{Na}_2\text{CO}_3$  with pH 4. ATPS method was performed to extract succinic acid using imidazolium-based ionic liquid in Chapter 4 and Chapter 5. In Chapter 4, salting-out extraction proceeded when adding salt into the solution by using imidazolium-based ionic liquids and alcohols. The succinic acid extracted to the upper phase was recovered as the precipitate

of succinate salt by using alkali hydroxide. In Chapter 5 the ATPS were successfully extracted the succinic acid using 1-propanol and  $[\text{C}_4\text{mim}][\text{CF}_3\text{SO}_3]$ . Generally the using of ionic liquid as solvent extractor in each system presented in this thesis were successfully improved the extractability of succinic acid.

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## **CHAPTER 2**

### **Permeation of Succinic Acid through Supported Ionic Liquid Membranes**

# 2

## Permeation of Succinic Acid through Supported Ionic Liquid Membranes

**Abstract:** Ionic liquids, which have negligible vapor pressure and whose solubility in the surrounding phases can be minimized by the appropriate selection of anions and cations, have potential application as supported ionic liquid membranes (SILMs) to separate succinic acid. It is found that succinate successfully permeated through an SILM containing Aliquat 336 and observed the uphill transport. By examining the effects of pH and sodium chloride concentration on the permeability and distribution ratio, the permeation mechanism of succinate using the solution-diffusion model was explained. It found that the succinate di-anion reacted with quaternary ammonium salt in the membrane during the SILM process.

### Introduction

A separation method for succinic acid has been intensively studied for cost-effectiveness and environmental safety [1,2,3,4]. Methods such as precipitation, sorption, ion exchange, electrodialysis, and liquid–liquid extraction have been proposed to recover succinic acid from the fermentation broth. Cheng et al. (2012) [5] discussed the technological development of succinic acid

purification, including direct crystallization, membrane separation, chromatography, and in situ separation.

In separation techniques, ILs are used as a new class of alternative nonvolatile solvents because of the environmental concerns and toxicity issues associated with the use of common volatile organic solvents. ILs play an important role in the separation process because of their unique and tunable miscibility and solubility properties [6]. ILs also have low vapor pressure, high thermal stability, high viscosity, good solvation ability, a wide liquid range, and tunable polarity [7].

Although supported liquid membranes have advantages such as simple scale-up, very low energy requirements, low capital and operating costs, and high separation factors [8], there are concerns regarding their stability and long-term performance in the supported liquid membrane processes. However, because of their negligible volatility, relatively high viscosity, and interfacial tension, ILs have recently been used as stable impregnated liquids in the supported liquid membrane process [8,9,10,].

In this study, supported ionic liquid membranes (SILMs) were used for the separation of succinic acid. The previous studies [11,12] showed that Aliquat 336 gave a good permeation rate for lactic acid; the permeation behavior was also qualitatively discussed. In this study, the mechanism underlying the permeation of succinic acid through an SILM was investigated. For this purpose, the permeations under various pH conditions and in the presence of sodium chloride in the feed phase were examined.

## Experimental Section

### Chemicals

Aliquat 336 (main component: trioctylmethyl-ammonium chloride) was used as an IL and was purchased from Strem Chemicals Inc., Newburyport. Succinic acid was purchased from Nacalai Tesque, Inc. Hydrophilic porous polyvinylidene fluoride (PVDF) membranes (Millipore Corp., Billerica) were used as the support to maintain the IL in the membrane; the membrane thickness was 125  $\mu\text{m}$ , and the pore size was 0.45  $\mu\text{m}$ . Concentrated hydrochloric acid and concentrated sodium hydroxide solutions (Wako Pure Chemicals, Ltd.) were used for preparing the permeate solution and for adjusting the pH of the feed phase. All the chemicals were used without further purification.

### Membrane preparation

A hydrophilic PVDF membrane was soaked in Aliquat 336 in a petri dish. The membrane was left in the soaked state for 24 h at room temperature, wiped, and dried in vacuum desiccators for 24 h. Next, it was used as the SILM for our transport experiment. The membrane had an effective area,  $A$ , of 12  $\text{cm}^2$  and was fixed in the apparatus shown in Figure 1. In some experiments, the membrane thickness was adjusted by increasing the number of the membrane.

### Permeation experiment

The apparatus shown in Figure 1 for the permeation experiment consists of feed and stripping phases. The feed solution was succinic acid (1 g/L) with the pH set at the desired value (from 3 to 9) by adding concentrated sodium hydroxide solution. The feed solution was adjusted at the optimum pH for

examining the effect of the membrane thickness. The stripping solution was 0.085 mol/dm<sup>3</sup> hydrochloric acid, whose pH was 1.1. This concentration ratio in the feed and stripping phases was based on a previous procedure [12]. Each compartment was filled with 100 mL of the respective solution at room temperature under the initiated conditions. The feed solution was maintained at a specific pH by adding concentrated sodium hydroxide solution during the experiment. The stirring speed of the magnetic bar in each cell was controlled at 300 rpm. Samples from both solutions were withdrawn at regular time intervals, and the pH in both cells was measured with a pH meter. Succinic acid concentrations were determined by HPLC (LC-10 ADvp) with a refractive index detector (RID-10 A, Shimazu Corp.) using a Shodex SUGAR SH-1011 (Showa Denko K. K.) column and 5 mmol/dm<sup>3</sup> sulfuric acid solution as the mobile phase.

### **Distribution experiment**

Liquid–liquid extraction experiments were performed to determine the distribution of succinic acid in Aliquat 336. 10 mL of aqueous succinic acid solution with the desired pH and 10 mL of Aliquat 336 without any dilution were mixed in a tube and placed in a thermo-mixer at 30°C until equilibrium was reached. The concentration of succinic acid and the pH of the aqueous solution were measured before and after equilibration. The distribution ratio,  $K_D$ , was defined as the ratio of the succinate concentration in the extract (Aliquat 336) phase to that in the raffinate phase.

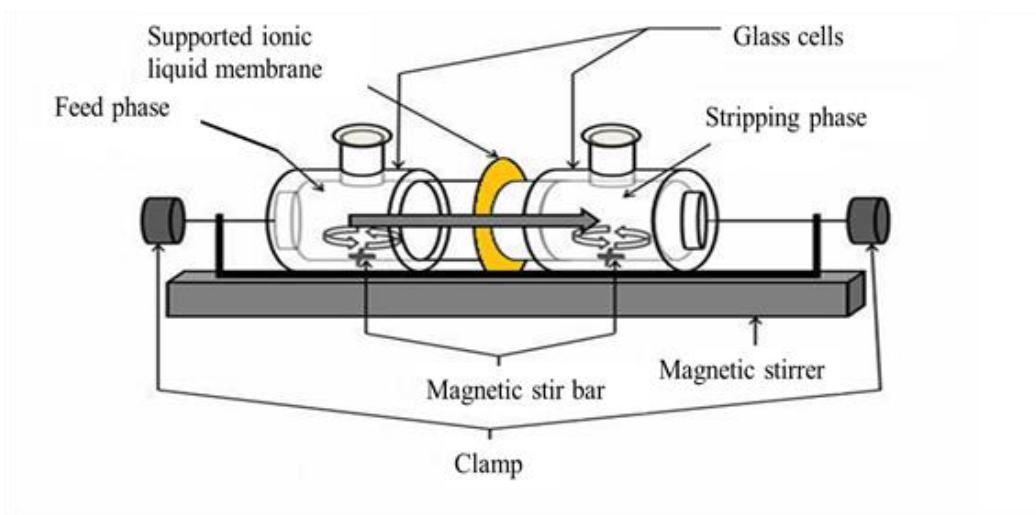


Figure 1. Experimental apparatus for studying the permeation of succinic acid through an SILM

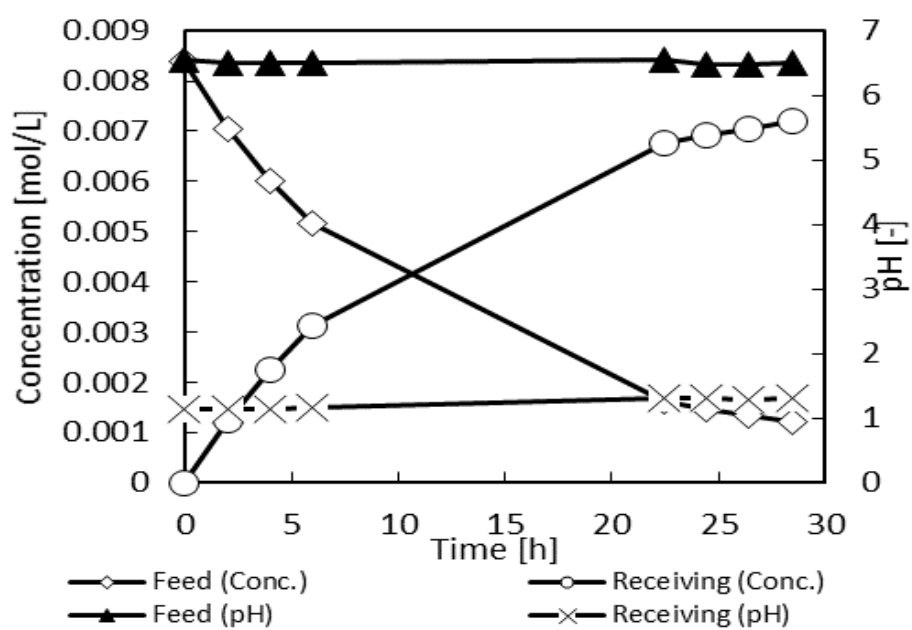


Figure 2. Typical result for the permeation of succinic acid through the SILM

## Results and Discussion

The typical results for the time courses of the succinic acid concentration and the pH of the feed and stripping solutions using Aliquat 336 are shown in Figure 2. Aliquat 336 was used as an IL because it showed an excellent permeation rate in lactate permeation [11;12].

The concentration of succinic acid in the feed phase decreases with time, while the concentration in the stripping phase increases. We observed the uphill transport of succinic acid. The succinate anion in the feed phase at pH 6.5 (the  $pK_{a1}$  and  $pK_{a2}$  values of succinic acid are 4.17 and 5.64) diffuses across the SILM into the HCl-containing stripping phase, where it reacts with hydrochloric acid to form undissociated succinic acid. Since this species is not soluble in the membrane phase, it is trapped in the stripping phase. Therefore, the permeation proceeds until the succinate concentration in the feed phase falls to zero.

### Effect of membrane thickness

Generally, the driving force for succinate transport is the gradient through the diffusional layers of the feed liquid film and the liquid membrane, because the succinate concentration in the stripping phase is assumed to be zero, as described above. Therefore, the succinate flux is expressed by the following equation [13]:

$$J = -\frac{V_f}{A} \frac{dC_f}{dt} = K_{ov} C_f \quad (1)$$

where  $K_{ov}$  is the overall mass transfer coefficient,  $V_f$  is the volume of the feed solution,  $A$  is the contact area between the feed and membrane phases, and  $C_f$  is the succinate concentration in the feed phase. Therefore,

$$\ln\left(\frac{C_f}{C_{f,0}}\right) = -a \cdot K_{ov} \cdot t \quad (2)$$



where the subscript 0 denotes the initial state and  $a (=A/V_f=12 \text{ m}^{-1})$  is the specific area. The  $K_{ov}$  values were obtained from the slopes of the straight line plots based on Eq. (2), as shown in Figure 3.

The overall resistance to mass transfer,  $1/K_{ov}$ , is considered the sum of the membrane resistance  $1/K_m$  and the feed boundary layer resistance  $1/K_L$  [14]:

$$\frac{1}{K_{ov}} = \frac{1}{K_L} + \frac{1}{K_m} = \frac{1}{K_L} + \frac{\delta}{P} \quad (3)$$

where  $P$  is the permeability and  $\delta$  is the membrane thickness. To evaluate the film mass transfer coefficient, the relation between the overall mass transfer coefficient and the membrane thickness was investigated (Figure 4).

From the intercept in Figure 4, the feed boundary layer resistance,  $1/K_L$ , was found to be 72.9 h/m. Next, the permeability obtained from Eq. (3) was discussed by assuming that feed boundary layer resistance is independent of the feed composition.

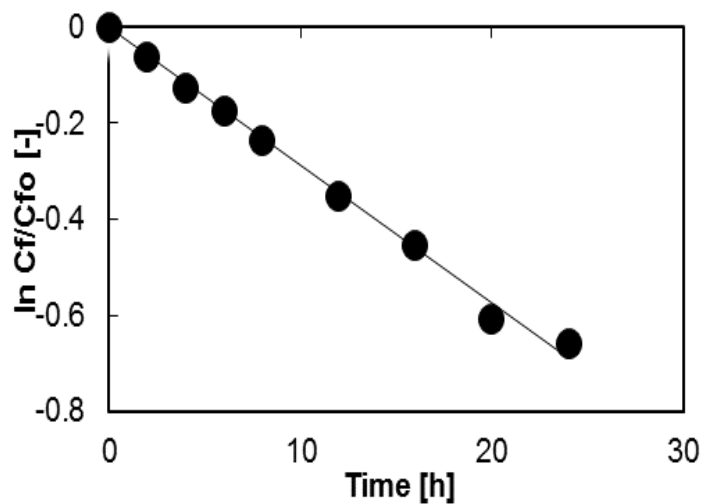


Figure 3. Typical result for the determination of the overall mass transfer coefficient from plots based on Eq. (2)

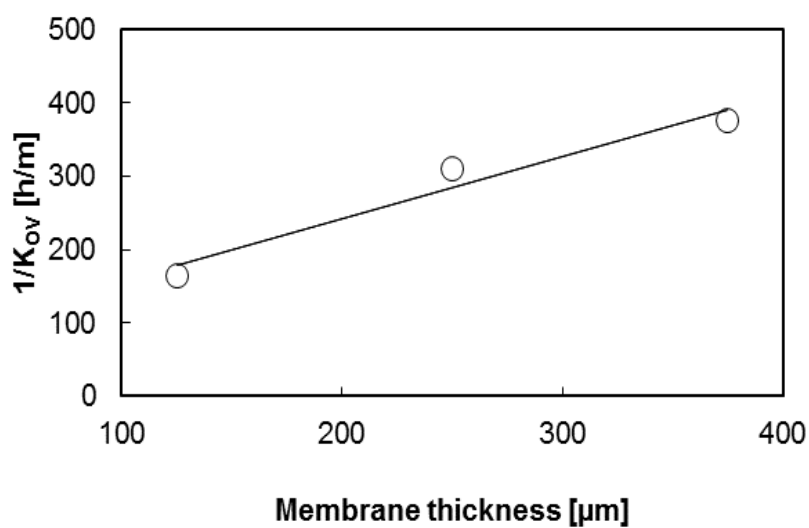


Figure 4. Effect of membrane thickness on the overall mass transfer coefficient

### Effect of pH

The pH effect on the permeability is presented in Figure 5. The maximum permeability was observed at pH 6.5, which was very convenient because the optimum pH in the fermentation broth was 6–7 [15]. As concluded by Song *et al.* (2006) [16], selective separation of a specific acid from a fermentation broth containing mixed acids can be achieved by adjusting the  $pK_a$  values of the acids and the operating pH.

In this case,  $pK_{a1}$  and  $pK_{a2}$  of succinic acid are 4.2 and 5.6, respectively. The pH of the feed solution should set at a larger value than  $pK_a$  of succinic acid to ensure better permeation, suggesting that the permeation species is anionic, that is, succinate.

Figure 4 also shows the pH effect on the succinate distribution ratio. A similar pH dependency indicates that the succinate distribution controls the permeation process.

### Effect of salt

The effect of salt added to the feed phase can be seen in Figure 6. The salt (sodium chloride) reduces the permeability of succinate because anion exchange in the feed phase with Aliquat 336 is blocked by the excessive chloride ions in the feed solution, suggesting that permeation proceeds via the anion exchange reaction. This is proved by the salt effect on the succinate distribution ratio (Figure 6).

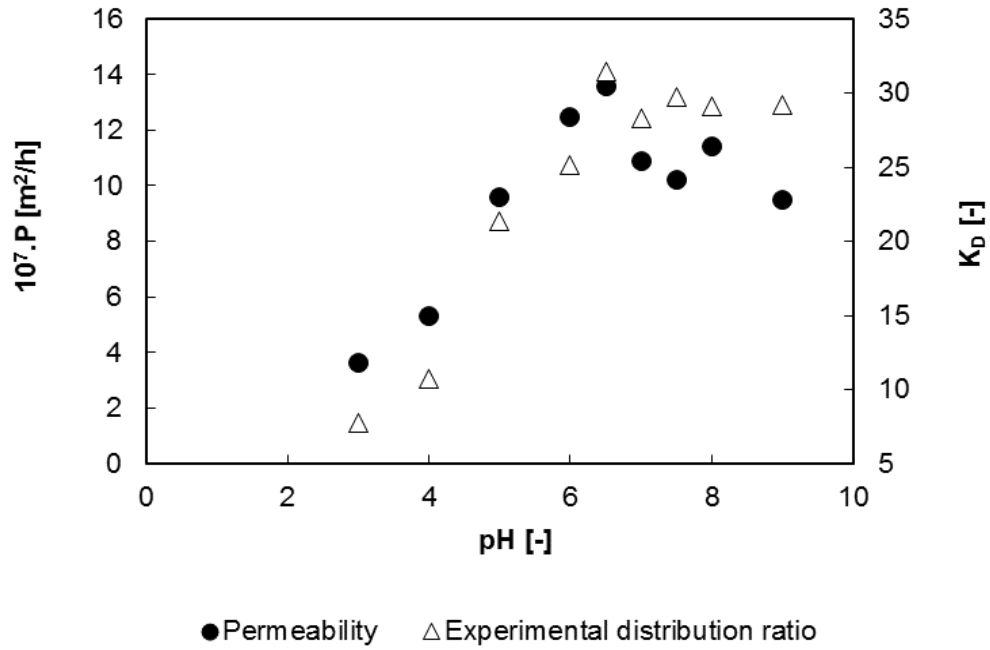


Figure 5. Effect of pH on permeability and distribution ratio

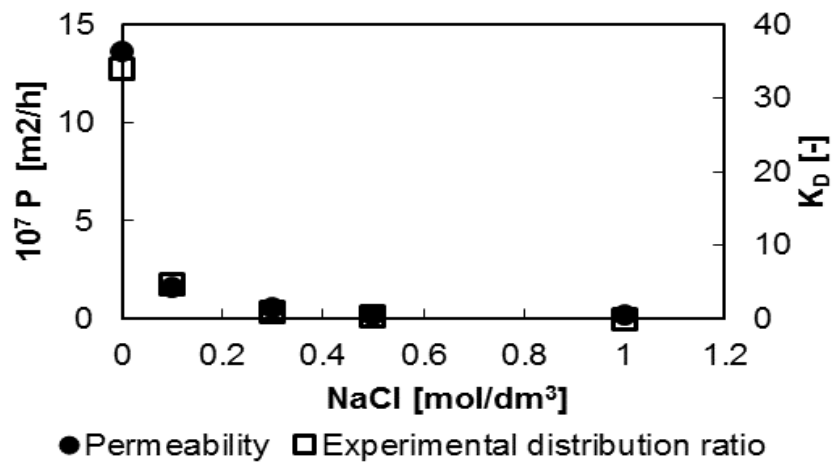


Figure 6. Effect of NaCl on permeability and distribution ratio

### Permeation mechanism

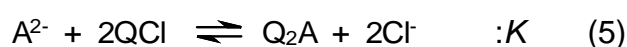
Based on the solution diffusion theory, permeability is expressed by the following equation:

$$P = D_f \cdot K_D \quad (4)$$

where  $D_f$  is the diffusivity of the succinate complex and  $K_D$  is the succinate distribution ratio.

If the diffusivities of the succinate complex with Aliquat 336 are kept constant under the experimental conditions in this study, the permeability should be proportional to the distribution ratio. This relation, which can be seen in Figure 7, was found to be linear. The diffusivity obtained ( $D_f = 1.2 \times 10^{-12} \text{ m}^2/\text{s}$ ) was similar to that in Aliquat 336-impregnated resin [17] and about 10 times greater than those in Aliquat 336/PVC membranes [14,18].

In our previous study [19], we found that the succinate di-anion ( $A^{2-}$ ) reacted with quaternary ammonium salt by an anion-exchange reaction based on Eq. (5). In this study, since we used pure Aliquat 336 without diluents, we confirmed the extraction equilibrium of succinate ( $A^{2-}$ ) with Aliquat 336 (QCl):



where  $K$  is the extraction equilibrium constant defined by Eq. (6):

$$K = \frac{[Q_2A]_{\text{org}} [Cl^-]^2}{[A^{2-}] [QCl]_{\text{org}}^2} \quad (6)$$

Equation (7) is derived from Eq. (6) using  $K_{a1}$  and  $K_{a2}$  :

$$\log K'_D = \log K + 2 \log \left( \frac{[QCl]_{\text{org}}}{[Cl^-]} \right) \quad (7)$$

where  $K_D'$  is the distribution coefficient of  $A^{2-}$  defined by Eq. (8):

$$K_D' = K_D \left( 1 + \frac{[H^+]^2}{K_{a1}K_{a2}} + \frac{[H^+]}{K_{a2}} \right) \quad (8)$$

We assumed that the Aliquat 336 and chloride concentrations at equilibrium equal the initial concentrations because the latter concentrations are much larger than the concentration of succinic acid.

Figure 8 shows the plot based on Eq. (7). A straight line with a slope of 2 was obtained. In this case, the experimental results satisfied Eq. (5). Therefore, the mechanism of permeation of succinic acid through the SILM is considered to involve the following steps

- 1) The succinate di-anion reacted with Aliquat 336 by an anion-exchange reaction at the interface between the feed and membrane phases.
- 2) The Aliquat 336-succinate complex diffused through the IL membrane.
- 3) The Aliquat 336-succinate complex reacted with hydrochloric acid to form undissociated succinic acid at the interface between the membrane and stripping phases.

Figure 9 shows a schematic representation of these steps.

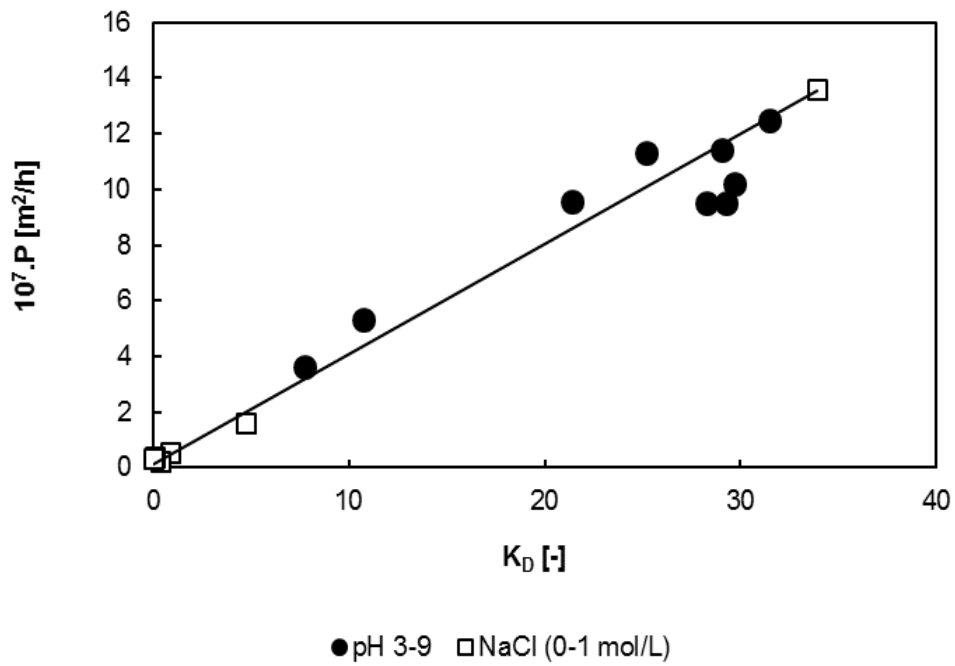


Figure 7. Relation between permeability and distribution ratio

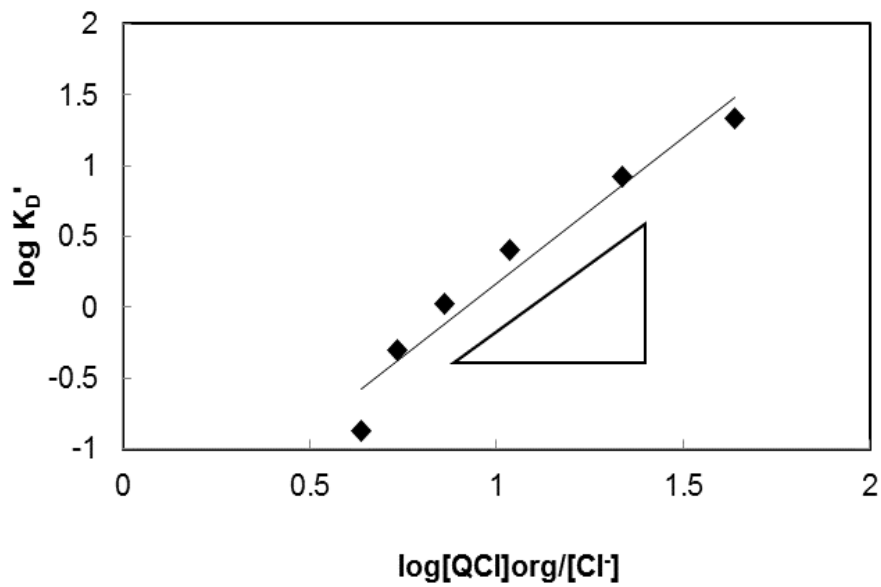


Figure 8. Extraction equilibrium of succinate with Aliquat 336 Plot

based on Eq. (7)

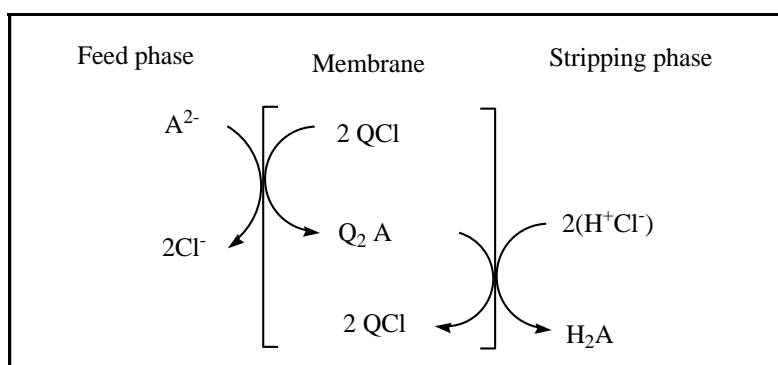


Figure 9. Transport mechanism for permeation of succinic acid by an SILM

( $H_2A$ : succinic acid,  $QCl$ : Aliquat 336)



## Conclusion

Permeation of succinate through an SILM using Aliquat 336 as an IL and hydrochloride acid as a receiving solution was successfully carried out against its concentration gradient. The optimum pH for the permeation of succinate coincided with that of the fermentation process.

The chloride ion in the feed phase depressed the permeability and distribution ratio of the succinate, suggesting that permeation proceeded by an anion-exchange reaction. From the distribution ratio experiments with succinate, we found that the di-anion species is involved in the permeation of succinic acid through the SILM. The proportional relation between the permeability and the distribution ratio was obtained and it is found that the permeation follows the solution diffusion mechanism.

These results suggest that future research must devoted toward finding a lifetime of use of the membrane and measuring the stability of the IL reaction to obtain a complete report of the uses of ILs as supported membranes for the permeation of succinic acid.

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## **CHAPTER 3**

### **Permeation Mechanism of Succinic Acid through Polymer Inclusion Membranes with Ionic Liquid Aliquat 336**

# 3

## Permeation Mechanism of Succinic Acid through Polymer Inclusion Membranes with Ionic Liquid Aliquat 336

**Abstract:** In this study, polymer inclusion membranes (PIMs) containing an ionic liquid, Aliquat 336, were applied to the separation of succinic acid. PIMs are formed by casting a solution containing a carrier, plasticizer, and base polymer (poly(vinyl chloride)) to form a thin, flexible, and stable film. It is found that succinate successfully permeated the PIM containing Aliquat 336 and observed the uphill transport using a feed solution with pH 4 and  $\text{Na}_2\text{CO}_3$  solution as the stripping phase. PIMs show complex permeation behavior involving undissociated, mono-anionic, and di-anionic forms of succinic acid as the species permeated. The mono-anionic form of succinic acid showed the largest permeability among the three forms, with the optimum pH range for succinic acid permeation being approximately 4–5.

### Introduction

Liquid membrane processes are interesting because they use less organic solvent. In the previous studies [1,2,3; Chapter 2] showed that Aliquat 336 as a membrane component and HCl as a stripping agent afforded a good permeation rate for lactic acid in the SILM and PIM, and that succinic acid

permeates successfully through the SILM. In this study, we applied a PIM containing IL for the separation of succinic acid. PIMs are formed by casting a solution containing a carrier, plasticizer, and base polymer such as cellulose triacetate (CTA) or poly(vinyl chloride) (PVC) to form a thin, flexible, and stable film [4]. It is well known that PIMs are more stable and have lower permeability than supported liquid membranes [5].

There are many studies about PIM, which are applied to separate metal ions such as Cr (IV) [6; 7]. Consequently modified PIM using new carrier as plasticizer has been developed as well [6; 8; 9]. The driving force that brings through the species across the membrane is the concentration gradient of the carrier complex or ion-pair formed within the PIM [10]. Therefore, PIM is considered to be a kind of liquid membrane.

Aliquat 336 as plasticizer for PIM has been well-known because of its properties, water insoluble quaternary ammonium salt composed of a large organic cation associated with a chloride [11] as shown in Figure 1.

Kagaya *et al.* [9] has been studied PIM using Aliquat 336 as carrier, however it was not applied into separation of bio-based yet. Therefore, in this study, the permeation mechanism of succinic acid through PIMs was examined.

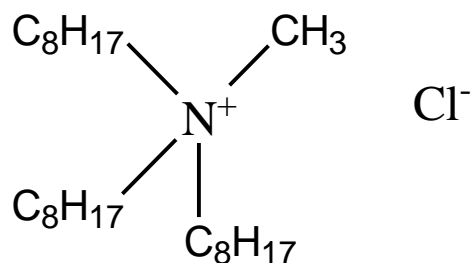


Figure 1. Aliquat 336

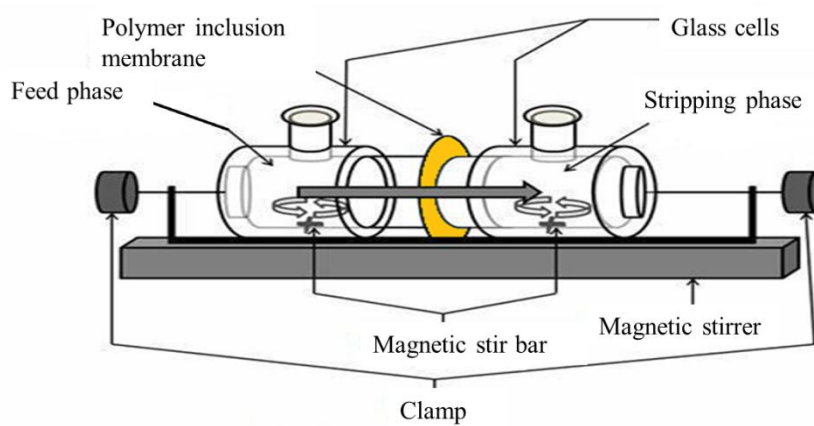


Figure 2. Experimental apparatus for studying the permeation mechanism of succinic acid through PIM



## Experimental Section

### Chemicals

Aliquat 336 (main component: trioctylmethyl-ammonium chloride) was used as an IL and was purchased from Strem Chemicals Inc., Newburyport. The base polymer used was PVC from Wako Pure Chemical Industries, Ltd., ( $n=1,100$ ). Succinic acid was purchased from Nacal Tesque, Inc. Concentrated hydrochloric acid and concentrated sodium hydroxide solutions (Wako Pure Chemical Industries, Ltd.) were used for preparing the permeate solution and for adjusting the pH of the feed phase. All chemicals were used without further purification.

### Membrane preparation

According to the procedure described in a previous paper [3], the PIM was prepared by the solution casting method. A polymer solution was prepared by dissolving the PVC and Aliquat 336 in 25 mL of tetrahydrofuran (THF). The solution mixture was stirred with a homogenizer and poured into a Petri dish. After evaporating THF for 24 h at room temperature, the resultant PIM was typically obtained with an average film thickness of 280  $\mu\text{m}$  using a micrometer (Digimatic, Mitutoyo). When examining the effect of the membrane thickness, the amount of the solution mixture was adjusted. The polymer membranes had an effective area,  $A$ , of 12  $\text{cm}^2$  and were fixed in the apparatus, as shown in Figure 1.

### Permeation experiment

The apparatus shown in Figure 2 for the permeation experiment consists of the feed and stripping phases. The feed solution was succinic acid (2 or 5  $\text{kg/m}^3$ ), with the pH set at the desired value (from 2 to 8) by adding concentrated

sodium hydroxide solution. The feed solution was adjusted at the optimum pH for examining the effect of the membrane thickness. The stripping solution was 0.5 mol/dm<sup>3</sup> hydrochloric acid or sodium carbonate solution. Each compartment was filled with 100 mL of the respective solution at room temperature under the initiated conditions. The feed solution was maintained at a specific pH by adding concentrated sodium hydroxide or hydrochloric acid solution during the experiment. The stirring speed of the magnetic bar in each cell was controlled at 300 rpm. Samples from both solutions were withdrawn at regular intervals, and the pH in both cells was measured with a pH meter. Succinic acid concentrations were determined using HPLC (LC-10 ADvp) with a refractive index detector (RID-10 A, Shimadzu Corp.) using a Shodex SUGAR SH-1011 (Showa Denko K.K.) column and 5 mmol/dm<sup>3</sup> sulfuric acid solution as the mobile phase.

The permeation percent was defined by Eq. (1):

$$\text{Permeation percent}[\%] = \frac{C_{S,24h}}{C_{F,0}} \times 100(1)$$

where  $C_{S,24h}$  is the concentration of succinic acid in the stripping phase after 24 h and  $C_{F,0}$  is the initial concentration of succinic acid in the feed phase.

### Stripping experiment

Liquid–liquid stripping experiments were performed to determine the effect of stripping agents. Ten mL of aqueous succinic acid solution with pH = 4 and 10 mL of Aliquat 336 without dilution were mixed in a tube and placed in a thermo-mixer at 30°C until equilibrium was reached. Four mL of succinic acid-loaded Aliquat 336 solution was taken in a tube and was mixed with 4 mL of hydrochloric acid or sodium carbonate solution at 30°C until equilibrium was reached. The concentration of succinic acid and the pH of the aqueous solution were measured

before and after equilibration. The extent of stripping,  $E$ , was defined as the ratio of the succinate concentration in the stripping solution to initial succinic acid concentration in succinic acid-loaded Aliquat 336 solution.

## Results and Discussion

### Effect of stripping agent on permeation

The time courses of the succinic acid concentration in the feed and stripping solutions for PIM permeation are shown in Figure 3, when hydrochloric acid was used as a stripping agent. In the previous studies on lactate permeation through SILM and PIM [2,3] and succinate permeation through SILM (Chapter 2), hydrochloric acid was found to be efficient as a stripping agent, and the permeation percents were over 80% after 24 hours. Unexpectedly, the permeation rate of succinate in PIM was lower than those for the lactate permeation through SILM and PIM and the succinate permeation through SILM.

Therefore, this permeation behavior should be elucidated. In SILM permeation, di-succinate anion ( $A^{2-}$ ) reacted with Aliquat 336 (QCl) to form the bulky ternary complex  $Q_2A$  (Chapter 2). The diffusion coefficient in PIM, after normalization, was reported to be much lower than in the corresponding SLM[12]. The bulky tertiary complex,  $Q_2A$ , unlike lactate mono-anion binary complex, may be difficult to diffuse through PIM. If mono-anion of succinate ( $HA^-$ ) is alternative species permeated, it may be possible to observe rapid permeation. Therefore, we examined composition of feed and stripping solutions. First, two stripping agents,  $Na_2CO_3$  and HCl, were examined Table 1 shows the extent of back-extraction from succinate-loaded Aliquat 336 phase to the stripping phase using  $Na_2CO_3$  and HCl as stripping agent.

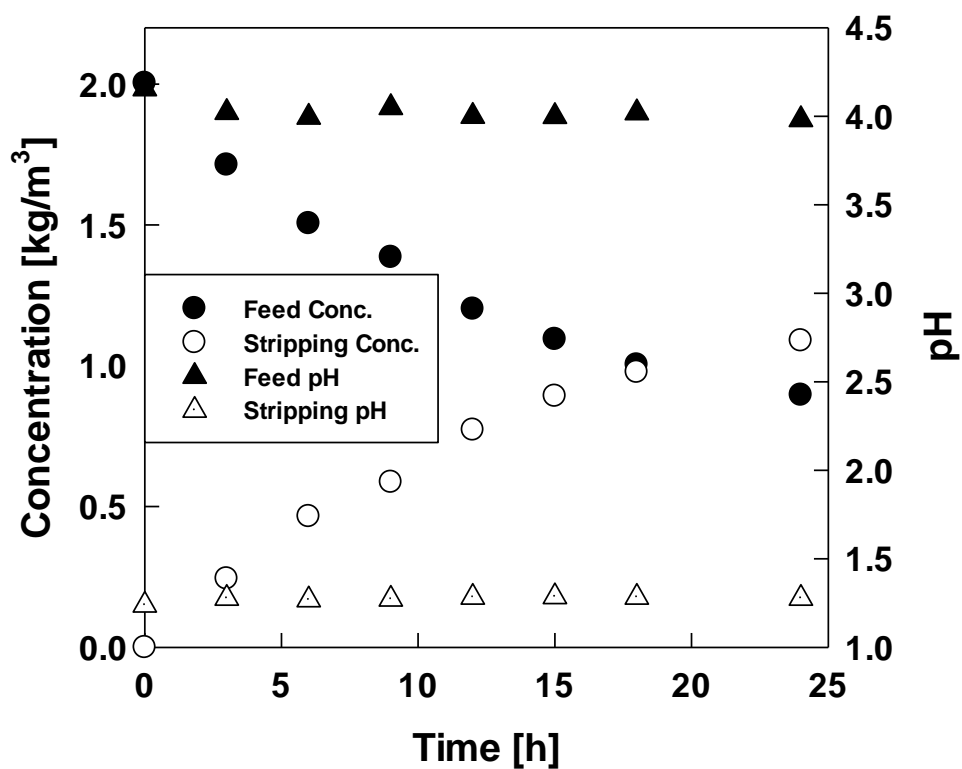


Figure 3. Permeation of succinic acid through PIM using hydrochloric acid  
(membrane thickness, 135  $\mu\text{m}$ )

Table 1. Effect of  $\text{Na}_2\text{CO}_3$  and HCl concentration on stripping of succinic acid

Concentration [ $\text{mol}/\text{dm}^3$ ]	$E$ [%]	
	$\text{Na}_2\text{CO}_3$	HCl
1	75.7	-
0.5	79.1	4.17
0.25	65.0	17.15
0.1	51.9	15.75

$\text{Na}_2\text{CO}_3$  at  $0.5 \text{ mol/dm}^3$  or over as the stripping agent was given the higher stripping capacity. In lactate permeation [2,3], hydrochloric acid was used as the stripping agent, although sodium carbonate may be a better stripping agent. It is because enriched and desalinated lactate solution, which could be simultaneously accomplished using hydrochloric acid, was very convenient for subsequent polymerization process.

Figure 4 shows the time courses of the succinic acid concentration in the feed and stripping solutions for PIM permeation when using sodium carbonate as a stripping agent. The succinate rapidly permeating to the stripping phase contrasts with the result of Figure 3. This overall permeation rate of succinate through PIM was similar to that of SILM (Chapter 2) and larger than that of lactate through PIM because of using the hydrochloric acid as a stripping agent in lactate permeation through PIM as described above.

### **Effect of feed pH on permeability**

The permeability,  $P$ , from the effect of membrane thickness to the overall mass transfer coefficients (data not shown) was calculated according to the procedure described in our previous papers [3 and Chapter 2]. The pH effect on the permeability is presented in Figure 5.

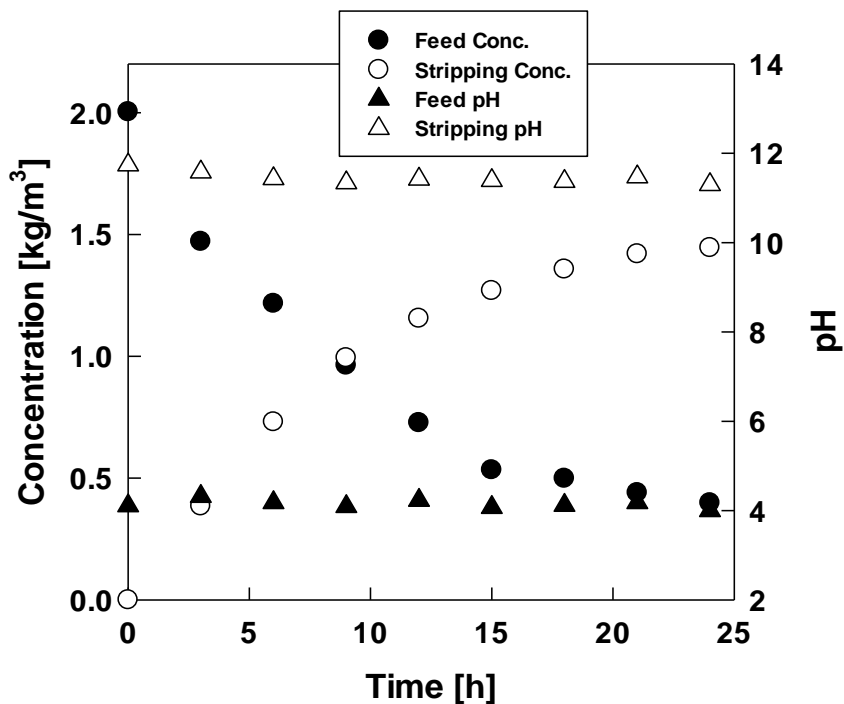


Figure 4. Permeation of succinic acid through PIM using sodium carbonate (membrane thickness, 132  $\mu\text{m}$ )

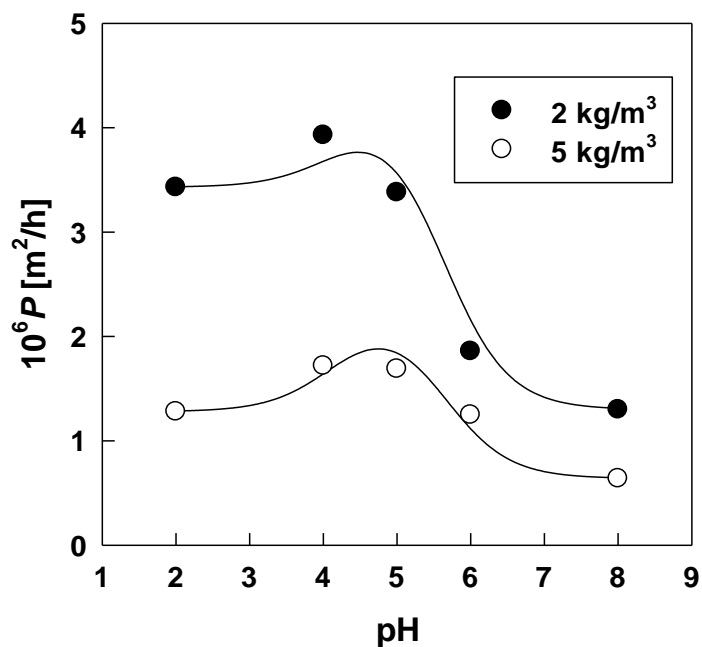


Figure 5. Effect of pH on permeation of succinic acid through PIM at concentrations of 2  $\text{kg/m}^3$  and 5  $\text{kg/m}^3$ . Solid lines are calculated by Eq. (1) (average membrane thickness, 280  $\mu\text{m}$ )

The maximum permeability was observed at pH 4 under this experimental condition, in spite of optimum pH of 6.5 in SILM (Chapter 2). This is due to the difference in permeation mechanism between SILM and PIM. From Figure 5, un-dissociated, mono-anionic and di-anionic forms of the succinic acid concern the permeation of the succinic acid because the  $pK_{a1}$  and  $pK_{a2}$  values of succinic acid are 4.17 and 5.64.

Based on the effects of stripping solution and feed pH, the following permeation mechanism is considered. The succinate mono-anion in the feed phase diffuses across the PIM into the  $\text{Na}_2\text{CO}_3$ -containing stripping phase, where it reacts with sodium carbonate to form di-anionic succinate. The succinate di-anion in the feed phase diffuses across the PIM according to its concentration gradient. Furthermore, succinic acid permeated at feed pH =2, at which un-dissociated succinic acid is the dominant species. This suggests that physical dissolution of un-dissociated succinic acid to the PVC membrane is another permeation route. Therefore, it was examined whether succinic acid in the feed solution at pH 4 permeated the PVC membrane in the absence of Aliquat 336. However, succinate rarely permeated the above membrane, suggesting that un-dissociated succinic acid directly interacts with Aliquat 336. In the previous study on the extraction of carboxylic acids with quaternary ammonium salt [13], Aliquat 336 extracted both dissociated and un-dissociated forms of the acids. Therefore, the un-dissociated succinic acid in the feed phase diffuses across the PIM into the  $\text{Na}_2\text{CO}_3$ -containing stripping phase, where it reacts with sodium carbonate to form di-anionic succinate. The permeability is expressed by the following equation.

$$P = P_{H_2A}f_{H_2A} + P_{HA^-}f_{HA^-} + P_{A^{2-}}f_{A^{2-}} \quad (2)$$

where  $f$  denotes the mole-fraction of acid forms and is a function of pH. If values of  $P_{H_2A}$  and  $P_{A^{2-}}$  can be assumed to be values at pH 2 and 8, respectively, a parameter in Eq. (2) to be obtained is only  $P_{HA^-}$ . Estimated values from the results shown in Figure 5 are summarized in Table 2. Solid lines in Figure 5 are calculated from the values in Table 2, and optimum pH is found to be 4.6~4.8.

Based on the solution diffusion theory, permeability is expressed by the following equation:

$$P = D_f \cdot K_D \quad (3)$$

where  $D_f$  is the diffusivity of the succinate complex, and  $K_D$  is the succinate distribution ratio. As expected from Figure 5, permeability of the mono-anionic form of succinic acid is largest. This may be because of smaller diffusivity of bulky di-anionic complex with Aliquat 336 and smaller distribution ratio of undissociated succinic acid to the membrane.



Table 2. Permeability of acid forms

	$10^6 P$ [ $m^2/h$ ]	
	2 kg/ $m^3$	5 kg/ $m^3$
Un-dissociated	3.4	1.3
Mono-anionic	4.1	2.2
Di-anionic	1.3	0.64

## Conclusions

Succinic acid successfully permeated PIM, which has different optimum conditions from SILM. PIM was successfully used to permeate succinic acid by using  $\text{Na}_2\text{CO}_3$  as the stripping agent. While SILM with hydrochloric acid as a stripping agent showed a permeation mechanism in which ion exchange reaction of succinate di-anion played a role in the permeation of succinic acid, PIMs showed complex permeation behavior involving un-dissociated, mono-anionic and di-anionic forms of succinic acid as the species permeated. Permeability of the mono-anionic form of succinic acid was largest among these species, and the optimum pH range for succinic acid permeation was found to be around 4 to 5.

Comparison between SILM and PIM from the viewpoints of permeation flux and life-time remains as future work, although the succinic acid permeations through SILM and PIM have different optimum conditions.

The succinic acid concentration produced from fermentation ranges from 10 to 100  $\text{kg/m}^3$  [14]. In this study, succinic acid concentration was lower than those from fermentation, because the main purpose is to clarify the permeation mechanism. To treat high concentration of succinic acid, the membrane configurations with high ratio of contact area to unit volume such as spiral-wound, hollow fiber will be utilized.

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## **CHAPTER 4**

### **Salting-out for Succinic Acid Extraction by Aqueous Two-Phase System using Alcohols and Ionic Liquids**

# 4

## Salting-out for Succinic Acid Extraction by Aqueous Two-Phase System using Alcohols and Ionic Liquids

**Abstract:** Production of a fermentation-derived succinic acid (bio-succinic acid) has recently emerged as a potential green technology. For bio-succinic acid production, 50 to 60% of processing costs are attributed to recovery and purification processes. In this research, aqueous two phase systems (ATPS) using water-miscible alcohols/salts and ionic liquids/salts was examined to extract succinic acid. From binodal curves, the phase separation abilities of solvents are in the order *t*-butanol > 1-propanol > HmimBr > 2-propanol  $\approx$  OmimBr > BmimBr > ethanol. Succinic acid was successfully extracted in both ATPSs. In the ATPS with alcohols, undissociated succinic acid was extracted, and it was critical to control the pH of the salt solution. In the ATPS with ionic liquids, the extraction behaviors of succinic acid were affected by the salts used. An ATPS with an OmimBr and  $(\text{NH}_4)_2\text{SO}_4$  system gave highest quantitative extraction. Extracted succinic acid was quantitatively precipitated by adding sodium hydroxide to the extracted phase and recovered as crystallization of sodium succinate.

### Introduction

Previously, in Chapter 2 and Chapter 3, liquid membrane systems containing ionic liquid for succinic acid separation were proposed. Although considerably

stable liquid membranes containing ionic liquid were obtained compared with conventional liquid membranes, membrane performance was gradually impaired over the long term. Recently, Sun *et al.* [1] reported an acetone/ $(\text{NH}_4)_2\text{SO}_4$  aqueous two-phase system (ATPS) for recovery of bio-succinic acid.

The ATPS has been well known as one extraction technique for biomolecules [2]. Recent research has focused on ATPSs that were formed by water-miscible organic solvent or ionic liquid with salt [2,3]. Most of the polymers used in ATPSs, so far, have high viscosity and tend to form a cloudy solution [4] because of low interfacial tension. Therefore, a polymer/salt ATPS has difficulty isolating the extracted biomolecules from the viscous polymer phase by back-extraction. An ATPS composed of hydrophilic organic solvents and salts attracted much attention because of low extraction cost, easy solvent recovery by evaporation and easy scale up [2]. An ATPS composed of hydrophilic ionic liquids and salts was also interesting because of faster phase separation, reduction in viscosity, and tailoring of the polarity of the coexisting phase [5].

In this study, water-miscible alcohol/salt and ionic liquid/salt ATPSs for the extraction of succinic acid was examined. As mentioned, water-miscible alcohol is easily recovered by evaporation. 1-Alkyl-3-methylimidazolium bromide was used as ionic liquid. It is known that the ionic liquids with  $\text{Br}^-$  anion more easily form two phases compared to those with  $\text{Cl}^-$  anion because of the different Gibbs energies of hydration of  $\text{Cl}^-$  ( $-340$  kJ/mol) and  $\text{Br}^-$  ( $-315$  kJ/mol) [6]. Finally succinic acid from the extracted phase was recovered by direct precipitation with alkali hydroxide.



## Experimental Section

### Chemicals

1-Butyl-3-methylimidazolium bromide [BmimBr] was purchased from Merck, 1-hexyl-3-methylimidazolium bromide [HmimBr] and 1-octyl-3-methylimidazolium bromide [OmimBr] were purchased from Tokyo Chemical Industries, Ltd, and these were used as received.  $K_2HPO_4$ ,  $K_3PO_4$ ,  $K_2CO_3$ , KF,  $(NH_4)_2SO_4$ ,  $C_6H_5Na_3O_7 \cdot 2H_2O$ ,  $Na_2CO_3$ , NaCl,  $MgSO_4$ , and  $NH_4NO_3$  of G.R. grade were used as salts. 1-Propanol (1-ProOH), 2-propanol (2-ProOH), *t*-butanol (*t*-BuOH) and ethanol (EtOH) were used as water-miscible organic solvents.

### Preparation of phase diagram

The binodal curves were determined by turbidimetric titration as described in a previous paper on an ATPS using water-miscible organic solvent and salt [7]. A  $K_2HPO_4$  solution of known concentration was placed in a 100 mL conical flask, and ionic liquids (BmimBr, HmimBr and OmimBr) were added drop-wise to the flask until the clear solution turned turbid or two-phase systems were formed. Then, de-ionized water was added drop-wise to the flask to obtain a clear one-phase system, and more ionic liquids were added again to produce two-phase systems. The composition of this mixture was noted and the experiments were repeated to obtain the binodal curve.

### Extraction of succinic acid using ATPS

Solid inorganic salts and 5 mL of water-miscible organic solvents or ionic liquids were added into the 5 mL solution including succinic acid (20 g/L) to form the ATPS consisting of 25-150 g-salt/100 g-water in a calibrated test tube. The solution was mixed thoroughly and left standing for more than 6 h in a

thermostated bath at 25°C. The volumes of the top and bottom phases were recorded. The concentrations of succinic acid and the water content in both phases were analyzed by HPLC as described in previous chapters (Chapter 2 and 3) and Karl Fischer's method. The pHs of the aqueous solutions before and after equilibration were determined by a Horiba F-71 pH meter. The extractability ( $E$ ) of succinic acid was defined as,

$$E [\%] = \frac{C_T V_T}{C_0 V_0} \times 100 \quad (1)$$

where  $C$  and  $V$  are the concentration of succinic acid and the solution volume and subscripts T and 0 denote the top phase and initial state.

### **Precipitation of succinate from extracted phase**

After succinic acid was extracted by ATPS containing ammonium sulfate of 20 g/100mL, the aliquot of the top phase was taken from the test tube for extraction into the flask tube. The precipitants, NaOH, NH<sub>4</sub>OH and Ca(OH)<sub>2</sub>, were added until the precipitate formed. Precipitates were filtered. The amounts of unrecovered succinic acid in the filtrate were measured with HPLC.

## Results and Discussion

### Phase diagram for ATPS consisting of water-miscible organic solvent or ionic liquid, and dipotassium hydrogen phosphate

Figure 1 shows the effects of water-miscible solvents on the binodal curves of the  $K_2HPO_4$ /solvent ATPS at 25°C plotted as mass fractions.

The closer the binodal curves are to the coordinate origin, the less solvent is required for the formation of the ATPS under the same concentration of salt.

From the figure 1, the phase separation abilities of solvents are in the order *t*-BuOH > 1-ProOH > HmimBr > 2-ProOH  $\approx$  OmimBr > BmimBr > EtOH. Pei *et al.* [6] reported that HmimBr has the best phase-forming ability among the 1-alkyl-3-methylimidazolium bromides. In this paper, the phase-forming ability of HmimBr was relatively high among the solvents used. The polarity of HmimBr ( $E_T^N = 0.59$  [8]) exists between those of 1-ProOH (0.62) and 2-ProOH (0.55). A relatively small polarity of HmimBr led to good phase-forming ability.

### Extraction of succinic acid with ATPS containing water-miscible alcohol

Table 1 shows the extractability of succinic acid by ATPS using water-miscible alcohol and  $(NH_4)_2SO_4$ .

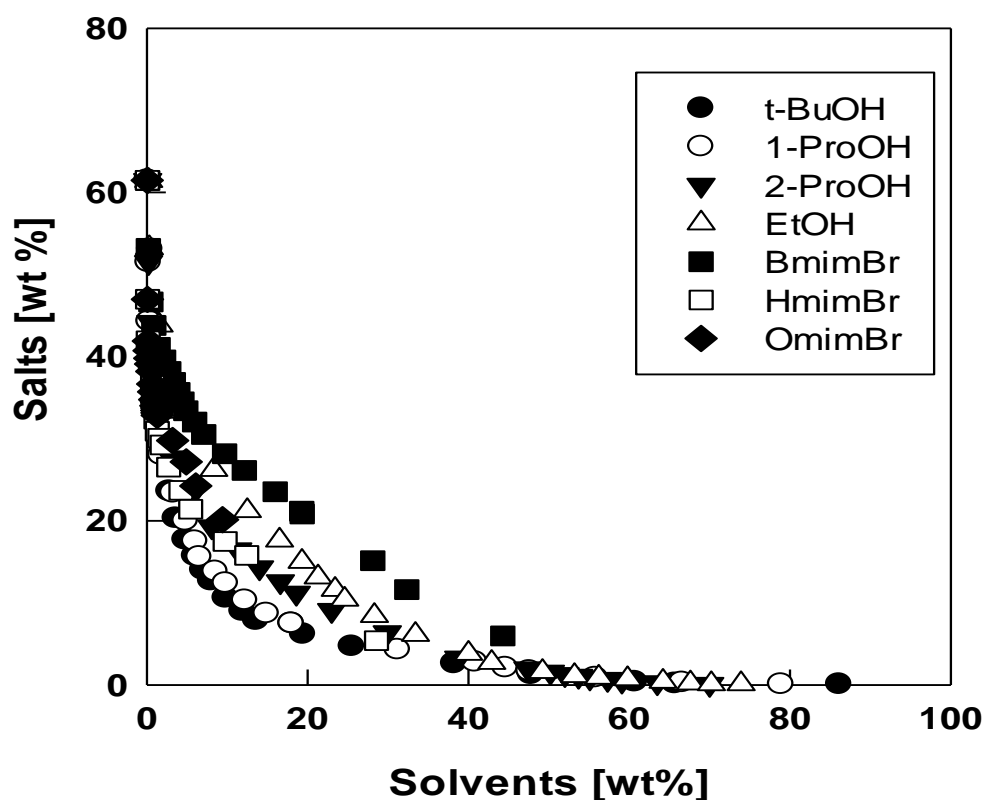


Fig. 1 Binodal curves of solvent (alcohol and ionic liquid) +  $K_2HPO_4$  aqueous two phase system

Table 1. Effect of water-miscible alcohol on extractability of succinic acid by ATPS with  $(NH_4)_2SO_4$

Alcohol	Salt concentration [g/100mL-water]	Top phase* [mL]	Bottom phase* [mL]	Water content [%]	E [%]
<i>1-ProOH</i>	20	6.2	3.8	24.4	70.8
	40	5.8	4.1	18.2	64.8
<i>2-ProOH</i>	20	6.8	3.2	33.0	78.7
	40	6.0	4.0	23.8	68.1
<i>t-BuOH</i>	20	6.2	3.8	25.8	76.3
	40	5.6	4.4	17.3	67.5
	60	5.5	4.5	13.0	65.0
<i>EtOH</i>	20	8.0	2.0	42.6	86.9

\*Initial volumes of both phases were 5 mL.

The extractability and the water content in the top phase decreased with increasing salt concentrations in all cases. The volume of top phase using EtOH was much higher than those with other solvents, suggesting that EtOH has lower phase-forming ability. The highest extractability except EtOH in this paper was 78.7 %. Sun *et al.* [1] reported 90 % recovery of succinic acid with ATPS using 30% acetone and 20% ammonium sulfate. However, direct comparison of their data with ours was difficult because composition of the top phase was not described in their paper [1]. Extractabilities were found to be roughly correlated with water content in the top phase. This might be caused by succinic acid being co-extracted with water to the alcohol-rich phase.

Table 2 shows the effect of salt on the extractability and volumes of both phases. Volumes of both phases were almost identical, suggesting that the phase-forming ability of each salt is similar.

Figure 2 shows the relation between the equilibrium pH of the bottom phase and the extractability. Evidently, lower pH caused the high extractability and, neutral and acidic salts were found to be favorable to the extraction of succinic acid. Because the pKa values of succinic acid were 4.2 and 5.6, extracted species in the alcohol-rich phase were undissociated forms of succinic acid. Undissociated succinic acid may be integrated in the hydrogen-bonding network [9] in the alcohol-rich phase.

Table 2. Effect of salt on extractability of succinic acid by ATPS with 1-propanol

Salts [20 g /100mL-water]	Top phase* [mL]	Bottom phase* [mL]	pH	E [%]
$K_2HPO_4$	6.2	3.8	7.47	3.6
$(NH_4)_2SO_4$	5.8	4.2	3.39	70.8
$MgSO_4$	6.4	3.6	2.95	36.8
$K_3PO_4$	6.0	4.0	12.3	0.0
$K_2CO_3$	6.0	4.0	10.6	1.4
$KF$	5.6	4.4	6.27	0.9
$NH_4NO_3$	6.2	3.8	2.75	35.3
$C_6H_5Na_3O_7 \cdot 2H_2O$	6.5	3.5	5.88	8.7
$Na_2CO_3$	6.0	4.0	10.2	1.4
$NaCl$	5.4	4.6	2.66	72.1

\*Initial volumes of both phases were 5 mL.

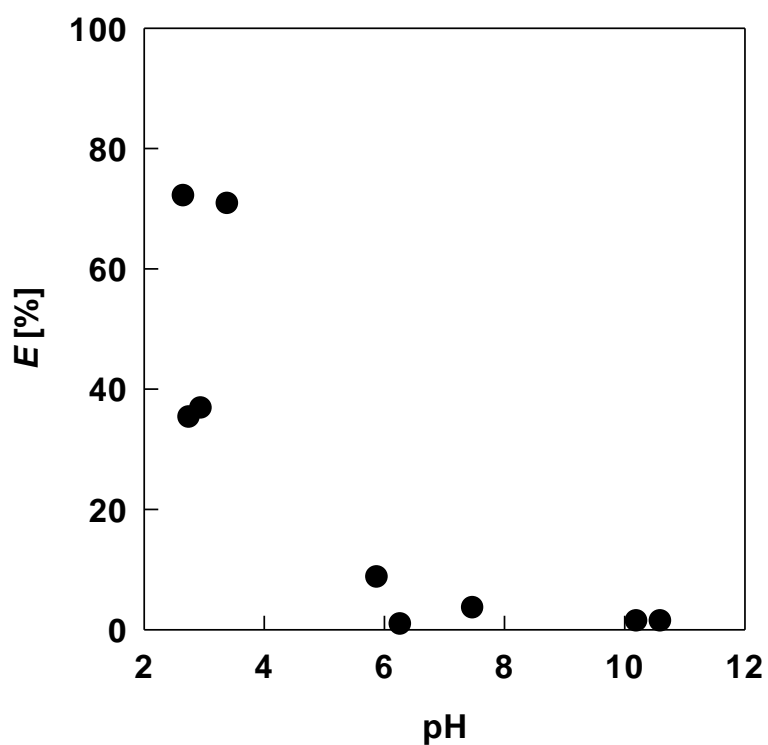


Figure 2. Relationship between equilibrium pH of bottom phase and extractability for alcohol + salt aqueous two phase system

### **Extraction of succinic acid with ATPS containing water-miscible ionic liquids**

Table 3 shows the extractability of succinic acid by ATPS using water-miscible ionic liquids and  $K_2HPO_4$ . From Table 3, BmimBr gave the highest extractability among the solvents, although BmimBr had a relatively small phase forming ability as mentioned above. Extractability was independent of water contents unlike ATPS with alcohol and water contents generally were higher than those with alcohols.

Table 4 shows the effect of salt on the extractability and volumes of both phases. In the cases of using  $MgSO_4$ ,  $NH_4NO_3$ ,  $Na_2CO_3$  and  $NaCl$ , ATPS was not formed under the condition of 20 g-salt/100mL-water. Two salts,  $(NH_4)_2SO_4$  and  $K_2CO_3$ , had high extractability, while for volume change of both phases between before and after equilibrium,  $(NH_4)_2SO_4$  had a largest effect.

Figure 3 shows the relation between the equilibrium pH of the bottom phase and the extractability for BmimBr, HmimBr and OmimBr. Unlike ATPS with alcohols, extractability depended on not pH but salt used. Over pH 6, succinate di-anion was also distributed to ionic liquid phase. Water aggregation in non-polar solvent such as reversed micelle was influenced by the water activity, which was determined by the salt used. Therefore, we guess the formation of water pool in ionic liquid and the distribution of succinic acid and succinate to the water pool.

Table 3. Effect of water-miscible ionic liquids on extractability of succinic acid by ATPS with  $K_2HPO_4$ 

Ionic liquid	Salt concentration [g/100mL-water]	Top phase* [mL]	Bottom phase* [mL]	Water content [%]	E [%]
<i>BmimBr</i>	40	1.6	4.2	56.5	37.4
	60	1.4	4.4	55.7	48.1
	80	1.2	4.6	54.8	47.7
	100	1.0	4.8	35.5	36.2
<i>HmimBr</i>	40	1.5	4.3	46.3	32.3
	60	1.2	4.6	35.2	33.4
	80	1.0	4.8	28.9	29.1
	100	1.0	4.8	23.2	23.7
<i>OmimBr</i>	40	1.4	4.4	41.0	37.4
	60	1.2	4.6	30.0	42.7
	80	1.0	4.8	24.7	36.8
	100	1.0	4.8	21.4	32.5

\*Initial volumes of water and ionic liquid phases were 5 mL and 0.8 mL, respectively.

Table 4. Effect of salt on extractability of succinic acid by ATPS with *HmimBr*

Salts [20 g/100mL-water]	Top phase** [mL]	Bottom phase** [mL]	pH	Extractability [%]
$K_2HPO_4$	6.3	2.0	7.40	58.7
$(NH_4)_2SO_4$	7.2	1.3	3.43	85.5
$K_3PO_4$	6.0	2.4	11.6	60.3
$K_2CO_3$	5.8	2.5	10.5	82.8
<i>KF</i>	5.0	3.0	6.97	16.0
$C_6H_5Na_3O_7 \cdot 2H_2O$	6.5	1.9	5.72	58.6

\*\*Initial volumes of water and ionic liquid phases were 5 mL and 3.1 mL, respectively.



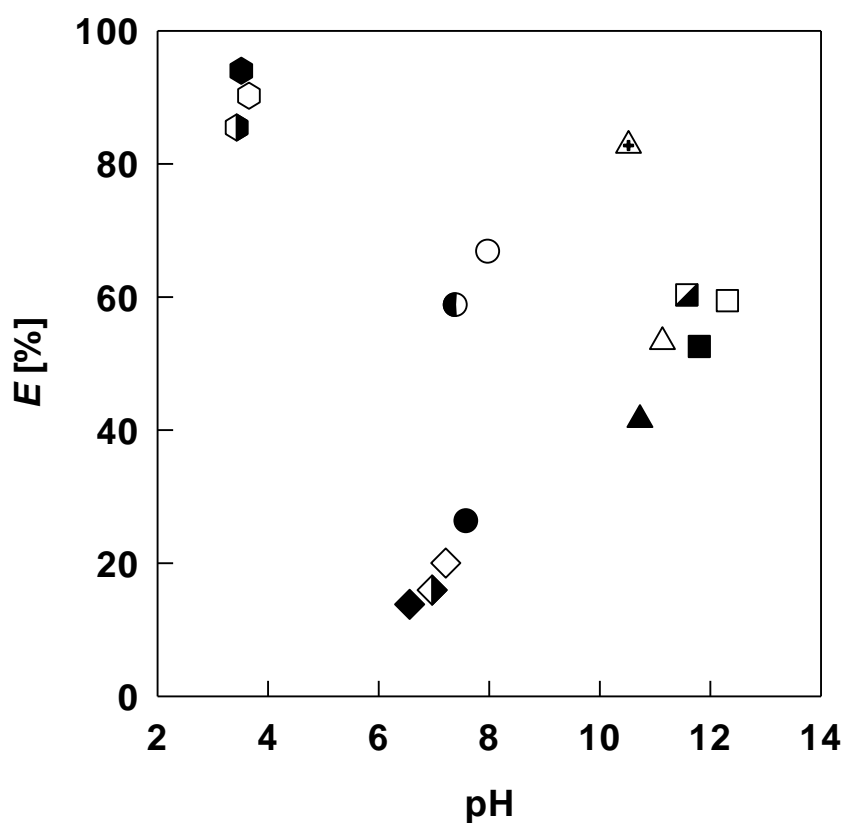


Figure 3. Relationship between equilibrium pH of bottom phase and extractability for ionic liquid + salt aqueous two phase system. Symbols are as follows; circle:  $K_2HPO_4$ , hexagonal:  $(NH_4)_2SO_4$ , square:  $K_3PO_4$ , triangle:  $K_2CO_3$ , diamond: KF, and open: BmimBr, right half black and +: HmimBr, filled: OmimBr.

**Table 5** Recovery of succinate from top phase in ATPS

Alkali	HmimBr	OmimBr	1-propanol
$NH_4OH$	0	0	0
NaOH	71.1%	31.2%	100%
$Ca(OH)_2^*$	$\approx 0$	$\approx 0$	84.9%

\*Two-phase formation was not observed.

### **Precipitation of succinate from extracted phase**

The recovery of succinic acid from loaded organic solvents was important for process development. In the reactive extraction with a water-immiscible solvent and an extractant like alkylamine, succinic acid was back-extracted by pH-swing [10]. In ATPS with acetone, acetone was recovered by vacuum distillation and then succinic acid was crystallized at a pH of 2.0 and a temperature of 4 °C [11]. In ATPS with ionic liquid, organic acid in ionic liquid phase was stripped by 10 wt% Na<sub>2</sub>CO<sub>3</sub> solution [12].

In this reserach, in order to recover the directly succinic acid from the extracted phase, we added the hydroxides, NH<sub>4</sub>OH, NaOH and Ca(OH)<sub>2</sub> to the extracted phase and the results were listed in Table 5.

It was found that sodium hydroxide was effective as the precipitant. Quantitative recovery, which is larger than 85 % with crystallization of succinic acid [11], was accomplished with crystallization of sodium succinate in the case of 1-propanol/ammonium sulfate ATPS. In the cases of ionic liquids/ ammonium sulfate ATPS, recoveries were lower than that of 1-propanol because ionic liquid-ATPS had the larger water content than 1-propanol-ATPS. In further investigation, recovery may be improved by the optimization of extraction and crystallization processes.

## Conclusion

In this research, we examined ATPS with water-miscible alcohols/salts and ionic liquids/salts to extract succinic acid. From binodal curves, the phase separation abilities of solvents are in the order  $t$ -BuOH > 1-ProOH > HmimBr > 2-ProOH  $\approx$  OmimBr > BmimBr > EtOH. Succinic acid was successfully extracted in both ATPSs. In ATPS with alcohols, undissociated succinic acid was extracted and it was critical to control the pH of the salt solution, suggesting that undissociated succinic acid may be integrated in the hydrogen bonding network in the alcohol-rich phase. In ATPS with ionic liquids, the extraction behaviors of succinic acid are affected by the salts used, suggesting that the formation of water pool in ionic liquid and the distribution of succinic acid and succinate to the water pool. ATPS with OmimBr and  $(\text{NH}_4)_2\text{SO}_4$  system gave highest extraction. Extracted succinic acid in 1-propanol was quantitatively precipitated by adding sodium hydroxide to the extracted phase and recovered as crystallization of sodium succinate.

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## **CHAPTER 5**

### **Sugaring-out for Succinic Acid Extraction by Aqueous Two-Phase System using Alcohol and Ionic Liquid**

# 5

## Sugaring-out for Succinic Acid Extraction by Aqueous Two-Phase System using Alcohol and Ionic Liquid

### Abstract:

Sugaring-out extraction of succinic acid using alcohol and ionic Liquid (IL) as water-miscible organic component were studied. Examining the pH effect on sugaring-out extraction of succinic acid, it was found that ionic liquid gave higher extractability than 1-propanol as organic phase and could extract 83% of succinic acid at pH 3, which is below  $pK_{a1}$  (=4.2).

### Introduction

As studied in Chapter 4, ATPS, which is one of an important technique in separation and purification of metals and biomolecules, could be formed by adding salt into aqueous solution of ionic liquid or alcohol. ATPS has been mainly applied for separation of protein[2]. Freire *et al.* [1] stated that two main applications of IL-based ATPS are extraction of biomolecules and other added-value compounds, and their use as alternative approaches for removing and recovering ILs from aqueous media.

In this Chapter, the separation of succinic acid as bio-based compound is studied with addition of sugar instead of salt to induce two-phase formation in ATPS techniques because sugar has less effect to microbes than salt. As novel separation techniques, condition of sugaring-out separation was previously

studied in acetonitrile-water system [3]. By addition of monomeric sugar or disaccharide in an acetonitrile (ACN)-water mixture, it formed two-phases with the upper ACN rich phase rich and lower aqueous phase. Hence this technique was applied to the separation of bio-based compound such succinic acid in this study.

Ionic liquids also meet requirements for the use of this technique: water stability at room temperature, low hydrogen bond basicity and easily salting-out or sugaring-out from aqueous solution [1]. Therefore in this study, 1-butyl-3-methylimidazolium trifluoromethanesulfonate [ $C_4mim$ ][ $CF_3SO_3$ ] and 1-propanol as a water-miscible ionic liquid and alcohol, were used for sugaring-out with monosaccharides and disaccharides.

Carbohydrates are non-charged, biodegradable, nontoxic, and a renewable feedstock as biomass. The mono and disaccharides are polyhydroxy aldehydes or ketones with a high affinity for water and sugaring-out aptitude (several  $-OH$  groups with dual donor/acceptor character that are involved in hydrogen bonding) [4].

In this research the goal is to find mechanism reaction between succinic acid and alcohol or ionic liquid. There were few studies on ATPS using sugaring out and therefore, it was applied to separation and purification of succinic acid as well-known bio-based compound for the industrial application.



## Experimental Section

### Chemicals

1-Butyl-3-methylimidazolium trifluoromethanesulfonate [C<sub>4</sub>mim][CF<sub>3</sub>SO<sub>3</sub>] was purchased from Merck, Germany, and used as received. Monosaccharides and disaccharides of G.R. grade were used as sugar. 1-Propanol (1-ProOH), from Wako Pure Chemical Ltd., was used as water-miscible organic solvents.

### Sugaring-out of succinic acid by ATPS IL-based

At first 5 mL of succinic acid solution of 20 g/L was added into vial tube and followed by adding sugar at various amount. All components were mixed until mixtures from clear solution. After that, 4 g of alcohol or IL were added to the mixture and then mixed. The vial tube was put into thermo-mixer for one hour to obtain the equilibrium between aqueous phase and alcohol or IL phase at 298 K. Before and after extraction process, the succinic acid concentration in the aqueous phase was determined by HPLC, volumes of both phases were measured by graduated tube, and the water contents in alcohol or IL phase were determined by Karl-Fischer method. The extraction of succinic acid were calculated by equation (1),

$$\text{Extractability [\%]} = \frac{C \cdot V}{C_0 \cdot V_0}$$

where  $V$  and  $V_0$  are the volume of organic phase and initial volume of aqueous phase respectively.  $C$  is the concentration of succinic acid in alcohol or ionic liquid phase and  $C_0$  is the initial concentration of succinic acid in aqueous phase.

## Results and Discussion

### Extraction of succinic acid with ATPS containing water-miscible alcohol

In order to confirm possibility of extraction of succinic acid by sugaring-out based ATPS with alcohol, extraction of succinic acid was carried out using 1-propanol and sucrose. Figure 1 shows extraction of succinic acid in 1-propanol with disaccharide, sucrose, of various concentrations from 40 g/100 mL-water until 100 g/100 mL-water. However two-phase was not formed at concentration 40 g/100 mL-water. As is evident from Figure 1, it was found that succinic acid was successfully extracted with sugaring-out based ATPS.

Table 1 shows the abilities of various sugars to induce ATPS for extraction of succinic acid at 60 g/100 mL-water of sugar and pH = 3. Although lactose and mannose could not give two-phase formation, the remaining saccharides have ability for two-phase. Maltose gave highest extractability compared with other saccharides in 72%. The water contents in the cases of maltose and sucrose were almost similar in 30%.

Because maltose gave high extractability, Figure 2 shows the pH effect at maltose concentration in 60 g/ 100 mL and 80 g/100 mL-water. The extractability decreased with increasing pH, as well as salting-out ATPS with alcohol. Therefore, undissociated succinic acid may be extracted. Moreover, higher maltose concentration gave higher extractability of succinic acid. Extractability reached 83 % at 80 g/100 mL and pH = 3.

Figure 3 shows the extraction with 2 different sugar, maltose and sucrose, at 60 g/100 mL-water. As described above, maltose gave higher extractability at pH 3, although at pH over 5, both extraction behaviors were almost similar.

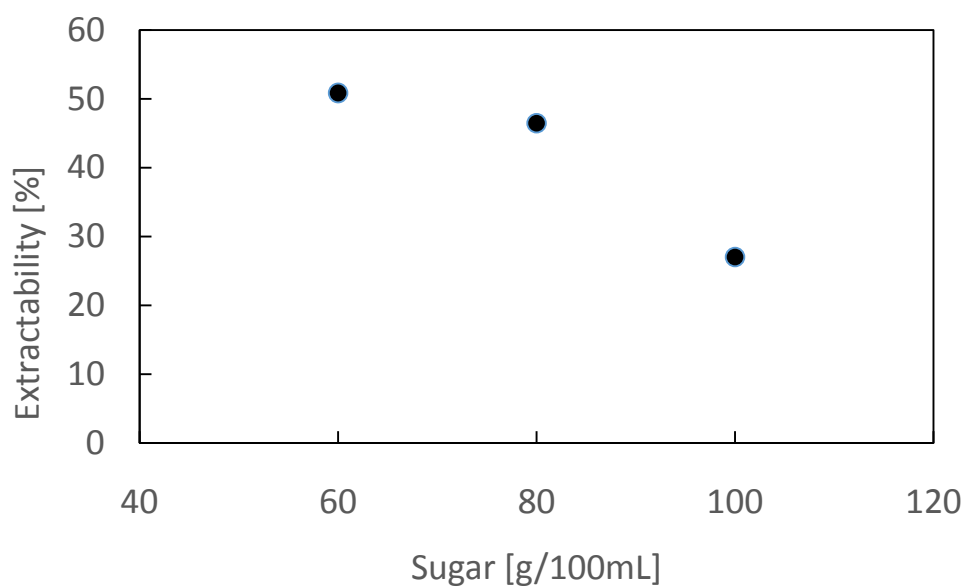


Figure 1. Extraction of succinic acid using 1-propanol with sucrose (60 g/100 mL)

Table 1. Extraction of succinic acid using 1-propanol with sugars (60 g/100 mL)

Sugar	Extractability (%)
Glucose	33
Fructose	31
Galactose	35
Lactose	X*
Maltose	72
Sucrose	51
Mannose	X*

X\* : can not form ATPS

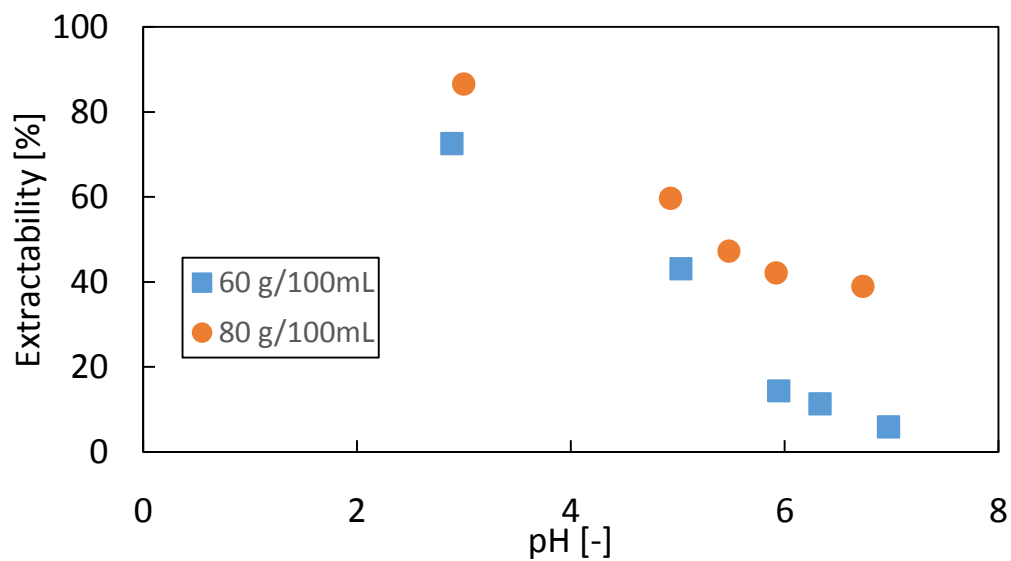


Figure 2. Extraction of succinic acid using 1-propanol and maltose in different concentration

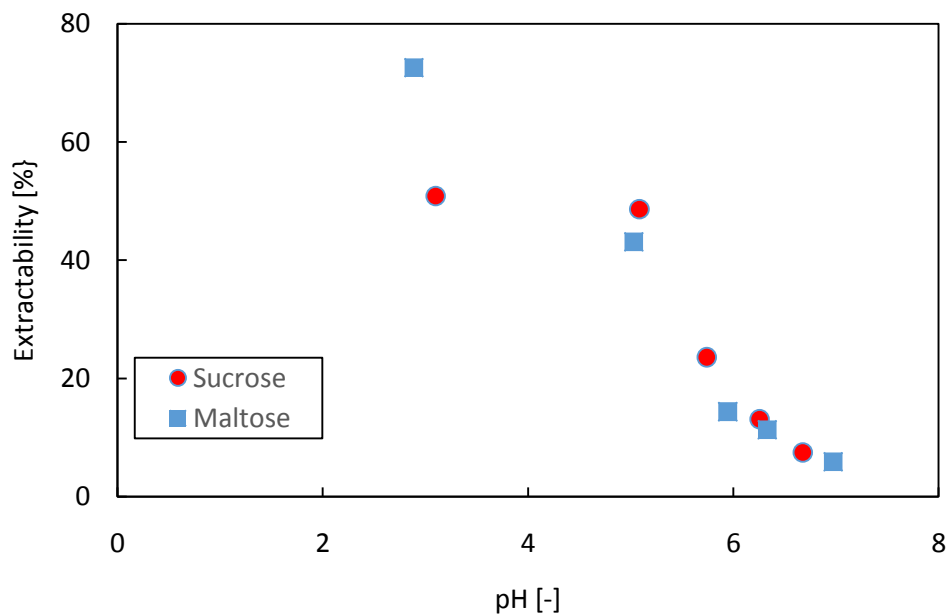


Figure 3. Extraction of succinic acid using 1-propanol with different sugar (60g/100mL)

## Extraction of succinic acid with ATPS containing water-miscible ionic liquids

As mentioned in the previous paper [5], the functionalized salts can be used in ATPS using ionic liquids which support for separation from aqueous solution. Moreover, Freire *et al.* [4], mono- and disaccharides instead of salts were used for formation of ATPS using a water-soluble ionic liquid  $[\text{C}_4\text{mim}][\text{CF}_3\text{SO}_3]$  and  $[\text{C}_4\text{mim}][\text{BF}_4]$ . However  $[\text{C}_4\text{mim}][\text{BF}_4]$  had several lack which are suffer in hydrolysis even at moderate temperature [4].

Figure 4 shows the pH effect on the extraction of succinic acid at various concentration with ionic liquid  $[\text{C}_4\text{mim}][\text{CF}_3\text{SO}_3]$ . It was shown that concentration sugar at 60 g/100 mL was the optimum condition for extraction of succinic acid. The increase in the sugar concentration caused an increase in the water content in the ionic liquid phase, suggesting hydration of sugar in aqueous solution as a driving force of two-phase formation.

As well as Figure 2, in Figure 4, the extractability decreased with increasing pH. This was suggested that undissociated succinic acid may be extracted, unlike ATPS using ionic liquids and salts as described in Chapter 4.

Figure 5 shows extractability for different organic solution under the 60 g/100 mL in 1-propanol and  $[\text{C}_4\text{mim}][\text{CF}_3\text{SO}_3]$ . At pH lower than  $\text{pK}_{a1}$ , the extractability of succinic acid gave higher extraction for both cases using sugaring-out. In the pH higher than  $\text{pK}_{a1}$ , the extractabilities of 1-propanol and  $[\text{C}_4\text{mim}][\text{CF}_3\text{SO}_3]$  were almost same.

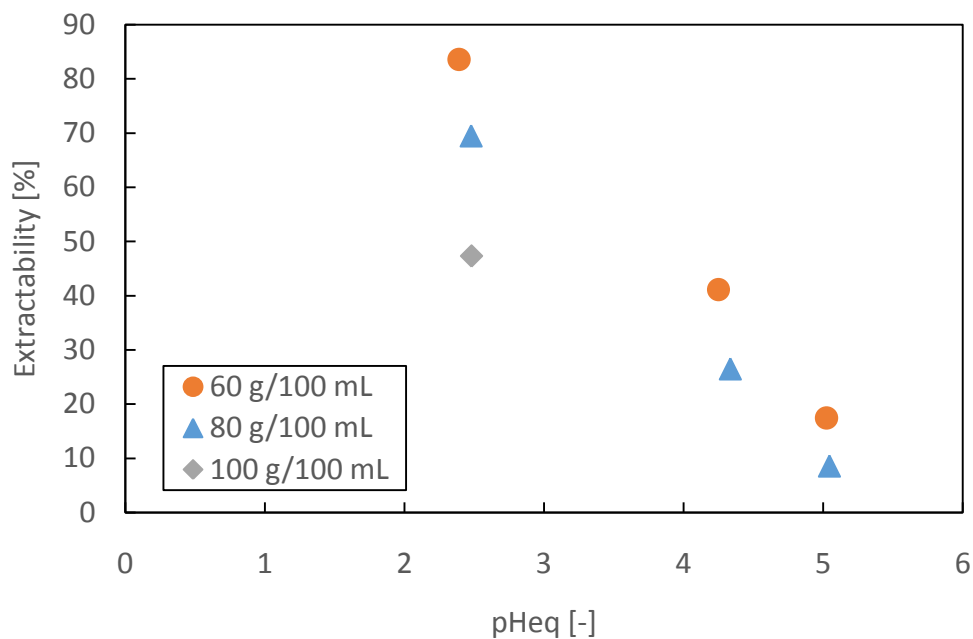


Figure 4. Extraction of succinic acid using ionic liquid  $[C_4mim][CF_3SO_3]$  and maltose in different concentration

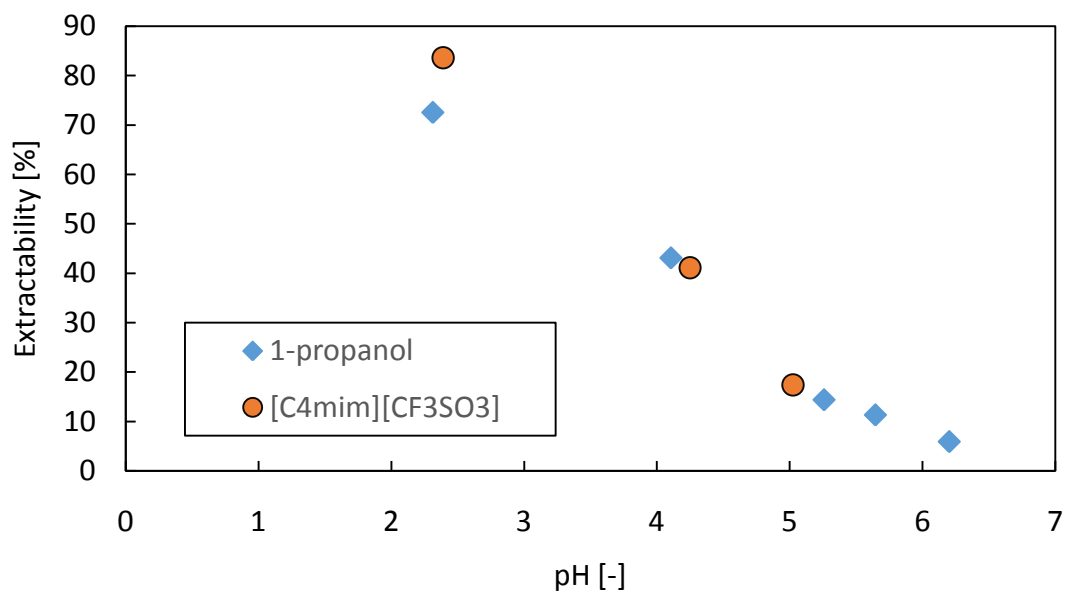


Figure 5. pH effect on extraction of succinic acid with 1-propanol and  $[C_4mim][CF_3SO_3]$

## Conclusion

Succinic acid was successfully extracted with ATPS using sugar to extract using alcohol (1-propanol) and ionic liquid ( $[\text{C}_4\text{mim}][\text{CF}_3\text{SO}_3]$ ). It was found that the extracted species in both solvents were undissociated form of succinic acid. Maltose has higher ability to induce ATPS than sucrose and other sugars (mono- and disaccharides) with 1-propanol. The pH condition of ATPS technique was really important in order to get high extractability.

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## **CONCLUSION**

## CONCLUSION

Succinic acid is widely used in many industrial sectors; pharmacy, textile, food, and etc. Hence the production of succinic acid were developed through fermentative production because of the low-cost raw material and the by-product from the fermentative production itself. Ethanol, acetate, malate, pyruvate, lactate, malate, and formate were known as by-product of fermentative production of succinic acid. Therefore the extraction and separation techniques such succinic acid by liquid membranes (LM) and aqueous two-phase system (ATPS) containing ionic liquids (ILs) were conducted and successfully extracted in this research.

In Chapter 2, supported liquid membranes including IL, Aliquat 336, was successfully permeated succinic acid using hydrochloric acid as receiving solution. The pH in optimum condition of permeation was coincided with the fermentation process. From the permeability and distribution ratio approach, it is suggested that permeation proceeded via an anion-exchange reaction. The di-anion species was involved in the permeation of succinic acid through the SILM. From the proportional relation between the permeability and the distribution ratio, the permeation obeys the solution diffusion mechanism.

In Chapter 3, PIM successfully permeated succinic acid by using sodium carbonate as the stripping agent. PIMs showed complex permeation behavior involving un-dissociated, mono-anionic and di-anionic forms of succinic acid as the species permeated. Permeability of the mono-anionic form of succinic acid was largest among these species, and the optimum pH range for succinic acid

permeation was found to be around 4 to 5. SILM and PIM had the different optimum condition.

ATPS with water-miscible alcohols/salts and ionic liquids/salts was examined to extract succinic acid (Chapter 4). The phase separation abilities were  $t\text{-BuOH} > 1\text{-ProOH} > \text{HmimBr} > 2\text{-ProOH} \approx \text{OmimBr} > \text{BmimBr} > \text{EtOH}$  in order. In the cases of alcohols, undissociated succinic acid was considered to be extracted because it was critical to control the pH of the salt solution, suggesting that undissociated succinic acid may be integrated in the hydrogen bonding network in the alcohol-rich phase. On the other hand, the extraction behaviors of succinic acid with ATPS using ionic liquids are affected by the salts used, suggesting that the formation of water pool in ionic liquid and the distribution of succinic acid and succinate to the water pool. ATPS with OmimBr and  $(\text{NH}_4)_2\text{SO}_4$  system gave highest extractability. Extracted succinic acid in 1-propanol was successfully quantitatively precipitated by adding sodium hydroxide to the extracted phase and recovered as crystallization of sodium succinate.

ATPS using sugar to extract succinic acid was examined using alcohol (1-propanol) and Ionic Liquid ( $[\text{C}_4\text{mim}][\text{CF}_3\text{SO}_3]$ ) (Chapter 5). It was found that the extracted species was undissociated form of succinic acid. Maltose has higher ability to induce ATPS than sucrose and other sugars (mono- and disaccharides) with 1-propanol. The pH condition of ATPS technique is really important in order to get high extractability.

In conclusion, each technique of extraction of succinic acid by LM and ATPS has different optimum condition. Among SILM and PIM, SILM is suggested for separation succinic acid from fermentation broth because the simple reaction

was occurred during permeation and the optimum pH of 6.5 coincided that of fermentation. The ATPS with IL is also suggested to separate succinic acid from fermentation broth because the ability to process in large volume and quantitative extractability. Yet to consider the effect to the process or equipment/container, the IL-based ATPS with sugar (maltose) was better because of less impact on the environments.

### List of Publication

Permeation of Succinic Acid through Supported Ionic Liquid Membranes,

Aulia Indah Pratiwi, Michiaki Matsumoto, and Kazuo Kondo.

*Journal of Chemical Engineering of Japan*. Vol. 46 (2013) No. 6 p. 383-388.

(Chapter 2)

Permeation Mechanism of Succinic Acid through Polymer Inclusion Membranes with Ionic Liquid Aliquat 336.

Aulia Indah Pratiwi, Michiaki Matsumoto, and Kazuo Kondo.

*Journal of Chemical Engineering of Japan*. Vol. 47 (2014) No. 4 p. 314-318.

(Chapter 3)

Separation of Organic Acids through Liquid Membranes Containing Ionic Liquids.

Aulia Indah Pratiwi and Michiaki Matsumoto.

*Ionic Liquids in Separation Technology, 1<sup>st</sup> Edition*. Editor: Perez De Los Rios & Hernandez Fernandez. Elsevier. (2014) pp.189-206.

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**Aulia Indah Pratiwi**

*Graduate School of Science and Engineering*

*Doshisha University*

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