Research Article

Separation of Itaconic Acid from the Aqueous Phase using Phosphoric Extractant

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ABSTRACT

The technique for the separation of itaconic acid from fermented broth has a substantial impact on overall manufacturing costs. To make biorefineries sustainable and profitable, optimization and highly efficient downstream processes are technological hurdles. Itaconic acid has previously been separated using processes like crystallization, extraction, electrodialysis, diafiltration, precipitation, adsorption, and pertraction. Crystallization is a common way to separate itaconic acid from fermented broth, but other methods, viz., reactive extraction and membrane separation, show promise as recovery processes that could be used with fermentation to increase the yield of the process. In this study, the distribution coefficients were obtained from 1.89-4.05 with extraction efficiencies of 65.35-80.20% at 10–50 vol% of tri-n-butyl phosphate with iso-octanol. The maximum separation of itaconic acid was observed with 50% tri-n-butyl phosphate at 0.051 mol.L^{-1} of itaconic acid. The loading ratio was less than 0.5, showing that the complex was formed 1:1 (acid: extractant) in the organic phase. The results indicate that iso-octanol with tri-n-butyl phosphate could be further used as a solvent to the separation of itaconic acid.

1. Introduction

Itaconic acid (IA), or 2-methylidenebutanedioic acid, is a kind of unsaturated dicarbonic acid. According to the U.S. Department of Energy, it is considered in the top twelve biochemicals of the world. [1–2]. Itaconic acid is a white, solid powder that has a strong smell and the ability to attract water. It will melt between 167 and 168°C and boil at 268°C. Water solubility is 83.1 g/L, while the pH of an itaconic acid solution (80 mg/L) in clean water is 2.0. Itaconic acid has a density of 1.63 (20°C). Itaconic acid two dissociation stages have pKa values of 3.84 and 5.55 at 25°C [3]. It is a dicarbonic, unsaturated acid. It is a building element for a multitude of compounds, including paints, resins, acrylic plastic, superabsorbents, and anticalin agents [4]. Itaconic acid can be used in various industries, viz. foods, textiles, and pharmaceuticals, for the manufacturing of food packing materials, paints, carpets, coating materials, etc. (Figure 1).

Microorganisms can produce itaconic acid, which has commercial value as a precursor to polymers, compounds, and fuels. It is interesting to use microbes to transform biomass into useful compounds like itaconic acid. The primary producer of itaconic acid is the fungus Aspergillus terreus, which can generate up to 86 g/L.
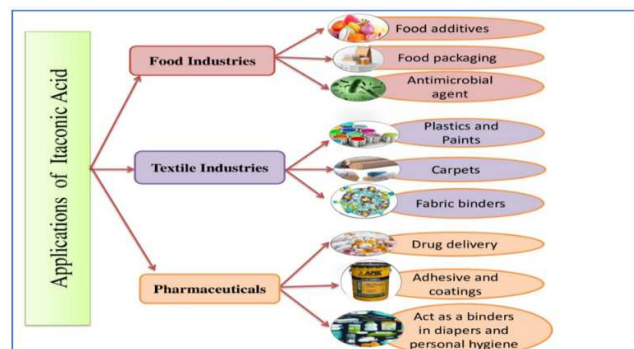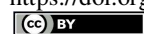

Figure 1. Applications of Itaconic acid in various sectors [5]

Some microorganisms can produce itaconic acid through fermentation. The majority of these are fungi, including Ustilago zeae, Ustilago maydis, Candida species, and Rhodotorula. Aspergillus terreus, which can generate up to 86 g/L, is the principal producer. This may seem excessive, but it is not as high as the citric acid levels that can be produced, which can reach up to 200 g/L [6].

Itaconic acid is manufactured similarly to citric acid through batch fermentation. The carbon supply should be readily metabolizable (glucose syrup, crude starch hydrolysates, and molasses) and diluted to a 10% by weight concentration. [7].

For the separation of bioactive compounds, volatile organic compounds, and fiber various methods like β-Cyclodextrin, ultrasound, and headspace solid-phase microextraction were used [8-11]. According to the industry, the IA separation technique is complex and expensive. Various techniques, including adsorption, distillation, evaporation, chromatography, absorption, liquid-liquid extraction, and reactive extraction, can be used to separate IA from synthesis and fermentation broth [12-18]. Some disadvantages of these methods include contamination issues, cost, and energy consumption [19–23]. Therefore, IA separation required low-cost, simple, and environmentally favorable methods. The most economical, simple, effective, and environmentally benign alternative method is reactive extraction. Because it can generate less waste than other separation methods and the

solvent can be reused. This method for recovering carboxylic acid from the fermentation fluid has become more established in recent years compared to other methods. [24-25]. Various extractants like trioctyle amine, Aliquat 336, and tributylphosphate, were used to increase the separation efficiency of valuable chemicals [26-28].

Itaconic acid is extracted from the aqueous phase using iso-octanol with tri-n-butyl phosphate (TBP) in this work. The findings of several parameters such as extraction efficiency *(E%)*, distribution coefficient *(K_D)*, extraction equilibrium constant *(K_E)*, and loading ratio (Z) have been investigated and predicted.

2. Materials and Method
2.1. Materials

Itaconic acid (IA) with 98% purity and 1140 kg/m^3 density was purchased from Himedia Pvt. Ltd., India. Tri-n-butyl phosphate (TBP) (99% purity) was used as an extractant and purchased from Spectrochem Pvt. Ltd., India. Iso-octanol (99% purity) was used as a solvent and supplied by Loba Chemie Pvt. Ltd., India. The NaOH flakes used to titrate the aqueous solution were supplied by Merck Specialities Pvt. Ltd., India. Fisher Scientific (India) provided phenolphthalein indicator (pH 8–10) for the titrations. To standardize the NaOH solution, oxalic acid was used and purchased from SD Fine Chem Ltd., India. All chemicals were used directly without any pretreatment in the experimental study. Table 1 provides a summary of the details of all chemicals.

Table 1 List of chemicals used in the present study

Chemicals	Purity (%)	MW (g/mol)	IUPAC name	Viscosity (cP)	Density (g.cm^{-3})	Supplier
Itaconic acid	98	130	4-oxopentanoic acid	_	1.632	Himedia Pvt. Ltd.
Iso-octanol	99	130.23	2- ethylhexanol	7.36	0.832	Loba Chemie Pvt. Ltd.
TBP	99	266	Tributyl phosphate	3.39	0.975	Spectrochem Pvt. Ltd.

*MW=molecular weight

2.2 Sample Preparation

For the aqueous phase solution, itaconic acid was diluted in distilled water and prepared from 0.051-0.398 mol.L^{-1} concentration solutions. The concentrations were chosen based on the fermentation broth concentration present downstream [6]. Tri-n-butyl phosphate was diluted in iso-octanol from 10 to

50 vol% in the organic phase to prepare organic phase solutions.

2.2 Experimental Procedure
To prepare the samples, a 100-ml conical flask was filled with a 1:1 volumetric mixture of the aqueous and organic phases. Then, the samples were kept and shaken in orbital incubator (REMI India) for 4 hours at 200 rpm at 298 ± 1 K to reach equilibrium. After

that, the samples were placed in 15-ml centrifuged plastic tubes and centrifuged at 4000 rpm for 5 minutes to separate the clear aqueous and organic phases using a bench-top tape centrifuge (R-4C REMI, India). After separation, the volume of both phases changed very little, according to a check. The organic and aqueous phases were separated for the analysis.

2.3 Analytical Method
The concentration of IA in the aqueous phase was determined by titrating the aqueous phase solutions with newly prepared 0.1 N NaOH solutions with the help of phenolphthalein indicator. Through mass balance calculations, the concentration of the IA in the organic phase was determined.

2.4.1   Standard uncertainty analysis
To minimize human and instrument errors, the experiments were repeated 2-3 times. Repeated experiments for the uncertainty analysis revealed error within 2% for virtual findings. The Standard uncertainty within x ± 0.001 was calculated using the calculation below. [29].

$$\mu(x) = \sqrt{\frac{\sum_{i=N}^{N}(x_i - \bar{x})^2}{(N-1)}} \qquad (1)$$

where x_i is an experimental value, $\bar{x}$ is the experiment's mean value, and N is the total number of experiments run.

3. Results and Discussion
The previous study result showed that physical extraction efficiency was 47.62% with iso-octanol [17]. As a result, TBP is utilized as an extractant to boost IA extraction performance. In this research, 0.051-0.398 mol.L^{-1} was the initial concentration of IA in the aqueous phase and iso-octanol in the organic phase with 10-50% TBP. Figure 2 depicts the chemical equilibrium between the aqueous and organic phases.
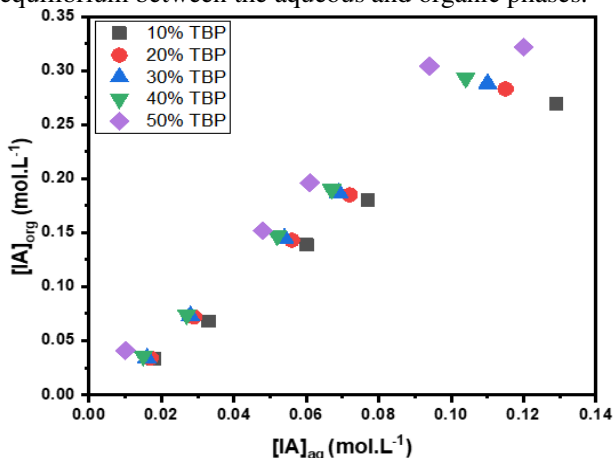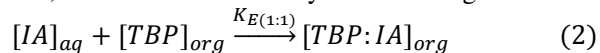

Figure 2. Chemical equilibrium for the separation of IA with 10-50% TBP at 298.15 ± K.

The following equations can be used to explain the reactive extraction of IA:
In the organic phase, acid forms a combination with TBP, which can be shown by the following:

$$[IA]_{aq} + [TBP]_{org} \xrightarrow{K_{E(1:1)}} [TBP\!:\!IA]_{org} \qquad (2)$$

$$K_E(n\!:\!1) = \frac{[TBP\!:\!(IA)_n]_{org}}{[TBP]_{org}[IA]_{aq}n} \qquad (3)$$

Where, $[IA]_{aq}$ is the aqueous phase concentration of IA; $[TBP]_{org}$ is the TBP concentration in organic phase.

3.1 Distribution coefficient (K_D)
The distribution coefficient can be defined as the equilibrium ratio between IA concentration in organic phase and its corresponding concentration in aqueous phase. It can be written as:

$$K_D = \frac{[IA]_{org}}{[IA]_{aq}} = \frac{[IA.TBP]_{org}}{[IA]_{aq} + [I^-]_{aq}} \qquad (4)$$

The highest K_D of IA was observed as 4.05 at 50% of TBP. Table 2 illustrates a positive correlation between the concentration of TBP in the organic phase and the corresponding distribution coefficients. When the concentration of TBP was augmented by 10–50% (Figure 3), it was observed that the K_D values exhibited an increase in correlation with the starting concentration range from 0.05–1.398 mol.L^{-1} of IA.
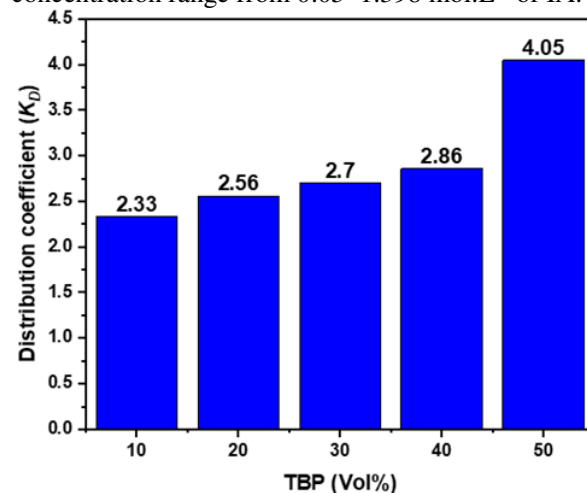

Figure 3. Distribution coefficient values increase with the concentration of TBP in the organic phase.

3.2 Extraction efficiency (E%)
In terms of K_D, the extraction efficiency (E%) is described as follows:

$$E\% = \left(\frac{K_D}{1 + K_D}\right) \times 100 \qquad (5)$$

The efficiencies were increased from 65.35 to 80.20% with increased TBP from 10% to 50% as an extractant in iso-octanol solvent (Figure 4). Because TBP has a P-(O)-OH molecular structure and presents phosphorus-bonded oxygen donors and acceptors of

both electrons, pure TBP gave a maximum efficiency of 80.20% for the separation of IA. Iso-octanol provided the higher distribution coefficient and extraction efficiency, due to strong polarity and its capacity to donate hydrogen atoms connected to O-(O-H).
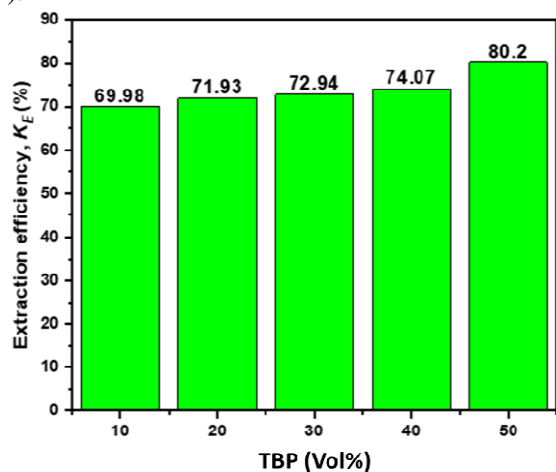

Figure 4. Variation of extraction efficiency with TBP.

3.3 Loading ratio (Z)

The loading ratio is the ratio of the initial concentration of TBP in the organic phase to the concentration of IA.

$$Z = \frac{[IA]_{org}}{[TBP]_{in,org}} \qquad (6)$$

Loading increases with increasing extractant content in iso-octanol as the solvent becomes a more effective solvating agent since the non-polar extractant alone is a reasonably powerful solvating medium for the polar complexes. This suggests that a stronger extractant concentration could be able to separate more IA.

3.4 Extraction equilibrium constant (K_E)

The extraction equilibrium constant, K_E, describes how complexes between IA and TBP evolve. According to the current study, the complex developed in a 1:1 ratio during the organic phase. As a result, to get the extraction equilibrium constant, the plot graph using the following equation:

$$\frac{Z}{1-Z} = K_E [IA]_{aq} \qquad (7)$$

Table 2. Separation of itaconic acid using iso-octanol with TBP at 298 ± 1 K

TBP (%)	$[IA]_{in}$ (mol.L^{-1})	$[IA]_{aq}$ (mol.L^{-1})	$[IA]_{org}$ (mol.L^{-1})	K_D	E (%)	Z	K_E
10	0.051	0.018	0.033	1.89	65.35	0.090	11.02
	0.101	0.033	0.068	2.10	67.70	0.187	
	0.199	0.060	0.139	2.32	69.85	0.380	
	0.257	0.077	0.180	2.33	69.98	0.490	
	0.398	0.129	0.269	2.09	67.61	0.734	
20	0.051	0.017	0.034	2.03	66.98	0.046	5.03
	0.101	0.029	0.072	2.45	71.04	0.098	
	0.199	0.056	0.143	2.54	71.73	0.195	
	0.257	0.072	0.185	2.56	71.93	0.252	
	0.398	0.115	0.283	2.46	71.07	0.386	
30	0.051	0.016	0.034	2.14	68.12	0.031	3.06
	0.101	0.028	0.073	2.59	72.15	0.066	
	0.199	0.054	0.145	2.69	72.86	0.132	
	0.257	0.069	0.187	2.70	72.94	0.170	
	0.398	0.110	0.288	2.61	72.33	0.262	
40	0.051	0.015	0.036	2.37	70.30	0.024	2.31
	0.101	0.027	0.074	2.74	73.27	0.051	
	0.199	0.052	0.147	2.85	74.05	0.101	
	0.257	0.067	0.190	2.86	74.07	0.130	
	0.398	0.104	0.293	2.82	73.80	0.200	
50	0.051	0.010	0.041	4.05	80.20	0.022	1.91
	0.199	0.048	0.152	3.19	76.13	0.083	
	0.257	0.061	0.196	3.20	76.22	0.107	
	0.398	0.094	0.304	3.24	76.42	0.166	
	0.442	0.120	0.322	2.68	72.83	0.176	

*Standard uncertainties (μ) are μ(IA) = ± 0.001 mol.L^{-1} ; μ (T) = ±1 K.
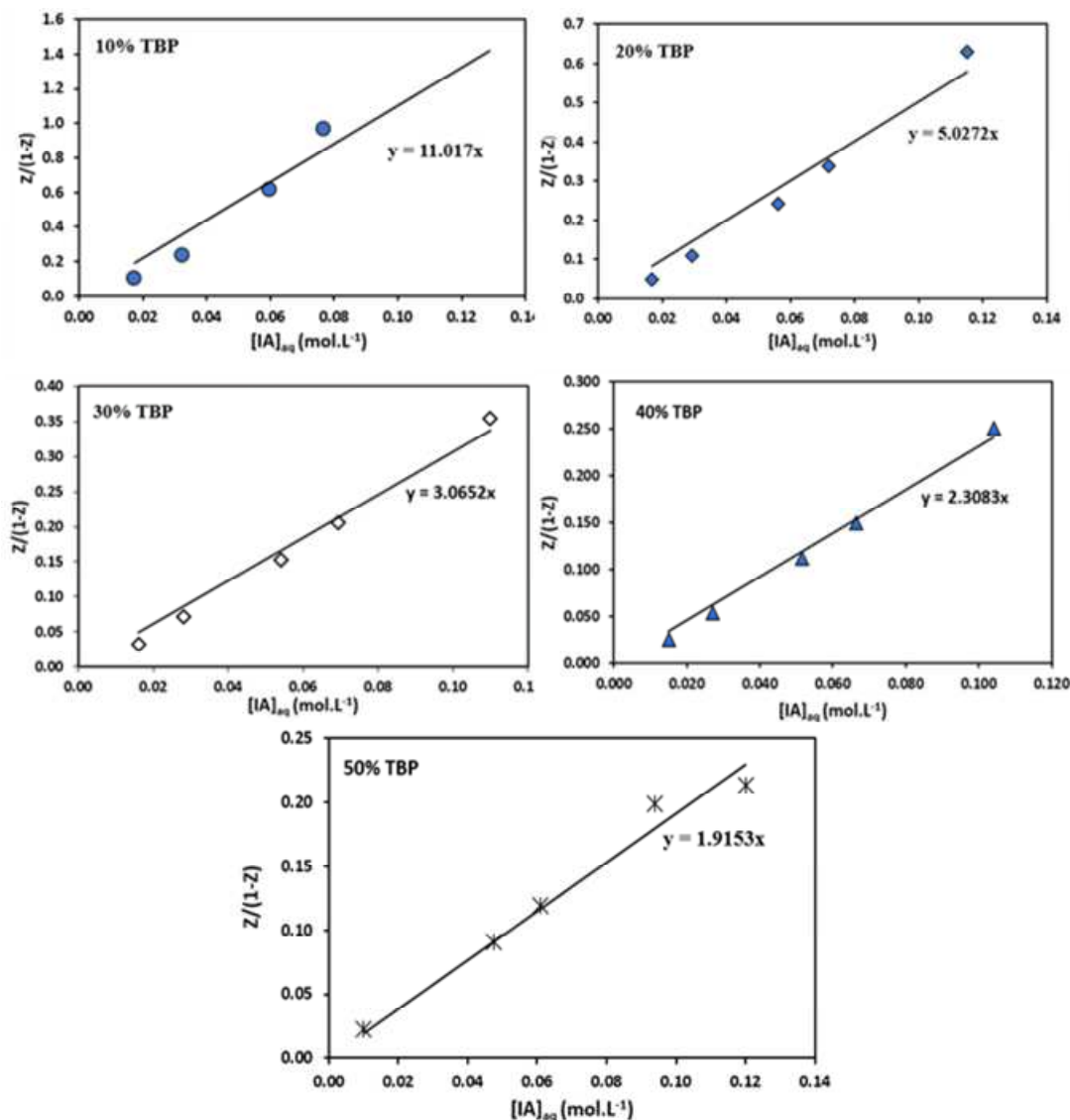
Figure 4. Graph plotting between Z/(1-Z) and [IA]$_{aq}$ to calculate extraction equilibrium constant.

The experimental results for 10-50% TBP reveal that the loading ratios are less than 0.5, validating the (1:1) complex formed in the organic phase. The loading ratio was more than 0.5 for a single point at a higher concentration of IA (0.398 mol.L^{-1}) with a lower concentration of TBP (10%). Otherwise, it is less than 0.5 for all TBP values. When IA is extracted from iso-octanol using TBP, the linear relationship between Z/(1-Z) and [IA]$_{aq}$ is shown in Figure 4. Using 10-

5. Conclusion

Itaconic acid separation from the aqueous phase using iso-octanol with TBP by the reactive extraction method was studied. In the experiment, it was found that 50% TBP in iso-octanol led to a better separation of IA. The higher extraction efficiency was determined to be 80.20% with a 4.05 distribution coefficient, respectively. To understand the reaction, complex loading ratios were calculated and found to

50% TBP, the K_E (1:1), was calculated to be 11.02, 5.03, 3.06, 2.31, and 1.91 respectively.

Iso-octanol is an aliphatic hydrocarbon solvent, so it provides more extraction efficiency, because of its linear chemical structure and capacity to establish hydrogen bonds with TBP in the organic phase [30]. As a result, itaconic acid extraction had higher distribution coefficient and extraction efficiency. TBP can be regarded as an effective extractant when used with iso-octanol to separate itaconic acid. be less than 0.5, which means that a 1:1 complex of IA and TBP forms in the organic phase. To understand the reaction, the extraction equilibrium constant was calculated as 1.91 with 50% TBP, which provided higher extraction efficiency. According to the results, TBP can be used for the separation of IA with iso-octanol. This study can be used to design instruments like column design, mixer settlor reactor, etc. to

develop a continuous method for the separation of itaconic acid.

Acknowledgments

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