International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.7, No.4, pp 1889-1894, 2014-2015

Separation of Pyruvic Acid Using Reactive Extraction: Back Extraction and Effect of P^H

Dharm Pal*, Amit Keshav

Department of Chemical Engineering, NIT Raipur- 492010 C.G. India

Abstract: Downstream (separation and purification) processing are important mainly due to the specialization, complexity and strict quality requirements of products. Therefore, the cost of downstream processing contributes heavily in the whole bioprocess. A reasonable design can help reduce production cost of the target products and achieve successful commercial production. In recent years, fermentation technology has started to replace conventional synthesis for the production of organic acids. Even though, recovery of pure product from fermentation broth is still a challenging task.

The aim of this study was to investigate the effectiveness of reactive extraction for the separatation of pyruvic acid from the fermentation media. For this purpose, reactive extraction was investigated from aqueous solutions using tributylamine (TBA) as an extractant. The effects of different diluents (n-decane,n-decanol,1,2-dichloroethane), the stoichiometry of pyruvic acid to TBA, and the initial pH of the aqueous phase on the extraction process are studied. Back extraction was also studied using sodium hydroxide (NaOH). The extraction parameters were optimized in terms of distribution coefficient, extraction efficiency, and initial P^H .Conditions obtained from the aqueous solutions based on experiments proved efficient. Around 76 % complete of pyruvic acid was obtained.

Key Words: Reactive extraction, Pyruvic acid, Diluents, Extractant, Extraction equilibrium.

1. Introduction:

Pyruvic acid, also known as 2-oxopropanoic acid is the most important α-oxocarboxylic acid. Pyruvic acid is a weak acid having P^{Ka} value 2.49. Pyruvic acid plays a central role in energy metabolism in living organisms apart from its significance in metabolism. Pyruvic acid has been used as a valuable starting material for the synthesis of many drugs and agrochemicals, presently, in the food industry as a fat burner.[1,2]

Large scale production of pyruvic acid is done by dehydration and decarboxylation of tartaric acid. However, due to high energy consumption chemical synthesis is a costly approach. Pyruvic acid is can act as a promising alternative for imparting a sour taste to foods, but due to high cost, it is hardly ever used to replace other organic acids. An alternative approach to reduce production cost and to fulfill environmental constrains at the same time is the use of biotechnological methods. In last few years, biocatalytic reactions and fermentation technology is being used to replace conventional synthesis for the production of carboxylic acid. However, downstream processing steps (separation and purification) are still a major challenge in biochemical processing. Very few methods have been studied and reported in the literature to recover pyruvic acid from fermentation broth. Generally used methods include organic precipitation, solvent extraction, reverse osmosis method and ion exchange process. Among these methods, reactive extraction has been proposed to be a promising method with its high distribution coefficient, simplicity, low energy demand, and ineffectuality to thermal stability of the products. Recovery of carboxylic acid by reactive extraction is an active area of research.[3] Recently, various study on reactive extraction of pyruvic acid have been reported.[4,5,6] In this recovery technique, tertiary amines with long aliphatic chains[7] such as tri-n-octanylamine (TOA)[4,5,8], and Alamine 336[3] and phosphourous based extractants; TBP[6] were usually employed as an extractant. However, very limited studies have reported in the literature to recover particularly pyruvic acid using reactive extraction.

The extraction parameters such as suitable diluents, initial pH of the aqueous phase, and stoichiometry of TBA to pyruvic acid were optimised by calculating distribution coefficient (D), and extraction efficiency (E).

2. Theory

2.1 Reactive Extraction

Reactive extraction strongly depends on various parameters such as the distribution coefficient, degree of extraction, loading ratio, complexation equilibrium constant, types of complexes (1:1, 2:1, etc.), rate constant of acid-extractant reaction, properties of the solvent (extractant and diluents).The most suitable extractants are the phosphorous-based oxygen extractants and amine based extractants. Tertiary amine extractants are effective, with K_D strongly dependent upon the nature of the diluents used and the concentration of amine in that diluent. The amine extractants are usually dissolved in a diluent in order to dilute the extractants up to desired composition and also to controls the viscosity and density of extractant phase. Tertiary amines are found to be effective in extracting acids and active diluents with functional groups like alcohols, ketomes and esters are among the best diluents.

2.2 Mathematical Background of Reactive Extraction:

Based on Nernst's distribution law and mass action law the reactive extraction betweenTributylamine; TBA, and the pyruvic acid; HA, can be treated as an acid base-type reaction.[7] The typical interfacial extraction reactions with aminic extractants can be represented as Eq. (1) as follows,

$$\alpha\ HA_{aq} + \beta\ TBA_{org} \leftrightarrow HA_{\alpha}TBA_{\beta,org} \qquad (1)$$

The equilibrium extraction constant (K_E) can be formulated as Eq. (2)

$$K_E = \frac{[HA_{\alpha}TBA_{\beta,org}]}{[HA_{aq}]^{\alpha}[TBA_{org}]^{\beta}} \qquad (2)$$

Pyruvic acid ionization in aqueous phase can be presented as Eq. (3)

$$HA_{aq} \leftrightarrow H^{+} + A^{-} \qquad (3)$$

$$K_a = \frac{[H^{+}][A^{-}]}{[HA_{aq}]} \qquad (4)$$

Where K_a represents ionization constant of the pyruvic acid and squar brackets denotes the concentrations of the respective species.

Distribution coefficient; K_d , and extraction efficiency, E, can be defined by Eq.(5) and Eq. (6) , and could be estimated from equilibrium experiments.

$$K_d = \frac{[HA_{t,org}]}{[HA_{t,aq}]} \qquad (5)$$

$$E = \frac{[HA_{t,org}]}{[HA_{i,aq}]} \times 100 \qquad (6)$$

Where, total acid in the organic phase, in the aqueous phase, and the initial acid in the aqueous phase are represented by where $HA_{t,org}$, $HA_{t,aq}$ and $HA_{i,aq}$, respectively.

3. Materials and Methods

3.1 Materials:

To prepare an aqueous phase the pyruvic acid was diluted in de-ionized ultrapure water (Millipore). Tri-*n*-butyl amine (TBA) was taken as extractant .Both TBA and pyruvic acid (98 % pure) was obtained from SRL Pvt. Ltd., Mumbai, India. Different diluents (*n*-decane, decanol, and 1,2-dichloroethane) were obtained from Merck Specialist Pvt. Ltd., Mumbai; India. Sodium hydroxide (NaOH) used for titration and back extraction was obtained from Ranbaxy, India and used for titration. Before use NaOH was first standardized with 99.8 % purity oxalic acid supplied by S. d. fine-Chem. Ltd., India. Phenolphthalein (pH range 8.2 to 10.0) was used as indicator obtained from Ranbaxy, India. All reagents used were of analytical grade and were utilized without any further treatment.

3.2 Methods.

3.2.1. Equilibrium experiments:

Experiments for equilibrium studied were performed by shaking equal volumes (0.02 dm^3) of aqueous and organic phases (V_{org} /V_{aq}=1) in a 100 mL Erlenmeyer glass made flask for 4 h (equilibrium time) at fixed conditions (T=308.2 ± 1 K and at atmospheric pressure) at 100 rpm in a temperature controlled reciprocal water bath shaker (REMI instruments (P) Ltd., India). For complete separation of both phases' equilibrated samples were kept for settling for at least 2 h at constant condition. Analysis of aqueous phase acid concentration was carried out by titration using fresh and standardized (with oxalic acid) NaOH (0.025 N). Mass balance was used to determine organic phase acid concentration. All weighing was done by AG Gottingen Germany, made weighing balance (Soritorious - CP 2245) with accuracy of 0.0001 g. The pH of the aqueous phase was determined by pH meter.

3.2.2. Back Extraction:

Back extraction efficiency $(BE)_{PA}$ which is the molar ratio of the aqueous phase pyruvic acid to that in the forward organic phase, was used to investigate the back extraction. Back extraction results were optimized by shaking forward organic phase with an equal volume of sodium hydroxide (NaOH) solution of various concentrations for 2 hr. After settling and complete phase separation of both phases, the concentrations of acids in the aqueous phase and pH of the aqueous phase were obtained.

3.2.3. Experimental Uncertainty:

Uncertainty analysis in the present work was performed by repeated experiments in triplicate and consistency of replicated experiments was within $\pm 2\%$. Standared uncertainity was evaluated to be ± 0.001.

4. Result and Discussion:

4.1. Effect of Diluents:

Initially extraction experiment of TBA alone was carried outbut due to high viscosity of TBA, an undesirable second organic phase (third phase) formation was observed.In order to overcome the third phase formation further exytrtaction experiments were performed by dissolving TBA in difeerent diluents namely, *n*-decane, decanol, and 1,2-dichloroethane. Third phase formation was minimized by addition of the diluent and the two phases can easily be separated. TBA and diluents systems were used in equal stoichiometry ratio with pyruvic acid. Distribution coefficient along with dielectric constants and E_T values are tabulated in Table 1. From Table 1, it can be seen that extraction efficiencies closely reassembles with dielectric constants and E_T values of diluents used. TBA in n-decanol gave highest distribution coefficients for pyruvic acid. The order of extraction efficieny is found as n-decanol >1,2- dichloroethane> decane.

Addition of diluents is helpful in modifying the basicity of TBA and hence stability of the complex formed. According to Kertes and King, equilibrium conditions are dictated by the stability acid extraction.[7] When TBA dissolved in n-decanol distribution coefficient; $K_{DPA,}$ improves significantly. This is attributed to the higher polarity as n-decanol has higher dielectric constant as well as E_T value than other diluents, n-decane and 1,2 dichloroethane examined in this study. Polar diluents are more favorable than the non-polar diluents as polar diluents can stablised the polar acid-amine complex formed through hydrogen bonding.[6,7] Hydrogen bond is possible between the –OH group of the n-decanol and the –C=O group of the pyruvic acid in the complex. According to results obtained n-decanol was found as most appropriate diluents and was utilized for further studies.

4.2. Effect of Stoichiometry Ratio:

Extraction was found to affect with stoichiometry ratio of the initial TBA in the organic phase to the initial aqueous pyruvic acid. The effect of stoichiometry ratio on the extraction was presented in terms of parameter $K_{D,PA}$ and E_{PA} in Fig. 1 and Fig. 2, respectively. From Fig. 1 and Fig. 2 it can be observed that both $K_{D,PA}$ and E_{PA} increases with the increase of stoichiometry ratio. In both cases maxima lies at stoichiometry ratio between 1 to 1.5.Hence, stoichiometry ratio 1 was chosen as optimim ratio for further investigations.

Table 1: Extraction parameters of various diluents systems

Extraction System	Dielectric constant (ε) of diluent	E_T Value[9]	Distribution Coefficient of Pyruvic Acid ($K_{D,PA}$)
TBA	--	--	7.31
TBA+ 1,2-dichloroethane	10.3	41.3	10.14
TBA+ n-decane	2.0	31.0	2.01
TBA+ n-decanol	8.1	47.7	15.30

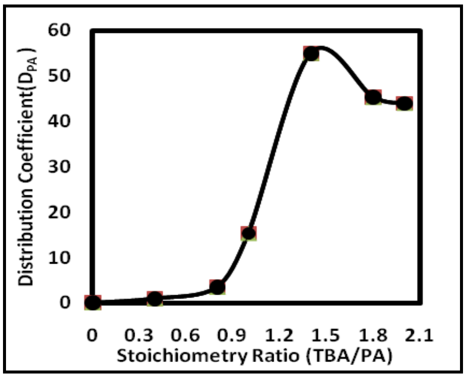

Fig. 1: Effect of stoichiometry Ratio of TBA to pyruvic acid on the distribution coefficient

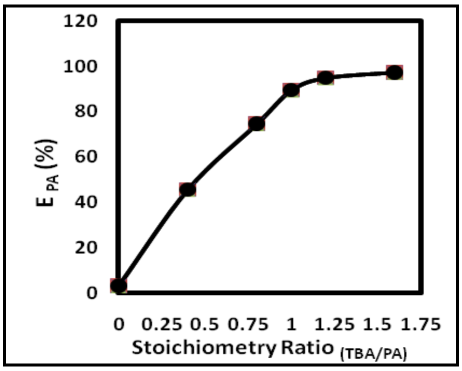

Fig. 2: Effect of stoichiometry Ratio of TBA to pyruvic acid on the extraction efficiency

4.3. Effect of pH:

Variation of distribution coefficient ($K_{D,PA}$) and Extraction efficiency (E) with pH of the aqueous phase are shown in Fig. 3 and Fig, respectively. Both D and E of pyruvic acid decreases with the increase in initial pH. This trend can be justified on the ground that the aminic extractants can extract only unionized acid. Acid transfered to the organic phase or complexed with the extractant in a molecular form.[8] At higher P^H particularly when $P^H > P^{Ka}$ of the pyruvic acid (P^{Ka} =2.49) the most of the acid will get ionized and availability of unionized will be less and hence, extraction will be lower.

4.4. Back Extraction Study:

For the development of complete recovery process it recycle of the extractant is necessary, and hence an efficient and economic back extraction is required. Various technique used for back extraction includes concentration swing, temperature swing and pH swing[10], and so forth. Fig. 5, show the effect of stoichiometry ratio of NaOH and pyruvic acid for the back extraction using pH swing regeneration. As the concentration of NaOH increased back extraction efficiency keep on rising till the stoichiometry ratio reached 1 when the back extraction was most efficient.

At pH > pK_a of the acid formation of the acid–amine complex is unfavourable, this can be employed for the back extraction of pyruvic acid from the organic phase to regenerate TBA. Acid-amine complex can be decomplexed by using aqueous NaOH, Na_2CO_3, or NaCl.[11] Back extraction efficiency of pyruvic acid increased with the increase of stoichiometry of the NaOH to pyruvic acid. Nevertheless, it is not recommended to use excess free base in the aqueous phase as it leads to decreased back extraction efficiency, because due to special molecular structure pyruvic acid has tendency to polymerize in basic alkaline medium (Kimura 1998).

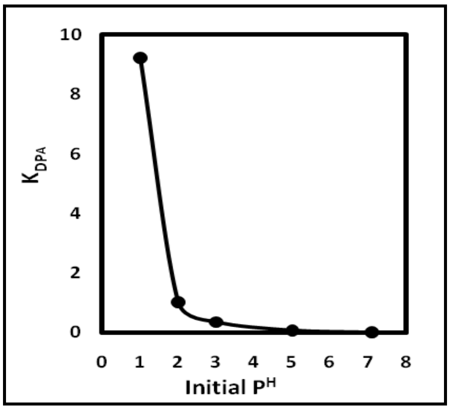

Fig.3: Effect of initial pH of aqueous phase on distribution coefficient

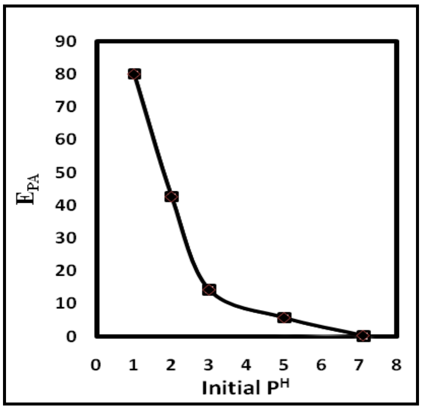

Fig.4: Effect of initial pH of aqueous phase on extraction efficiency

Experimental results indicate that, n-decanol is the best diluent, and the initial pH of the aqueous phase should be kept below P^{Ka} pyruvic acid (P^{Ka} =2.49). The optimum stoichiometry of TBA to the initial pyruvic acid and NaOH to pyruvic acid in the organic phase was 1.0. Above results confirmed that pyruvic acid could be efficiently and economically recovered from fermentation broth by using reactive extraction.

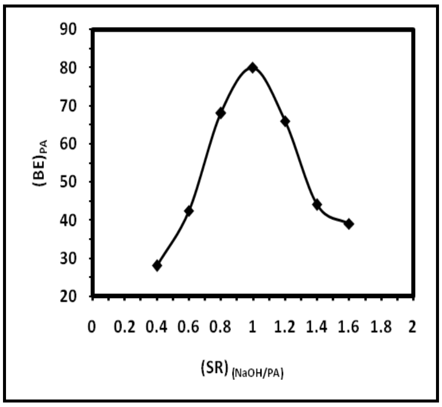

Fig.5: Effect of stoichiometry of NaOH to pyruvic acid on back extraction.

5. Conclusion:

Based on the experimental findings of high distribution coefficient and extraction efficiency, it is concluded that reactive extraction can be effectively and economically utilized for recovery of biotechnologically produced pyruvic acid. Process investigated in this study provided a total recovery of around 76% of pyruvic acid in single step. The best stoichiometry of TBA to the initial pyruvic acid and NaOH to pyruvic acid in the organic phase was found unity.Extraction was favourable at low P^H and it was recommended that for efficient recovery, P^H should be kept below P^{Ka} of the target acid. It was also found that addition of diluents and stoichiometry play an important role and influence the extraction greatly. Polar diluents are more favourable for recovery of pyruvic acid with tributylamine; TBA, as an extrantant. The data obtained are useful for design of extractor to achieve specific objective.

Notes

The authors declare no competing financial interest.

Nomenclature:

[HA]	Concentration of pyruvic acid in the aqueous phase
$K_{D,PA}$	Distribution coefficient of pyruvic acid
E_{PA}	Extraction efficiency of pyruvic acid
HA_{aq}	Undissociated acid in the aqueous phase
$HA_{i,aq}$	Initial acid in the aqueous phase
$HA_{t,aq}$	Total acid in the aqueous phase
$HA_{t,org}$	Total acid in the organic phase
K_a	Dissociation constant of acid
α	Number of acid in the acid–amine complex
β	Number of TBA in the acid–amine complex
$(BE)_{PA}$	Back extraction efficiency of pyruvic acid
$(SR)_{NaOH/PA}$	Stoichiometry of NaOH to pyruvic acid loaded in the organic phase
$(SR)_{TBA/PA}$	Stoichiometry of TBA to pyruvic acid
TBA	Tributylamine
E_T	Dimroth-Reichardt parameter
ε	Dielectric constant

References

1. Rosche B. Leksawasdi N., Sandford V., Breuer M., Hauer B., Rogers P., Enzymatic (R)-phenylacetyl carbinol production in benzaldehyde emulsions. Appl. Microbiol. Biotechnol.,2002, 60:94–100
2. Uchio R., Kikuchi K., Hirose Y., Process for producing pyruvic acid by fermentation. US Patent.,1976, 3,993,543.
3. Wasewar K.L., Heesink A.B.M. Versteeg G.F., Pangarkar V.G., Reactive extraction of lactic acid using alamine 336 in MIBK:equilibria and kinetics. J. Biotechnol.,2002 97,59–68.
4. Ma C.Q., Li J.C., Qui J.H.,Wang M., Xu, P., Recovery of pyruvic acid from biotransformation solutions", App. Microbio. Biotechnol.,2006,70,308-314.
5. Marti, M. E.; Gurkan, T.; Doraiswamy, L.K., Equilibrium and Kinetic Studies on Reactive Extraction of Pyruvic Acid with Trioctylamine in n-Octanol. Ind. Eng. Chem. Res. 2011, 50, 13518–13525.
6. Pal D., Keshav A., Extraction Equilibria of pyruvic acid using tri‑n‑butyl phosphate: influence of Diluents, J. Chem. Eng. Data., 2014**,**59, 2709 –2716.
7. Kertes, A.S., King, C.J., Extraction chemistry of fermentation product carboxylic acids, Biotechnol. Bioeng.,1986,28, 269–282.
8. Poposka F.A., Nikolovski K., Tomovska R., Kinetics, mechanism and mathematical modelling of extraction of citric acid with isodecanol/n-paraffins solutions of trioctylamine. Chem. Eng. Sci., 1998, 53,3227–3237.
9. Reichardt C., Solvatochromic Dyes as Solvent Polarity Indicators Chem. Rev., 1994, 94, 2319-2358.
10. Poole L.J., King C.J. Regeneration of carboxylic acid–amine extracts by back-extraction with an aqueous solution of a volatile amine. Ind. Eng. Chem. Res., 1991, 30,923–929.
11. Tong Y.P., Hirata M., Takanashi H., Hano T., Back extraction of lactic acid with microporous hollow fiber membrane. J. Membr. Sci., 1999, 157,189–198.
12. Kimura H., A simple method for the anionic polymerization of a-carbonyl acids in water. J. Polym. Sci. A. Polym. Chem.,1998, 36:189–193.

*****