REACTIVE EXTRACTION OF PYRUVIC ACID FROM AQUEOUS SINGLE AND MIXED ACID SOLUTIONS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

MUSTAFA ESEN MARTI

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
CHEMICAL ENGINEERING

JULY 2010

Approval of the thesis:

REACTIVE EXTRACTION OF PYRUVIC ACID FROM AQUEOUS SINGLE AND MIXED ACID SOLUTIONS

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ABSTRACT

REACTIVE EXTRACTION OF PYRUVIC ACID FROM AQUEOUS SINGLE AND MIXED ACID SOLUTIONS

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July 2010, 149 pages

Interest in recovery of carboxylic acids from their aqueous solutions and production media has been growing with the improvements in the production techniques. Reactive extraction is proposed as a promising method to achieve high distribution coefficients with good selectivity. In the present study, equilibrium and kinetic studies, needed for the design of a recovery unit were carried out. Reactive extraction of pyruvic, acetic and lactic acids from their single and mixed acid solutions were carried out with tertiary amines, Alamine 336 and trioctylamine, dissolved in diluents, 1-octanol and oleyl alcohol. The kinetic parameters such as reaction order and rate constant were calculated by using the aqueous solution of pyruvic acid with 1-octanol solution of trioctylamine.

The distribution coefficient of pyruvic acid was obtained as 0.30 and 0.07 with 1-octanol and oleyl alcohol respectively. The trend did not change after the addition of the extractants to the diluents. As expected, the more polar diluent extracted more acid than the less polar one. The extractant was observed to be the limiting reagent of the reversible complexation reaction in the organic phase. It was seen that loading ratio was not affected by the concentration of the extractant in the organic phase but increased with the equilibrium concentration of the acid in the aqueous phase. The results showed that mainly (1-1) acid-amine reaction occurred in the organic phase. Because tertiary amines can react with only undissociated acids, the increase in the initial pH of the aqueous phase caused a decrease in the distribution coefficients of the carboxylic acids studied. The effect of the concentration of the organic phase disappeared when the initial pH of the aqueous phase was 4.0 and a distribution coefficient value of 0.1 was achieved for all concentration levels of organic phase. Similar results were obtained for acetic and lactic acids and this behavior was used to propose a selective recovery of the acids from their mixed acid solutions.

The presence of acetic acid prevented the extraction of pyruvic acid. The increase in the concentration of the extractant in the organic phase caused an increase in the distribution coefficient of pyruvic acid. During the extraction process, lactic acid could not be recovered from ternary acid solutions because of its high hydrophilicity. The results of reactive extraction of lactic and pyruvic acids from their binary acid solutions showed that at higher concentration of lactic acid, the distribution of pyruvic acid was negatively affected. On the other hand, the presence of lactic acid at a low concentration level did not affect the distribution of pyruvic acid.

It was found that the reaction between pyruvic acid and trioctylamine in 1-octanol was first order with respect to the reactants with a second order rate constant of 0.94 L mol^{-1} s^{-1}. The enhancement factor of the system was obtained as 25.

Keywords: Reactive extraction, pyruvic acid, equilibrium studies, kinetic studies, trioctylamine, mixed acid solutions

ÖZ

PİRÜVİK ASİTİN TEK VE KARIŞIK SULU ASİT ÇÖZELTİLERDEN TEPKİMELİ ÖZÜTLENMESİ

Martı, Mustafa Esen

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Temmuz 2010, 149 sayfa

Karboksilik asitlerin sulu çözeltilerinden ve üretim ortamlarından geri eldesine olan ilgi, üretim metotlarındaki gelişmelerle birlikte artmaktadır. Tepkimeli özütleme, iyi bir seçicilikte yüksek dağılım katsayılarına ulaşılabilecek, ümit vadeden bir yöntem olarak önerilmektedir.

Bu çalışmada, bir ayırma ünitesinin tasarımı için gerekli olan denge ve kinetik incelemeler gerçekleştirilmiştir. Denge deneylerinde pirüvik, asetik ve laktik asitlerin tek ve karışık asit çözeltileri kullanılırken, kinetik çalışmalarda pirüvik asitin tek asit çözeltileri kullanılmıştır. 1-oktanol ve oleyil alkol içinde çözünmüş üçüncül aminler, Alamine 336 ve trioktilamin, organik faz bileşenleri olarak asitlerin sulu fazdan özütlenmesi için seçilmiş ve karşılaştırılmıştır. Dağılım ve yükleme oranları, fazların derişimleri, sulu fazın başlangıç pH değeri gibi denge parametrelerinin özütlemeye olan etkisini incelemek için kullanılmıştır. Diğer asitlerin pirüvik asitin dağılımına etkisi üçlü ve ikili asit karışımları kullanılarak incelenmiştir.

Tepkime derecesi ve sabiti gibi kinetik özellikler, kinetik veriler kullanlarak bulunmuştur.

Organik fazın düşük derişim seviyelerinde bir başka asitin varlığının pirüvik asitin dağılımını engellemiştir. Organik fazın derişimindeki artış pirüvik asitin dağılımında artışa neden olmuştur. Laktik asit, yüksek su-severliği nedeniyle üçlü asit çözeltilerinden çok düşük oranlarda özütlenmiştir. Laktik ve pirüvik asitlerin ikili karışımlarının tepkimeli özütleme sonuçlarının gösterdiği gibi; laktik asitin yüksek derişim seviyelerinde, pirüvik asit dağılımı olumsuz şekilde etkilenmiştir. Diğer yandan, laktik asitin düşük derişim seviyelerindeki varlığı, pirüvik asit dağılımını etkilememektedir. Organik fazdaki yüksek derişim seviyelerinde küçük sinerjistik etkiler gözlemlenmiştir.

Pirüvik asitin 1-oktanol içerisindeki trioktlilamin ile özütlenmesi ile ilgili kinetik veriler bir karıştırmalı hücre kullanılarak elde edilmiştir. Pirüvik asit ve trioktilamin arasındaki tepkimenin her iki kimyasala göre birinci dereceden olduğu, ve ikinci dereceden tepkime sabitinin 0.94 L mol^{-1} s^{-1} olduğu bulunmuştur. Sistemin hızlanma katsayısı 25 olarak bulunmuştur.

Bu çalışmada elde edilen denge ve kinetik verileri, pirüvik asitin sulu çözeltilerinden ya da üretim ortamından kazanımını sağlayacak bir tepkimeli özütleme ünitesinin tasarımında kullanılabilir.

Anahtar Kelimeler: Tepkimeli özütleme, pirüvik asit, denge çalışmaları, kinetik çalışmalar, trioktilamin, karışık asit çözeltileri

To my family

ACKNOWLEDGEMENTS

I am very thankful to my supervisor Prof. Dr. Türker Gürkan, from whom I learned studying hard and acquired the skill of looking at things from different perspectives, for his endless support, trust and motivation.

I would also like to thank Dr. Cevdet Öztin for his valuable contributions and sharing his invaluable vision and wisdom.

I would also like to thank Prof. Dr. Ufuk Bakır and Prof. Dr. Mürvet Volkan for their valuable contributions.

Many thanks to Gülten Orakçı for teaching everything I know about HPLC and to Kerime Güney, Turgut Aksakal and Selahattin Uysal for sharing their experiences with me.

Last but not the least, I would express my gratitude to my family, who have supported me in all my academic studies so far and motivated me in times of difficulty.

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A_c	Cross sectional area of the stirred cell
A^-	Dissociated Acid
D_{HA}	Diffusivity of acid in solvent (1-octanol), $m^2\ s^{-1}$
D_{HP}	Diffusivity of pyruvic acid in solvent (1-octanol), $m^2\ s^{-1}$
H^+	Hydrogen ion
H_3O^+	Hydronium ion
HA_t	Total acid
$HA_{t,0}$	Total acid at time equals to zero (t=0)
HA	Undissociated Acid
HA_{aq}	Undissociated acid in the aqueous phase
HA_{org}	Undissociated acid in the organic phase
$(HA\text{-}TA)_{org}$	Undissociated acid- tertiary amine complex in organic phase
HP	Undissociated pyruvic acid
HP_{aq}	Undissociated pyruvic acid in the aqueous phase
HP_{org}	Undissociated pyruvic acid in the organic phase
HP_d	Undissociated pyruic acid diluted by the diluent
HP_t	Total pyruvic acid
$HP_{t,0}$	Total pyruvic acid at initial

$(HP\text{-}TOA)_{org}$	Pyruvic acid- trioctylamine complex in organic phase,
k_2	Second order rate constant, L mol^{-1} s^{-1}
k_L	Overall mass transfer coefficient, m s^{-1}
k_{mn}	Rate constant for a reaction that is mth order in pyruvic acid, nth order in trioctylamine, (L mol^{-1} s^{-1})$^{m+n-1}$ s^{-1}
K_A	Equilibrium constant of dissociation of the acid, mol L^{-1}
K_D	Distribution ratio, dimensionless
$K_{D,d}$	Distribution ratio referred to the diluent alone, dimensionless
K_E	Acid-amine equilibrium complexation constant L mol^{-1}
K_{En}	(n-1) Acid-amine equilibrium complexation constant L mol^{-1}
m	Order of the reaction in reactant HA
M	Molecular weight of the diluent
n	Order of the reaction in reactant TA
N	Speed of agitation, rev s^{-1}
P^-	Dissociated Pyruvic Acid
R_A	Specific rate of extraction of the solute A, mol m^{-2} s^{-1}
R_{HP}	Specific rate of extraction of pyruvic acid, mol m^{-2} s^{-1}
TA	Tertiary amine
TA_{org}	Tertiary amine in the organic phase
TOA	Trioctylamine
TOA_{org}	Trioctylamine in the organic phase

V	Molar volume of the component
V_{aq}	Volume of the aqueous phase, L
z	Loading ratio, mol L acid $^{-1}$ mol L lamine $^{-1}$
z_p	Loading ratio, mol L pyruvic acid $^{-1}$ mol L trioctylamine $^{-1}$
$\alpha_{A/B}$	Separation factor
ψ	Eluent association factor
η	Viscosity of the diluent
v	Volume fraction of diluent in the organic phase, dimensionless
Φ	Enhancement factor, dimensionless
$[H^+]$	Hydrogen ion concentration, mol L^{-1}
$[A^-]_{aq}$	Dissociated acid concentration in the aqueous phase, mol L^{-1}
$[HA]_{aq}$	Undissociated acid concentration in the aqueous phase, mol L^{-1}
$[HA]_{aq,0}$	Undissociated acid concentration in the aqueous phase at iniial, mol L^{-1}
$[HA_t]_{aq}$	Total acid concentration in the aqueous phase, mol L^{-1}
$[HA_d]_{org}$	Undissociated acid concentration extracted by the diluent in the organic phase, mol L^{-1}
$[HA_{ex}]_{org}$	Undissociated acid concentration reacted with the extractant in the organic phase, mol L^{-1}
$[HA_t]_{org}$	Total acid concentration in the organic phase, mol L^{-1}
$[HA_t]_{aq,0}$	Total acid concentration in the aqueous phase at initial, mol L^{-1}
$[(HA\text{-}TA)]_{org}$	Acid-amine complex concentration in organic phase,mol L^{-1}

$[HP]_{aq}$	Undissociated pyruvic acid concentration in the aqueous phase, mol L^{-1}
$[HP]_{aq,0}$	Undissociated pyruvic acid concentration in the aqueos phase at initial, mol L^{-1}
$[HP]_{org}$	Undissociated pyruvic acid concentration in the organic phase, mol L^{-1}
$[HP_{ex}]_{org}$	Undissociated pyruvic acid concentration reacted with the extractant in the organic phase, mol L^{-1}
$[HP^{*}]_{org}$	Equilibrium concentration of pyruvic acid in organic phase, mol L^{-1}
$[HP_{t}]_{aq}$	Total pyruvic acid concentration in the organic phase, mol L^{-1}
$[HP_{t}]_{aq,0}$	Total pyruvic acid concentration in the organic phase at initial, mol L^{-1}
$[HP_{d}]_{org}$	Undissocated pyruvic acid concentration extracted by the diluent in the organic phase, mol L^{-1}
$[HP_{t}]_{org}$	Total pyruvic acid concentration in the organic phase, mol L^{-1}
$[HP\text{-}TOA]_{org}$	Pyruvic acid-trioctylamine complex concentration in organic phase, mol L^{-1}
$[P^{-}]_{aq}$	Dissociated pyruvic acid concentration in the aqueous phase, mol L^{-1}
$[TA]_{org}$	Tertiary amine concentration in the organic phase, mol L^{-1}
$[TA]_{org,0}$	Tertiary amine concentration in the organic phase at initial,mol L^{-1}
$[TOA]_{org}$	Trioctylamine concentration in the organic phase, mol L^{-1}
$[TOA]_{org,0}$	Trioctylamine concentration in the organic phase at initial, mol L^{-1}

CHAPTER 1

INTRODUCTION

Pyruvic acid, also known as 2-oxopropanoic acid, *α*-ketopropionic acid or acetylformic acid, is one of the most important *α*-oxocarboxylic acids (Figure 1.1). Interest in pyruvic acid has been increasing due to an increase in its potential areas of use as well as its significant role in metabolic reactions (Zhang *et al.*, 2006). It is primarily used as a trigger in the biosynthesis of pharmaceuticals, agrochemicals and as a fat burner in the food industry (Ma *et al.*, 2006). It was mentioned that calcium pyruvate reduces fat by accelerating the metabolism of fatty acids in human body (Roufs *et al.*, 1996). Furthermore, it is an important precursor of some amino acids such as L-tryptophan, L-dihydroxyphenylalanine (Uchio *et al.*, 1976), L-phenylacetylcarbinol (Rosche *et al.*, 2002) and N-acetyl-D-neuraminic acid (Mahmoudian *et al.*, 1997).

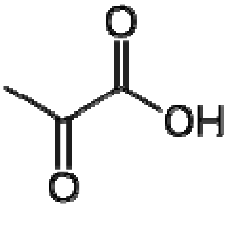

Figure 1.1 Pyruvic acid structure

In addition to the increase in usage areas, increase in production cost of conventional processes had caused researchers to consider novel production and recovery methods of pyruvic acid. Pyruvic acid has been produced by chemical methods such as dehydration and decarboxylation of tartaric acid in the industry. This process includes distillation of pyruvic acid from a mixture of tartaric acid and potassium hydrogen sulfates at 220^0C and then distillation of the crude acid obtained under vacuum. Although this process is simple to conduct, it is not cost effective which makes pyruvic acid difficult to be employed in the industry and commercialized. In recent years, biocatalytic reactions and fermentation technology have started to replace conventional synthesis (Li *et al.*, 2001). In the literature three different types of biological production methods were recommended for pyruvic acid:

(1) Enzymatic methods (Burdick and Schaeffer, 1987; Eisenberg *et al.*, 1997),

(2) Resting cells (Izumi *et al.*, 1982; Schinschel and Simon, 1993; Ogawa *et al.*, 2001),

(3) Fermentation processes (Yokota *et al.*, 1994; Li *et al.*, 2001a; 2001b; Causey *et al.*, 2004).

Enzymes and resting cells were suggested in order to achieve high selectivity and substrate yield with few by-products (Ma *et al.*, 2006). Moreover, in recent years, researchers have focused on optimization and scale-up problems (Zhang *et al.*, 2006).

Although the number of the areas, which they are applied to, is increasing, the influence of biotechnological methods on production is still restricted due to the limitations on recovery of products. Besides this, the separation of carboxylic acids from their dilute solutions still keeps its challenge. The conventional method of recovering low-volatile carboxylic acids from fermentation broths involves the formation of the calcium sulphate, which causes a major disposal problem for the environment. Calcium salt of carboxylic acid is formed by the addition of calcium hydroxide into the production medium. It is followed by filtration and addition of sulfuric acid to precipitate calcium sulfate before release of free acid. The dilute solution of carboxylic acid is then purified by activated carbon, evaporation and crystallization. The traditional methods, which were used to recover lactic and citric acids in the industry, should be replaced by novel techniques because of their high energy requirements and complexity. Several recovery approaches were attempted for the separation of carboxylic acids from their aqueous solutions. Some examples are:

- Ion Exchange Chromatography (Wang and Liao, 2004; Gao *et al.*, 2009)
- Adsorption (Huang *et al.*, 2007; Husson *et al.*, 1999)
- Capillary Zone Electrophoresis (Wang and Liao, 2005; Mato *et al.*, 2006)
- Electrodialysis (Siebold *et al.*, 1995; Zelic *et al.*, 2005; Biwer *et al.*, 2005)
- Anion Exchange (Peldszus *et al.*, 1996; Mancini *et al.*, 2000)
- Activated Carbon (Tedetti *et al.*, 2006)
- Liquid-Liquid Extraction (Biwer *et al.*, 2005)
- Membrane Separation (Moueddeb *et al.*, 1996; Juang *et al.*, 1997)
- Reactive Extraction (Tamada *et al.*, 1990; Wasewar *et al.*, 2002)

Among these techniques, 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 (Jarvinen *et al.*, 2000). Reactive extraction is a solvent or liquid-liquid extraction system with a chemical reaction that occurs between the extracted solute and the extractant present in the organic phase. The process is named as complex extraction (Ma *et al.*, 2006) as well as dissociation extraction (Malmary *et al.*, 2000, Doraiswamy, 2001) by some researchers.

Reactive extraction involves two stages: forward and backward extraction steps with one organic and two aqueous phases. The first aqueous phase, which consists of carboxylic acids, may simulate a biological production media or wastewater stream including the product to be removed. The organic phase includes an extractant and a diluent, where extractant is dissolved. In order to enhance the extraction power of the solvent phase, a modifier can be added to the organic phase. As can be seen in Figure 1.2, in the first stage, the carboxylic acid is transferred by the organic phase from the aqueous phase, and the extracted acid reacts with the extractant to form an acid-amine complex in the organic phase. In the second stage, the acid is stripped from the organic phase to the second aqueous phase. An important advantage of reactive extraction is the reusability of the extractant after releasing the acid. In the applications, the extractant is designed to be recycled to the production media and utilized for forward extraction again while carboxylic acid is back-extracted to the second aqueous phase. At the end of the process, aqueous solution of free or salt form of carboxylic acid is obtained depending on the type of back extraction method or stripping agent that is used.

In biological production systems, product inhibition occurs by the accumulation of the carboxylic acids in the production media. The reduction of the pH with the accumulation of the acid increases the inhibitory effect and toxicity on the microorganisms. It is well known that

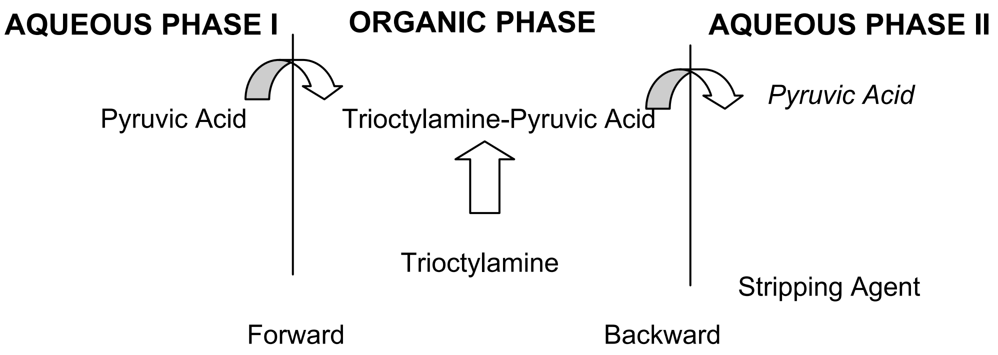

Figure 1.2 Schematic representation of the separation of pyruvate, from the fermentation broth to a second aqueous phase.

product inhibition prevents high yields. This problem can be solved by removing the product periodically from the production media. Therefore, the final product should be recovered *in situ* after reaching a level of concentration in order to eliminate product inhibition. Reactive extraction using appropriate extractants dissolved in appropriate diluents is a good choice to achieve the removal of the target product from the production media. This recovery can be performed in the production unit or in another separation unit coupled to the production media (Figure 1.3).

In a reactive extraction process, the purpose should be achieving a high distribution coefficient (K_D) with a good selectivity. This can be realized by utilizing an appropriate organic phase at optimum conditions. Kertes and King (1986) categorize the extractants as three major types:

- Conventional oxygen-bearing hydrocarbon extractants (MIBK, octanol, etc.)
- Phosphorus-bonded oxygen-bearing extractants (tributyl phosphate, etc.)

- High molecular weight aliphatic amines (Aliquat, Alamine, Trioctylamine)

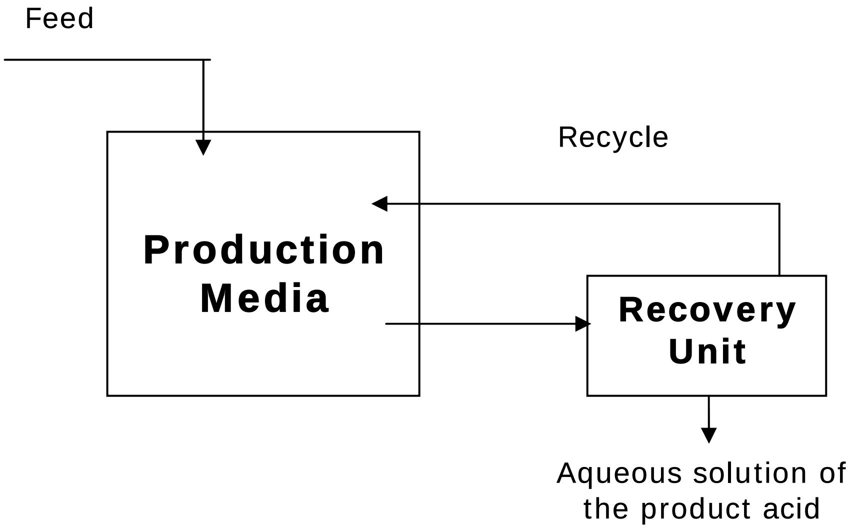

Figure 1.3 Schematic representation of a coupled production and recovery system

The first two categories are nonreactive solvents and extract the solute by forming donor bonds. In the literature, conventional extraction systems using water-immiscible solvents were proved to be inefficient for the recovery of carboxylic acids from their dilute solutions. On the other hand, the extractants in the third category, high molecular weight aliphatic amines, can react with the carboxylic acid and form acid-amine complexes. This causes a significant increase in the distribution coefficients of the carboxylic acids. (Wardell and King, 1978). Among aliphatic amines, primary alkyl amines were observed to be soluble in water while secondary amines caused the formation of a gel phase at the interface in some applications (Wennersten, 1983). Aliphatic tertiary amines, except for the ones with less than six carbon atoms per chain, were proposed as effective extractants at the extraction of carboxylic acids (Kertes and King, 1986).

The stability of the acid-amine complex is affected by the basicity of the amine and can be manipulated by using different types of diluents. Besides this, previous studies revealed that aliphatic amines dissolved in proper diluents yielded higher distribution coefficients than their pure form for lactic acid (San Martin *et al.*, 1996). Moreover, it is beneficial to use a diluent to control the viscosity. It is well known that, compared to the other diluents, polar diluents, i.e. alcohols, ketones, and ethers have higher solvation for the complex formed by the solute and extractant in the organic phase. Due to their ability to accept and donate protons, especially alcohols give the highest partition coefficients.

The pH of the solution is an important parameter for biological production and recovery systems. Selection of the pH is very critical for a production process because, as mentioned earlier, the decrease in pH value with the increase in the amount of carboxylic acid may be harmful to the microorganism producing the product. On the other hand, organic phase to be used for the extraction of carboxylic acids should be capable of extracting the product at the pH of the production media and should not be toxic to the microorganisms besides the necessity of yielding high distribution coefficients with a selective separation (Tong *et al.*, 1998).

It was stated that some organic phases are partially soluble in the aqueous phase which is undesirable because of causing problems during the recovery of the target product. The organic phase should be essentially water immiscible. Another problem observed during the reactive extraction is the formation of a third emulsion phase, which is generally seen during extraction with high molecular weight solvents. A significant amount of acid is kept by this emulsion phase.

Bart (2001) states that, for the design of reactive extraction unit, which is to be operated to recover carboxylic acids, initially equilibrium and kinetic data, should be obtained. This will be followed by the studies with the model equipment. In recent years, there is an increase in studies on recovery of carboxylic acids by reactive extraction.

In the present study, it was aimed to optimize the process conditions for the design of an industrial forward reactive extraction process for the recovery of pyruvic acid from a solution which has an equivalent carboxylic acid composition of a biological production medium containing pyruvic, lactic and acetic acids. Firstly, forward extraction experiments were carried out to examine the equilibrium conditions for single acid solutions of pyruvic, acetic and lactic acids. The organic phases with varying concentrations of extractants were contacted with the aqueous phases with varying concentrations at different initial pH values. These equilibrium experiments were followed by forward extraction experiments with a solution which has an equivalent carboxylic acid composition of a biological production media to observe the equilibrium conditions for a mixed acid solution. Finally, kinetics of reactive extraction of pyruvic acid by using trioctylamine dissolved in 1-octanol was studied. In this study, 1-octanol and oleyl alcohol were chosen as organic diluents. 1-octanol was chosen because of its capability of high solubility of complex (Wasewar *et al.*, 2004), whereas oleyl alcohol was selected because of its advantage of being a nontoxic solvent to many carboxylic acid producing microorganisms (Gu *et al.*, 1998). Tertiary amine extractants, trioctylamine and Alamine 336, were selected due to their successful usage for the extraction of mono-, di- and tricarboxylic acids (Tamada *et al.*, 1990; Siebold *et al.*, 1995; Poposka *et al.*, 1998; Canari and Eyal, 2003b & 2003c; Wasewar *et al.*, 2004). While loading ratios and distribution coefficients were obtained through equilibrium studies, reaction rates and orders were calculated with the kinetic data using a kinetic model proposed by Doraiswamy and Sharma (1984).

CHAPTER 2

LITERATURE SURVEY

Life science application of extraction dates back to about 3500 BC in order to produce products from various natural resources. At a Sumerian text dated 2100 BC, production of perfumes, pharmaceutical oils and waxes were documented. In medieval ages extraction was performed with ethanol and was applied in the field of hydrometallurgy by making use of mineral acids (Bart 2001). With the developments in thermodynamics, particularly the distribution law by Nernst in 1891 and design of an apparatus for extraction, significant improvements were accomplished in the late 19th century. Pfleidere (1898) patented the first liquid-liquid extraction column.

Metal salt extraction was started with uranium production during the Manhattan Project in 1940 and was followed by the extraction of vanadium, zirconium, hafnium, niobium, and tantalum (Bart 2001). Nowadays, besides the extraction of almost all metals in mining industries and environmental applications, extractants are widely used in the extraction of organic and inorganic acids, organic chemistry intermediates, and pharmaceuticals for the purposes of separation, purification or enrichment (Marr and Bart 1982).

Distribution coefficient and loading ratio are the two main parameters used to evaluate the extent of extraction. As shown in Equation E2.1, the ratio of the concentration of all forms of the solute in the organic phase to that in the aqueous phase gives the distribution coefficient. Loading ratio is the ratio of concentration of the solute that reacts with the extractant in the organic phase to the initial concentration of the extractant (Equation E2.2).

$$K_D = \frac{[HA_t]_{org}}{[HA_t]_{aq}} \qquad \text{(E2.1)}$$

$$z = \frac{[HA_{ex}]_{org}}{[TA]_{org,0}} \qquad \text{(E2.2)}$$

HA_t denotes all acid molecules including dissociated or undissociated, while HA_{ex} denotes the undissociated acid molecules reacting with the amine, which is present in the organic phase. TA denotes the tertiary amine molecules.

The main difference between reactive extraction and solvent extraction is the reaction between the extractant and the solute in the organic phase. Aliphatic amines and phosphoryl solvents were proposed as effective extractants by earlier researchers (Kertes and King, 1986). While extractants play the major role in the reaction, diluents also have a significant effect on the level of extraction. Non-aromatic, water-immiscible and polar solvents with intermediate molecular weights and high boiling points are commonly preferred for the extraction to have high distribution and selectivity (Holten 1971). Besides controlling viscosity and density of the solvent phase, the solvents affect the stability of the complex structure formed by the solute and the extractant.

2.1. Equilibrium Studies on Reactive Extraction of Carboxylic Acids

Several authors (Kertes and King, 1986, Tamada *et al.*, 1990a, Juang and Huang, 1997, Tong *et al.*, 1998, Wasewar *et al.*, 2002a, Keshav *et al.*, 2008a) have studied the recovery of carboxylic acids from their aqueous solutions by reactive extraction. These studies were on optimization of equilibrium conditions of the extraction systems, and the investigators examined the effects of the parameters on the distribution of the solutes between the phases. Some of these parameters were concentration and composition of the phases, types of the extractants and diluents, pH of the aqueous phase, temperature of the system, and toxicity of the solvent phase to the microorganism (Kertes and King, 1986, Tamada *et al.*, 1990a, Gu *et al.*, 1998, Tong *et al.*, 1998, Ma *et al.*, 2006). In addition to these experimental studies, possible reaction mechanisms were investigated using certain equilibrium and kinetic models (Poposka *et al.*, 1995, Wasewar *et al.* 2002a). Some experimental results showed synergistic and antagonistic effects on the extraction of the carboxylic acids when there was more than one acid in the aqueous phase or more than one extractant in the organic phase (Juang and Huang, 1997, Kirsch and Maurer, 1997 Canari and Eyal, 2003a). Some researchers tried to recover the carboxylic acids from production medium by extractive fermentation and investigated the process conditions affecting recovery during production (Siebold *et al.*, 1995, Tong *et al.*, 1998, Gu *et al.*, 1998, Ma *et al.*, 2006).

King and his group performed the pioneering studies on the equilibrium of reactive extraction of carboxylic acids. Besides carboxylic acids, they studied the extraction of chlorinated hydrocarbons and aromatics (Barbari and King, 1982), ethanol (Munson and King, 1984), ammonia (Mackenzie and King, 1985), and low molecular weight aliphatic alcohols (Kertes and King, 1987) from aqueous solutions.

In one of their earlier work, King and co-workers studied the extraction of acetic and formic acids from their aqueous solutions (Wardell and King, 1978). They used phosphoryl solvents - tributyl phosphate (TBP), dibutyl phosphonate (DBP), tributylphosphinoxide (TBPO) and triphenylphosphineoxide (TPPO) - and tertiary amine extractants - trioctylamine (TOA) and tri-iso-octylamine (TIOA) – as extractants. The extractants were dissolved in solvents and solvent mixtures of Chevron

25, chloroform, nitrobenzene, *n*-hexanol and *n*-heptane. The researchers aimed to reach high distribution coefficients to decrease the volume ratios of the organic and aqueous phases for the extraction process.

As a result of high polarity of the phosphoryl bond (P-O), which permits it act as a Lewis base, high distribution coefficients were achieved with phosphoryl compounds. The distribution coefficients obtained with phosphoryl compounds were in the decreasing order of phosphine oxide > phosphonate > phosphate. Among phosphine oxides, higher distribution coefficient values were obtained when tributyl and trioctylphosphine oxides were employed instead of the corresponding triphenyl compounds. The results indicated that with the increase in electronegativity, a decrease in the electron-donating ability and disappearance of Lewis basicity were observed.

The investigators reported the advantage of using long chain amines as extractants in the recovery of carboxylic acids. These extractants can form ammonium salts by reacting with carboxylic acids. It was found that distribution coefficients were not different for trioctylamine and tri-iso-octylamine. However, it was higher than that of tributylamine because of having a higher solubility of the acid-amine complex in the organic phase. The presence of the phenyl substituent caused a lower distribution value with triphenylamine. This group lowered the Lewis basicity, hence the distribution coefficient of the solute (Wardell and King, 1978).

When the distribution coefficients obtained with phosphoryl and conventional solvents were compared with those attained with trioctylamine, the advantage of using tertiary amines as extractant was clearly seen. It was observed that molecular weight did not cause any significant difference in the distribution coefficient values for phosphoryl solvents and high molecular weight amines. Moreover, Wardell and King (1978) stated that the extent of the extraction of acetic acid appeared to increase with an increase in the solubility parameter of the diluent besides the polarity. It was understood that both of the properties of the diluent affect the basicity of the amine and the distribution of the solute.

The first study of the King group was followed by that of Kertes and King (1986) which is a research report as well as a review. In this work, the authors reported the improvements in fermentation technology and the needs of commercialization. A chemistry background to extraction technology was provided to serve as a guide for researchers. The chemicals used in the literature for the extraction of carboxylic acids were classified by the authors as stated in the previous chapter. The researchers intended to improve the recovery technology of fermentation products and carried out the extraction of propionic, pyruvic, lactic, succinic, fumaric, maleic, itaconic, tartaric, citric and isocitric acids, which were obtained by aerobic fermentation of glucose via the glycolytic pathway and glyoxylate bypass.

They claimed that the most important variables in the recovery of the carboxylic acids are the nature of the acid extracted, the concentration of extractant, and the type of the diluent. The authors re-emphasized the ineffectuality of conventional solvents for the extraction of carboxylic acids and the importance of the development of novel and more powerful extractive agents. The investigators pointed out that the extracted acid into carbon-bonded and phosphorus-bonded solvent is the undissociated monomeric acid, which means that initial pH of the aqueous solution and the pK_A of the acid are very important in the extraction of corresponding acid. They added that the reaction that occurs between the carboxylic acid and the high molecular weight aliphatic amine is a kind of acid-amine reaction and takes place by ion pair formation. If the carbon-bonded oxygen donor or phosphorus-bonded oxygen donor extractants are used as extractant, extraction proceeds by solvation of the acid by donor bonds (Kertes and King, 1986).

Mass Action Law and Nernst Distribution Law were used in order to evaluate the data. The authors considered the dimerization of acids in the organic phase and reported the partition ratios of some acids as following by ranging from 0.003 for aliphatic hydrocarbons to 2-3 for aliphatic alcohols and ketones and to 10 or more for organophosphates. Similar as phosphorus solvents or higher values were obtained when long-chain tertiary amines were employed as extractants.

The advantage of using aliphatic tertiary amines with six carbon atoms per chain instead of primary and secondary amines, which were examined in their previous work, was reported in the review paper. In particular, monocarboxylic acids under comparable conditions were noted to be more easily extracted with an appropriate organic phase than di- or tricarboxylic acids (Kertes and King 1986).

Mutual solubility of the phases was observed as an important phenomenon in the extraction of carboxylic acids. The extent of this was explained in terms of the nature and the total concentration of the component in the phases. Weak carboxylic acids at high concentrations caused dramatic volume increases in the organic phase.

As stated earlier, diluents strongly affect the basicity of the amines, and consequently solvation and stability of the ion pair formed. The researchers mentioned that the stability of the ion pair formed is the determining factor in establishing the equilibrium conditions, particularly at low values of the loading ratio and aqueous phase concentrations. Due to this reason, polar diluents were preferable to aliphatic and aromatic hydrocarbons which have lower polarity and lower dielectric constant.

The King group continued their studies with a trilogy (1990). First, they studied the extraction of carboxylic acids with different pK_A values. Furthermore, they searched for the effect of functional groups other than the primary carboxyl group on extraction. They examined reactive extraction of acetic, lactic, succinic, malonic, fumaric and maleic acids using Alamine 336, which is a commercially available tertiary aliphatic amine extractant, dissolved in various diluents. Alamine 336, which is a mixture of trioctyl-, tridecyl- and trinanylamine with a molecular weight of 352 grams per mole, has been used (Rice and Ikwue, 1990; Shillington and Tait, 1991, Sastre *et al.*, 2000; Guibal *et al.*, 2003; Jyothi *et al.*, 2009) for the extraction of metals from their aqueous solutions. The diluents were chosen with different chemical characteristics: electron donating, electron accepting, polar and non-polar. Active diluents (1-octanol, methylene chloride, chloroform, methyl isobutyl ketone (MIBK), nitrobenzene) and an inert diluent, n-heptane, were utilized in order to observe the effects of various parameters and diluent-complex interactions on the equilibrium conditions.

The authors mentioned the possible formation of complexes with more than one mole of acid per mole of amine, which was particularly common for monocarboxylic acids. The ratio of (1, 1) to (2, 1) complex formation was diluent dependent with respect to the results. It was seen that halogenated hydrocarbons and alcohols inhibited overloading, while ketones enhanced it. The authors stated that the total amine concentration had no effect on the loading ratio for inert diluents. That was valid except for low acid concentrations. In this range, the loading ratio increased with an increase in the amine concentration. They added that at a given aqueous phase concentration of the acid, loading decreased with an increase in the total amine concentration at higher amine concentrations with active diluents.

The researchers indicated that solubility of the acid-amine complex in the solvent phase was decreased in the following order: alcohol ≥ nitrobenzene ≥ proton donating halogenated hydrocarbon > ketone > halogenated aromatic > benzene > alkyl aromatic > aromatic hydrocarbon. The authors explained that if the diluent has poor solvation power, polar complexes tend to cluster and separate from the bulk solvent. As a result of this, they observed aggregation with inert diluents.

They studied the effect of pK_A of the corresponding acid on the extent of extraction. They found out that the higher the pK_A value of the acid, the more it was extracted. The final pH values of the aqueous phases were compared with the values obtained by the calculation of the concentrations of the acids. The results revealed that the values experimentally determined were up to one pH unit higher than the expected values, and the difference was found to increase with an increase in total amine concentration and a decrease in the initial acid concentration.

In the second study of trilogy, Tamada *et al.* (1990b) investigated the chemical interactions between the components by using the results of mass action law analysis and the spectroscopic studies. Infrared spectroscopy of organic phase was performed to examine the stoichiometry of the acid-amine complex. They proved their proposal by these studies and emphasized that there is an ion pair formation between the amine and first acid molecule. The authors added that there is a hydrogen bond formation between the carboxyl of the second acid molecule and the carboxylate of the first when stoichiometry of the complex is (2,1) acid-amine.

The investigators proposed that the magnitude of (1,1) equilibrium constant is related to the pK_A value of the acid and for each unit decrease in pK_A of the acid, there is approximately one order of magnitude increase in the equilibrium constant. The authors noted that the ratio K_{21} / K_{11} decreased in the same ratio as the pK_A of the acids studied. The effects of hydrophobicity and acidity of the acids on the distribution coefficients were discussed. It was observed that acidity played a more important role than hydrophobicity. Spectroscopic studies showed that the tendency to overload is less for proton-donating diluents while there is a tendency to form aggregation for polar complexes in inert diluents as stated in the previous study (Kertes and King, 1990a).

In the last part of the trilogy, the King group measured the co-extraction of water with the acids by Alamine 336 dissolved in various diluents and they concluded that co-extraction of water was amine independent (Tamada *et al.*, 1990c). Water co-extraction was decreased in the order of 1-octanol > MIBK > nitrobenzene > methylene chloride > chloroform > heptane during the extraction of succinic acid. Co-extraction of water for different acids was compared. The results revealed that water co-extraction with monocarboxylic acids were much less than that with dicarboxylic acids.

The researchers investigated the effect of the temperature on the reactive extraction of carboxylic acid by Alamine 336. It was observed that the distribution coefficient decreased with the increase in the temperature of the system. They proposed that with the formation of the complex, the system became more ordered and entropy decreased. Consequently, the amount of acid extracted decreased with the increase in temperature.

Finally King and his co-workers managed to regenerate the acids from organic phase to aqueous phase through back-extraction by two approaches, swing temperature and swing diluent methods. They gave brief information about the two methods and suggested the use of both methods in combination to achieve a good recovery (Tamada *et al.*, 1990c).

Yang *et al.* (1991) studied the extraction of acetic, propionic, lactic and butyric acids with tertiary and quaternary amines at different pH values. It was noted that Aliquat 336, which is a quaternary amine salt, could extract both dissociated and undissociated forms of the acids. Hence, it was not affected by the pH of the aqueous solution like Alamine 336. As mentioned earlier, secondary and tertiary amines (Alamine 336, trioctylamine) can only extract undissociated portion of the acids. Therefore, the authors verified the advantage of using Aliquat 336 instead of Alamine 336 specifically under basic conditions. On the other hand, they stated that extractant regeneration with Aliquat 336 was very difficult, while Alamine 336 was easily regenerated with an alkaline solution. The researchers concluded that selection of the extractant should be based on the pH range of the aqueous solution of the acids and reusability of the extractant. The effect of the diluents, 2-octanol and kerosene was investigated. As explained earlier in this chapter, Alamine 336 gave high distribution ratios with polar diluents, while Aliquat 336 was not affected by the polarity of the diluent. The authors indicated that optimum pH value of the fermentation broth for the recovery of lactic acid from the fermentation broth was less than the pK_A of lactic acid. Two types of aqueous solution were used for the back extraction process. An alkaline solution was suggested as well as aqueous solution of trimethylamine for back extraction. Finally, the authors modeled the pH dependency of distribution coefficient using a three-parameter equation for high and low pH ranges.

Siebold *et al.* (1995) contributed to the literature by producing lactic acid by three different Lactobacilli and performing its recovery by electrodialysis and extractive fermentation. Four different amine extractants in kerosene, butyl acetate and Cyanex 923 (phosphinoxide extractant) with modifiers (long-chain alcohols, alkylphosphates and acidic organic compounds) were utilized to extract lactic acid from the production medium. The biocompatibility of kerosene and oleyl alcohol was noted by the investigators. They were not able to achieve the complete re-extraction of lactic acid from organic phase. The authors performed the back extraction of lactic acid with HCl or H_2SO_4, but added that this would not be economical. Like previous researchers, Siebold *et al.* (1995) explained that loading ratio was independent of amine concentration; while it was affected by the concentration of the modifier.

San Martin *et al.* (1996) studied the extraction of lactic acid from its aqueous solutions with Alamine 336 dissolved in toluene. Their experimental results showed the overloading and three different types

((1,1), (2,1), (3,1)) of acid-amine species in the organic phase. The authors investigated the effect of temperature on the loading and found that loading decreased with the increase in temperature. It was proved by the authors that, aliphatic amines diluted in appropriate solvents yielded higher distribution coefficients of lactic acid than their pure form (San Martin *et al.*, 1996). The complexation constant was calculated by the mass action law in a similar manner carried out by the King's group.

Juang and Huang (1997) studied the recovery of lactic acid with tri-n-octylamine (TOA) and/or D2EHPA in xylene in the temperature range 293-323 K. Like other researchers, they used mass action law to determine the stoichiometry of the reaction between the extractant and the solute. It was stated that the dominant species formed in the organic phase was (1,1) at low initial aqueous solution concentrations, whereas (3,1) at high aqueous phase concentrations. In addition to this, the authors noted the enhancing effect of D2EHPA on the distribution coefficient of lactic acid. They explained that organophosphorus compounds can be used as phase-modifiers in liquid-liquid extraction systems, and enhance the solubility of the acid-amine complex in the organic phase resulting in high distribution coefficient values. Juang *et al.* (1997) compared the supported liquid membrane method and reactive extraction for the recovery of citric and lactic acid from their mixed acid solutions. This study is further explained in Section 2.2.

In another important study, Tong *et al.* (1998) performed the production and recovery of lactic acid by extractive fermentation. They used a number of extractants dissolved in organic diluents at a wide range of pH of the aqueous phase. Their results revealed that tri-n-octylmethylammonium chloride (TOMAC), a quaternary amine salt and an anion-exchange extractant, dissolved in oleyl alcohol, butyl acetate, and a mixture of hexane and oleyl alcohol was the most suitable organic phases for the extraction of lactic acid in terms of high distribution coefficients as well as the simplicity of operation. Toxicity studies showed that TOMAC, hexane and butyl acetate were very harmful to the lactic acid producing bacteria, whereas oleyl alcohol had no detrimental effect. Thus, oleyl alcohol was considered as the most appropriate diluent for the recovery of lactic acid from production media. In spite of a small decrease in the production of lactic acid during the control run, extractive fermentation was achieved successfully with TOMAC dissolved in oleyl alcohol (Tong *et al.*, 1998).

Gu *et al.* (1998) studied the extractive fermentation of propionic acid using Alamine 304-2 (triaurylamine) in 2-octanol, 1-dodecanol and Witcohol 85 NF (oleyl alcohol). Although 2-octanol yielded the highest distribution coefficients among the diluents studied, due to its toxicity to propionibacteria, it was not considered as the most appropriate solvent. Solvent toxicity was overcome by two strategies:

1) By capturing the dissolved toxic solvent by vegetable oils.
2) By using Witcohol 85 NF (oleyl alcohol) instead of 2-octanol.

They found that optimum concentration of the extractant to achieve a high distribution coefficient was between 30 and 40 %. Lastly, they recovered the acids from the organic phase by vacuum distillation.

Hong and Hong (1999, 2000) examined the extraction of lactic and succinic acids using 1-octanol/*n*-heptane solutions of mixed tertiary amines in two different studies. In the first of these, they studied the lactic acid recovery with tripropylamine (TPA) and trioctylamine (TOA) at different weight ratios. They achieved highest distribution coefficient values with the ratio of 1.5 and 4 for lactic acid. After this study, the authors (2000) studied the extraction of succinic acid using 1-octanol/*n*-heptane solutions of mixed acid tertiary amines. They reached extraction extents above 90 % with a TPA/TOA ratio of 4. They achieved to overcome the third phase formation with the addition of TPA.

Jarvinen *et al.* (2000) studied the recovery of lactic acid from fermentation broth by extractive fermentation. They utilized tertiary amines as extractant, decanol as diluent and aqueous solution of trimethylamine (TMA) as the stripping solution. The optimum pH value for the forward reactive extraction process was found to be 2.2. The authors compared sulfuric acid with a cation exchanger (Dowex) for the acidification of the aqueous phase and noted the advantages of cation exchanger. Sulfuric acid was observed to compete with lactic acid, which was extracted to the organic phase. The investigators utilized the McCabe-Thiele procedure in order to observe the effect of the acidification on the estimation of the number of equilibrium stages needed to achieve 90% level of extraction. The results revealed that the number of equilibrium stages required was reduced by two with the acidification by ion exchange. They finally investigated the effect of acidification on the purity of the product. After re-extracting lactic acid by TMA solution, TMA-lactic acid complex in the aqueous phase was decomposed by evaporation. It was seen that the cations co-extracted with lactic acid were not detected in the case of acidification by ion exchange, which increases the purity.

Schügerl reviewed (2000) the techniques used for the recovery of alcohols, carboxylic acids, amino acids, antibiotics and proteins. Adsorption, pervaporation, extraction and electrodialysis were compared by presenting examples from the literature. He mentioned that because of the limited capacity of the adsorbents, adsorption can not be competitive with extraction and pervaporation. He claimed that the inhibitory effect of the product to the microorganism is due to the accumulation of the carboxylic acid and the decrease in the pH value in the production media. He mentioned that in the literature there are some methods checked in order to achieve a successful recovery without harming the microorganism producing the carboxylic acids: “contacting the immobilized cells with the extraction solvent, extraction with the solvent separated from the cells by a membrane, use of solid-supported liquid membrane extraction, aqueous two phase extraction, an extraction after conversion of the acid into a hydrophobic compound with a high solubility in the solvent” (Schügerl, 2000). He stated the advantage of using oleyl alcohol due to its non-toxicity. He explained that because the back extraction of free acid is very difficult, elevated pH values are preferable in order to obtain the salt form of the carboxylic acid. He also pointed out the successful use of tertiary amines; however he added that the production of the carboxylic acids declined owing to the toxicity of the tertiary amines. In order to prevent the microorganism, the combined use of electrodialysis and solvent extraction was proposed.

Senol (2001, 2006) studied the distribution of nicotinic acid and pyruvic acid using Alamine 336 dissolved in various (proton-donating, proton-accepting, polar, non-polar) conventional diluents to examine the effect of the solvent. He achieved the highest distribution coefficients with cyclic alcohol for both of the acids. The author investigated the effect of the functional groups on the extraction of carboxylic acids by extracting valeric, benzoic, formic, levulinic, acetic and nicotinic acids. It was concluded that the hydrophobicity of the acid has a very important enhancing role on the distribution of the corresponding acid (Senol, 2001).

Wasewar *et al.* (2002a, 2002b) extracted lactic acid by using Alamine 336 dissolved in methyl-*iso*-butyl-ketone (MIBK) and decanol in two different studies. The authors observed the formation of two acid-amine complexes with stoichiometries (1-1) and (2-1). Moreover they calculated the equilibrium complexation constants (Wasewar *et al.*, 2002a, Wasewar *et al.*, 2002b). They conducted the pioneering studies on the kinetics of reactive extraction of carboxylic acids using the theory of extraction accompanied by a chemical reaction, proposed by Doraiswamy and Sharma (1984). The conclusions of their kinetic studies are presented in Section 2.3.

Matsumoto *et al.* (2004) investigated how the extraction behavior and solvent toxicity were affected by the replacement of the conventional solvents with the ionic liquids, which are proposed as alternative solvents to the organic diluents used in liquid-liquid extraction systems. They recovered the carboxylic acids by *in situ* extractive fermentation with the ionic liquids. They performed back extraction with 2 M NaOH solution and analyzed for the concentration of the two aqueous phases with HPLC. The distribution coefficients of the acids with the imidazolium-based ionic liquids were low. It was seen that glycolic acid, which is the most hydrophilic one among acetic, glycolic, propionic, lactic, pyruvic and butyric acids, could not be removed from the aqueous phase. Hence, the researchers evaluated the use of ionic liquids as diluents with the extractants utilized for the reactive extraction of carboxylic acids in the literature. Tri-n-butylphosphate (TBP), tri-n-octylphosphine oxide and tri-n-octylamine were examined with the ionic liquids. Satisfying results were only obtained with TBP. Extractability with TOPO was poor, while TOA was not miscible with the ionic liquids. The toxicity of imidazolium-based ionic liquids to the microorganism producing carboxylic acids was evaluated. It was found that the microorganism continued to grow and consumed glucose in order to produce lactic acid in the presence of the ionic liquids. Although there is no significance difference between the distribution coefficient values, Hmim was suggested as the best diluent among the ionic liquids studied.

Inci *et al*. (2005a, 2005b) studied reactive extraction of gluconic and citric acids with TOMAC dissolved in various diluents. The investigators indicated that due to the chloride ion, the quaternary amine served as an anion exchange reagent while extracting the solute and polarity of diluents did not affect the distribution coefficient of the gluconic acid. Methyl isobutyl ketone (MIBK) was found to be a most suitable solvent for the removal of gluconic acid with TOMAC (Inci *et al*., 2005a). Besides MIBK, 2,2,4-trimethyl pentane was noted to be very effective in the recovery of citric acid (Inci *et al*., 2005b).

Uslu (2007) studied reactive extraction of tartaric acid with Alamine 336 dissolved in organic diluents. Highest and lowest distribution coefficient values were obtained with MIBK and hexane respectively. The author noted that for the diluents, only (1-1) acid-amine complex was observed, concentration of the extractant had no effect on the loading ratio. However loading ratio increased with the increase in the concentration of Alamine 336 when more than one amine molecule was observed in the acid-amine complex. The investigator finally modeled the distribution coefficient value of tartaric acid with the diluents by using the chemical properties of the solvents and linear solvation energy relationship (LSER), which was proposed by Kamlet *et al*. (1983).

Uslu *et al*. (2007) conducted reactive extraction of citric acid by quaternary amine in active diluents. They achieved highest distribution coefficient values with 1-propanol. Their results showed that extraction power of the diluents decreased with the increase in the number of the carbon atoms of the solvents. The investigators used the solvatochromic parameters of the solvents in order to correlate the distribution coefficient values using LSER model.

Uslu and Kirbaslar (2008) extracted levulinic acid with trioctylamine dissolved in five alcohols and two ketones. They stated that distribution coefficient values less than one were obtained with the conventional solvents. The positive effect of the extractant was observed on the transfer of the solute and distribution coefficient value of the acid increased with the increase in the concentration of the extractant in the organic phase. Isoamyl alcohol was noted to be the most effective diluent among the solvents studied. The distribution coefficient value was observed to decrease with the increase in the number of the carbon atoms in the solvent structure. The formation of (1-1) and (2-1) acid-amine complexes were estimated in the organic phases and the complexation constants were calculated. The investigators mentioned that the distribution coefficient values obtained using LSER method were close to the ones experimentally obtained.

Keshav *et al.* (2008a, 2008b) performed a series of studies on reactive extraction of propionic acid using the extractants TOA, TBP, TOPO, and Aliquat 336. The authors found that when TOPO was used as the extractant, hexane was the best diluent among n-heptane, light liquid paraffin, heavy liquid paraffin and hexane (Keshav *et al.*, 2008a). Then, they compared the 1-octanol solution of the extractants TOA, TBP and Aliquat 336, and found the highest distribution coefficient values with TOA (Keshav *et al.*, 2008b). They searched for the most appropriate solvent with trioctylamine for the reactive extraction of propionic acid with trioctylamine and observed that oleyl alcohol yielded the highest distribution coefficient value when compared with the diluents *n*-heptane, petroleum ether, and ethyl acetate.

In conclusion, although there are several equilibrium studies on reactive extraction of carboxylic acids using different kinds of extractants dissolved in various diluents, there are a few studies on the reactive extraction of pyruvic acid with tertiary amines dissolved in alcohols. Alcohols were chosen due to their higher polarity among various solvents. Tertiary amines were chosen due to several reasons. The primary reason is their potential in providing a high distribution coefficient value for carboxylic acids. Another reason is the ease of regeneration of the extractant. Additionally, since they react only with the undissociated portion of the acid, pH variation in the aqueous phase can be utilized as a parameter for the selective separation of the carboxylic acids from their mixed acid solutions.

2.2. Equilibrium Studies on Reactive Extraction of Mixed Acids

It is well known that biological production media of carboxylic acids generally consist of more than one type of acid. For the recovery of these acids selectively, some investigators performed equilibrium studies on the reactive extraction of mixed acids. Purification of the species in the production medium is often necessary to obtain a product of certain specifications demanded by the market. In addition to this, by-product acids can be recovered selectively and can also be marketed as product. Unconverted substrate can be recycled to the production media after the separation of the product acids.

Juang and Huang (1997) carried out one of the first studies on the reactive extraction of mixed acids. Citric and lactic acid were attempted to be extracted with tri-*n*-octylamine (TOA) selectively from their mixed solutions. In the study, the researchers compared the performance of supported liquid membranes with reactive extraction considering total acid concentration, concentration ratio of the acids, and the concentration of extractant as parameters. Their results showed that equilibrium distribution of each acid is strongly affected by the presence of the other acid. Synergistic and antagonistic effects were detected on the distribution of the acids depending on the total acid concentration and their ratios. It was seen that the existence of the second acid enhanced the transfer of citric acid whereas hindered the transfer of lactic acid (Juang and Huang, 1997).

Kirsch and Maurer (1997, 1998a, 1998b) conducted a series of studies on the distribution of binary mixtures of citric, acetic and oxalic acids using trioctylamine in diluents, toluene, MIBK and chloroform. Their model was in good agreement with the distribution ratios of citric and oxalic acids which were found experimentally. When acetic acid was extracted together with one of the other acids, distribution coefficient values for acetic acid were higher than the ones predicted. It was deduced that acetic acid was bound very strongly to the amine compared to the other two acids.

Canari and Eyal (2003a) studied the effect of pH on selective extraction of lactic, malic, glutaric, and maleic acids from their binary solutions with a C_{18}-C_{22} primary amine, Primene JMT dissolved in 1-octanol. They noticed that selectivity was strongly dependent on the pH of the aqueous solution. For example, one acid could be extracted easily at low pH values, whereas another one at higher pH values. The authors noted that distribution of the acids in a mixed acid solution was seen to be quite different from the distribution of the same acid when it is alone in the initial aqueous phase (Canari and Eyal, 2003a).

Huh *et al.* (2004) studied the selective removal of acetic acid from mixed acid solutions to obtain a pure solution of succinic acid using tri-n-octylamine dissolved in 1-octanol in a multistage batch extractor. The researchers used varying pH to achieve a successful separation. After performing reactive extraction as a primary separation method, crystallization was carried out for further purification of the samples (Huh *et al.*, 2004).

Hong and Hong (2005) studied the removal of acetic acid from mixed aqueous solutions of succinic acid and acetic acid using tri-octylamine under various pH values. As expected, loading ratio was observed to decrease with the increase in the initial pH value of the aqueous solution. Likewise loading ratio, the extent of extraction decreased with the increase in pH of the aqueous phase since tertiary amine could react only with the undissociated form of the acid.

In another study, Huh *et al.* (2006) pointed out the importance of the removal of the contaminants for a successful recovery of succinic acid from the fermentor. They executed extractive fermentation, vacuum distillation and crystallization in combination to achieve a cost-effective complex separation process for the removal of cells and protein-like impurities. Reactive extraction with tri-n-octylamine was applied to remove acetic and pyruvic acids, and salts from the production media.

The researchers accomplished to purify the contaminated solution and obtained pure succinic acid at a pH of 5.0.

A similar study to the present one was carried out by Ma *et al.* (2006). The researchers aimed to separate pyruvic acid from a mixed acid solution of 0.37 M pyruvic acid and 0.09 M lactic acid with tri-n-octylamine dissolved in various diluents by reactive extraction. The effects of concentration of organic phase and initial pH of the aqueous phase on the extraction were investigated. Back extraction of pyruvic acid was performed by using sodium hydroxide (NaOH) and trimethylamine (TMA). The authors verified the effect of the diluent on the alkalinity of the amine and the stability of the acid-amine complex. Like previous researchers, they stated the advantage of using polar diluents, n-octanol and ethyl-acetate, in order to have a selective and effective separation. The authors claimed that lactic acid is more hydrophilic than pyruvic acid because of its additional hydroxyl group, which hinders the transfer of the acid to the organic phase and consequently lowers the distribution coefficient values.

It is seen that there are various studies on reactive extraction of carboxylic acids from mixed acid solutions. Citric, succinic, acetic, lactic and pyruvic acids were extracted from mixed acid solutions to some extent. The results of these studies reveal that the existence of other acids may cause antagonistic or synergistic effects on the distribution coefficient values of the acids. As summarized above in a partially similar study (Ma *et al.*, 2006) to the present one, pyruvic acid with 0.37 M was successfully separated from binary solutions of pyruvic and lactic acids. In the present study pyruvic acid was attempted to be recovered from a ternary acid solution of acetic, lactic and pyruvic acids and binary solutions of lactic and pyruvic acids at various aqueous phase concentrations. In addition to this, in the present study the initial concentration of the pyruvic acid in the mixed acid solution was much higher than that in the study of Ma *et al.* (2006)

2.3. Kinetic Studies on Reactive Extraction of Carboxylic Acids

As stated earlier, kinetic studies are essential as equilibrium studies for the design of a reactive extraction unit for the recovery of carboxylic acids. Although the number of the kinetic studies is increasing in the last decade, the literature is still sparse on the kinetics of reactive extraction.

Poposka *et al.* (1998) studied the kinetics and mechanism of reactive extraction of citric acid with trioctylamine dissolved in isodecanol/n-paraffin solutions. The researchers carried out the kinetic experiments by using a highly agitated system (750 min^{-1}) at 25^0C. They evaluated the kinetic data using two models, a formal elementary kinetic model and a mechanism of two reactions of (1-1) and (1-2) acid-amine complexes. According to the results, the reaction between the acid and the tertiary amine depended on the composition of the organic and aqueous phases. They found that the order of the reaction was 0.73 with respect to the concentration of citric acid in the aqueous phase and first order with respect to the amine concentration. The order of the reverse reaction was found to be first order with respect to the concentration of the acid-amine complex. Poposka *et al.* (2000) continued their kinetic studies on reactive extraction of tartaric acid. Poposka and his friends carried out equilibrium and kinetic studies using tri-*iso*-octylamine (Hostarex A 324) in isodecanol/low aromatic kerosene mixtures. The researchers used the same experimental set-up and model they used in their previous study. Their kinetic results showed that the forward reaction between the components was of the order 0.7 with respect to tartaric acid in the aqueous phase and 1.5 with respect to amine concentration. The backward reaction was of the order 1.0 with respect to acid-amine complex in the organic phase.

Pangarkar and co-workers (Gaidhani *et al.*, 2002, Wasewar *et al.*, 2002a, Wasewar *et al.*, 2002b, Nikhade *et al.*, 2004, Keshav *et al.*, 2008a) performed important studies on kinetics of reactive extraction of carboxylic acids. In one of their first studies, Wasewar *et al.* (2002a) investigated equilibrium and kinetics of reactive extraction of lactic acid using Alamine 336 dissolved in MIBK. This study was followed by a similar one (Wasewar *et al.*, 2002b) with the same acid and extractant, but with a different diluent, decanol. In these studies the authors used a Lewis type stirred cell in order to obtain the kinetic data and theory of extraction accompanied by a chemical reaction in order to interpret the data, which were proposed by Doraiswamy and Sharma (Doraiswamy and Sharma, 1984). According to their results, reaction kinetics was not affected by the volume ratio of the phases and the speed of agitation, which meant that reaction between lactic acid and Alamine 336 in MIBK or decanol was falling in the range of Regime 3, extraction accompanied by a fast chemical reaction occurring in the diffusion film (Wasewar *et al.*, 2002a, 2002b). For both diluent systems, reaction between lactic acid and Alamine 336 was first order with respect to lactic acid. It was seen that there was no effect of concentration of the organic phase and the rate constant was 1.38 s^{-1} for MIBK and 0.21 s^{-1} for decanol.

Gaidhani *et al.* (2002) studied equilibrium and kinetics of reactive extraction of phenyl acetic acid (PAA) by Alamine 336 in different diluents. The results of their equilibrium experiments revealed that kerosene yielded higher distribution coefficient values compared to MIBK. Then they continued their kinetic studies with kerosene. The authors observed that the reaction fell in Regime 3 and, was found to be zero order with respect to Alamine 336 and first order with respect to phenyl acetic acid with a first order rate constant of 0.9 s^{-1}. When the researchers utilized MIBK, they found that the reaction fell in Regime 2, which is a diffusion controlled regime. This result verified that the rate constant attained with MIBK was lower than the constant obtained with kerosene. The investigators reasoned the advantage of using kerosene instead of MIBK due to a higher reaction rate constant in addition to higher equilibrium distribution coefficient values.

Nikhade *et al.* (2004) studied the extraction of citric acid using Alamine 336 dissolved in MIBK. According to the results of their kinetic studies, the reaction between the two reactants fell in Regime 1, which was referred as the kinetic-controlled slow reaction occurring in the bulk organic phase. The reaction between citric acid and Alamine 336 was found to be first order in both Alamine 336 and citric acid with a rate constant of 0.013 m^3 $kmol^{-1}$ s^{-1}. In the following study (Nikhade *et al.*, 2005), the diluent was changed to cyclohexanone and the reaction between the two components fell in the same regime. The reaction was first order in both reactants with a second order rate constant of 0.0088 m^3 $kmol^{-1}$ s^{-1}.

Jun *et al.* (2005) studied kinetics of reactive extraction of succinic acid with 1-octanol solution of tri-n-octylamine in a stirred cell with a microporous hydrophobic membrane. Like previous investigators, they obtained the intrinsic kinetics of the system by correlating the interfacial concentration of species. It was found that the formation reaction of the acid-amine complex was first order in the concentration of succinic acid, while was in the order of 0.5 with respect to that of tri-n-octylamine in the organic phase with a rate constant of $3.14 \pm 0.6 \times 10^{-8}$ $m^{2.5}$ $mol^{-0.5}$ s^{-1}. Besides the formation of the complex, they proposed the dissociation reaction of the acid-amine complex, with the order of 2 with respect to concentration of acid-amine complex in the organic phase and the order of -2 with respect to that of TOA in the organic phase with a rate constant of $1.44 \pm 1.4 \times 10^{-4}$ mol m^{-2} s^{-1}. They concluded that the overall extraction process was controlled by chemical reaction at the interface within the studied ranges.

In another study of Jun *et al.* (2007), the effects of pH, salt and contaminated acid on the kinetics of the reactive extraction of succinic acid with TOA in fermentation broth were investigated. The authors observed that the reaction rates were affected by pH because of the dissociation of succinic acid. The existence of contaminated acid and salts, due to their ability to react with the tertiary amine extractants, hindered the extraction of succinic acid. It was concluded that to have an effective separation of succinic acid from production media, the pH of the fermentation broth should be kept at values lower than the pK_{A1} of the extracted acid.

Keshav *et al* (2008a) studied the equilibrium and kinetics of the extraction of propionic acid with TOPO dissolved in n-heptane, light liquid paraffin, heavy liquid paraffin and hexane. Hexane was found to be the most appropriate solvent among them for kinetic studies. The reaction was found to be first order with respect to both of the reactants, propionic acid and TOPO, with a rate constant of 46.91 $(m^3/kmol)^2\ s^{-1}$.

It is clearly seen that the reaction between the extracted acid and the extractant present in the organic phase is dependent on the corresponding acid and the contents of the organic phase. In the present study, kinetic studies on the reactive extraction of pyruvic acid with trioctylamine in 1-octanol were carried out in addition to the equilibrium studies. There is a lack of data on the kinetics of the reactive extraction of pyruvic acid in the literature and the data obtained in the present study will be useful for the design of a unit for the recovery of the target acid from the production medium.

CHAPTER 3

THEORETICAL APPROACH

The theories of equilibrium and kinetics, utilized in order to interpret the data, are explained in this chapter. Mass Action Law is explained in Section 3.1 and was used to interpret the equilibrium data. Theory of kinetics, “Extraction Accompanied by a Chemical Reaction”, proposed by Doraiswamy and Sharma (1984), is described in Section 3.2.

3.1. Theory of Equilibrium-Mass Action Law

Equilibrium data was interpreted by Mass Action Law, which was proposed by Guldberg and Waage (1864). Kertes and King (1986) applied Mass Action Law for reactive extraction of carboxylic acids with a tertiary amine in a diluent. For practical applications, activity coefficients in the liquid phases were assumed to be equal.

Chemical equilibrium of the system is described by three main steps:

1- The ionization of carboxylic acid in the aqueous solution.

$$HA_{aq} + H_2O \leftrightarrow H_3O^+ + A^-{}_{aq} \qquad \text{(R3.1.1)}$$

2- The partition of undissociated acid between the two phases.

$$HA_{aq} \leftrightarrow HA_{org} \qquad \text{(R3.1.2)}$$

3- The complex formation between carboxylic acid and tertiary amine in the organic phase during reactive extraction

$$HA_{org} + TA_{org} \leftrightarrow (HA - TA)_{org} \qquad \text{(R3.1.3)}$$

where HA represents the undissociated carboxylic acid, and TA denotes the tertiary amine. According to reaction R3.1.3, the ammonium salt is formed by the tertiary amine and undissociated carboxylic acid in the organic phase.

The amount of undissociated portion of a weak acid in the aqueous phase, HA_{aq}, can be calculated by using the Henderson-Hasselbach Equation. The pH of the aqueous solution and the pK_A value of the corresponding acid are needed in this equation.

$$pH = pK_A + \log\left(\frac{\left[A^-\right]_{aq}}{\left[HA\right]_{aq}}\right) \qquad \text{(E3.1.1)}$$

Concentration of the organic phase is calculated by mass balance after analyzing the aqueous phase for the concentration of carboxylic acid, which is explained in Section 4.4. It is assumed that, the volumes of the phases do not change during the extraction and equal to the initial volumes. Therefore, when the volumes of the phases are equal, the concentration of the organic phase is calculated by using Equation E3.1.4.

$$\left[HA_t\right]_{aq} = \left[HA\right]_{aq} + \left[A^-\right]_{aq} \qquad \text{(E3.1.2)}$$

$$V_{aq,0} * \left[HA_t\right]_{aq,0} = V_{aq} * \left[HA_t\right]_{aq} + V_{org} * \left[HA_t\right]_{org} \qquad \text{(E3.1.3)}$$

$$\left[HA_t\right]_{aq,0} = \left[HA_t\right]_{aq} + \left[HA_t\right]_{org} \qquad \text{(E3.1.4)}$$

As stated in the previous chapter, experimentally measured distribution coefficient is the ratio of total acid concentration of the organic phase to that of the aqueous phase, as shown in Equations E3.1.5 and E3.1.6. Total acid concentration includes both undissociated and dissociated portions of the acid.

$$K_D = \frac{[HA_t]_{aq,0} - [HA_t]_{aq}}{[HA_t]_{aq} * \frac{V_{org}}{V_{aq}}} \qquad \text{(E3.1.5)}$$

$$K_D = \frac{[HA_t]_{org}}{[HA_t]_{aq}} \qquad \text{(E3.1.6)}$$

It is well known that diluents with functional groups such as alcohols, ketones and ethers can interact with the solute and extract the undissociated carboxylic acid. Hence, as King and co-workers stated, the concentration of undissociated acid reacted with the extractant, HA_{ex}, should be calculated by considering the amount of the undissociated acid extracted by the diluent (Tamada *et al.*, 1990a). In order to determine the concentration of the carboxylic acid extracted physically by the diluent, Equation E3.1.8, which is derived from E3.1.3, was used.

$$[HA]_{org} = [HA_d]_{org} + [HA_{ex}]_{org} \qquad \text{(E3.1.7)}$$

$$[HA_d]_{org} = \frac{K_{D,d}[HA]_{aq,0}}{(1 + vK_{D,d})} \qquad \text{(E3.1.8)}$$

where v is the volume fraction of the diluent in the organic phase. It is also equal to the ratio of the volume of the organic phase to that of the aqueous phase. $K_{D,d}$ is the diluent/water distribution coefficient of the acid.

The equilibrium complexation constant of the reaction R3.1.4 can be calculated by Equation E3.1.9. This is for (1-1) type of acid-amine complex.

$$HA_{aq} + TA_{org} \Leftrightarrow (HA - TA)_{org} \qquad \text{(R3.1.4)}$$

$$K_E = \frac{[(HA - TA)]_{org}}{[HA]_{aq}[TA]_{org}} \qquad \text{(E3.1.9)}$$

Connors (1987) stated that if the chemical interactions between the components are stronger than the physical interactions in the system, the formation of complexes with various stoichiometries is possible. In order to evaluate reactive extraction equilibrium, in addition to (1-1) acid-amine complex, complexes with various stoichiometries should also be taken into account. In the literature, it can be seen that, some organic systems could take up acids more than required for the neutralization of the amines (Kertes and King, 1986; Yang *et al.*, 1991). The formation and equilibrium constant calculation of these complexes are also explained by the mass action law with *m* acid molecules and one amine molecule.

$$HA_{aq} + (HA - TA)_{org} \Leftrightarrow (HA_2 - TA)_{org} \qquad \text{(R3.1.5)}$$

$$HA_{aq} + (HA_{n-1} - TA)_{org} \Leftrightarrow (HA_n - TA)_{org} \qquad \text{(R3.1.6)}$$

giving

$$K_{En} = \frac{[(HA_n - TA)]_{org}}{[HA]_{aq}[(HA_{n-1} - TA)]_{org}} \qquad \text{(E3.1.10)}$$

The formation of these complexes was confirmed by the spectroscopic studies. Investigators conducted these studies by checking the type of the bonds that occur between the extracted acid and the amine. As stated earlier and shown in Figure 3.1, the first acid molecule and the amine molecule form an ion-pair. This can be followed by hydrogen bonding between the OH of the carboxyl of the second acid and the conjugated CO of the carboxylate of the first acid for the formation of (2-1) acid-amine complex (Tamada *et al.*, 1990b).

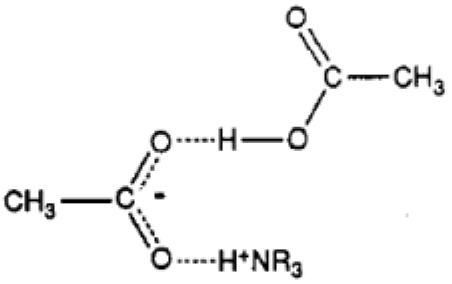

Figure 3.1 Structures of (1-1) and (2-1) acid-amine complexes of an undissociated acid and a tertiary amine

King and co-workers defined loading ratio, which is the ratio of the concentration of the acid reacted with the extractant in the organic phase to the initial concentration of the amine, to determine the magnitude of the load of the extractant (Kertes and King, 1986). This ratio is beneficial in order to clarify the stoichiometries of the complexes formed by acid and amine in the organic phase. The plot of loading ratio versus aqueous phase concentration or logarithm of that with amine concentration gives an estimation of types of complexes formed in the organic phase during reactive extraction of the acid. Besides, the effect of concentration of the components in the aqueous and organic phases can be seen.

$$z = \frac{[HA_{ex}]_{org}}{[TOA]_{org,0}} \qquad \text{(E3.1.11)}$$

As shown in Equation E3.1.9, equilibrium complexation constant, K_E, can be calculated using the concentration of the components at equilibrium. Equation E3.1.13, which is the combination of Equation E3.1.6, E3.1.9 and E3.1.12, signifies that equilibrium complexation constant can be calculated by a plot of logarithm of distribution ratio versus logarithm of amine concentration with the use of equilibrium data. Earlier investigators noted that if the slope of this graph is less than unity, this result implies that organic phase extracts more acid than is needed for the neutralization of carboxylic acid. In other words, there is a possibility of formation of additional complexes in the organic phase besides (1-1) acid-amine complex.

$$K_{D,ex} = \frac{[HA_{ex}]_{org}}{[HA_t]_{aq}} \qquad \text{(E3.1.12)}$$

$$\log K_{D,ex} = \log K_E + \log([TA]_{org}) \qquad \text{(E3.1.13)}$$

Hence, for organic phases, which are not highly concentrated, i.e. Z<0.5, King and co-workers proposed a combined equation of E3.1.9 and E3.1.11 to calculate the equilibrium complexation constant using loading ratio and equilibrium concentration of the aqueous phase. It was asserted that a straight line of the plot of Equation E3.1.14 passing through the origin, yields the equilibrium complexation constant of (1-1) acid-amine complex (Kertes and King, 1986). For higher values of loading ratios and “n” values greater than unity, complex (n-1) is formed as shown in reaction, R3.1.6, and a plot of Equation E3.1.15 yields a straight line, whose slope gives the equilibrium complexation constant for the (n-1) complex, K_{En}.

$$\frac{z}{1-z} = K_{E1}[HA]_{aq} \qquad \text{(E3.1.14)}$$

$$\frac{z}{n-z} = K_{En}[HA]^n{}_{aq} \qquad \text{(E3.1.15)}$$

3.2. Theory of Kinetics-Extraction Accompanied by a Chemical Reaction

Doraiswamy and Sharma (1984) proposed a comprehensive study on the theory of extraction accompanied with chemical reaction to determine the effect of chemical reaction on the specific rate of mass transfer. The reaction between the acid and amine forms an acid-amine complex in the organic phase.

$$mHA_{aq} + nTA_{org} \leftrightarrow \left(HA_m - TA_n\right)_{org} \qquad \text{(R3.2.1)}$$

They used the film and renewal theories with physico-chemical and hydrodynamic parameters and classified the systems into four regimes depending on their relative diffusion and reaction rates:

- Very Slow Reactions
- Slow Reactions
- Fast Reactions
- Instantaneous Reactions

Doraiswamy and Sharma (1984) have given a guide for the researchers to determine the mechanism of the extraction accompanied with a chemical reaction in a stirred cell. Table 3.1 summarizes the guidelines given by the authors. As can be seen, the speed of agitation, volume ratio of the phases and the order of the reaction are required to estimate the mechanism of the reaction during extraction in a Lewis type stirred cell. The parameters and the information about the cell used to obtain kinetics data are explained in Section 4.3

In the present study, specific rate of extraction was calculated using the concentration change of pyruvic acid in the organic phase with time, which was calculated by mass balance after analyzing the aqueous phase for the concentration of pyruvic acid. only initial rates were taken into consideration.

As is shown in Sections 5.2.3 and 5.2.4, the specific rate of extraction was not affected by the change of speed of agitation and the volume change, which means that the reaction between pyruvic acid and trioctylamine in 1-octanol falls in Regime 3, extraction accompanied by a fast chemical reaction occurring in the diffusion film in the range of the parameters studied. For Regime 3, specific rate of transfer of carboxylic acid is given by the Equation 3.2.1

$$R_{HA} = \left[HA^{*}\right]_{org} \sqrt{\frac{2}{m+1} D_{HA} k_{mn} \left[HA^{*}\right]_{org}^{\;m-1} \left[TA\right]_{org}^{\;n}} \qquad \text{(E3.2.1)}$$

Table 3.1 Regime of extraction with reaction in a stirred cell

Effect on the specific rate of extraction				
Regime	$[HA^*]_{org}^{m}$	$[TA]_{org}^{n}$	**Speed of agitation**	**Volume Ratio of the Phases**
1	$\alpha\ [HA^*]^{m}$	$\alpha\ [TA]_{org}^{n}$	None	α Volume of the organic phase
2	$\alpha\ [HA^*]^{m}$	None	Increases with increase in the speed of stirring	None
3	$\alpha\ [HA^*]^{(m+1)/2}$	$\alpha\ [TA]_{org}^{n/2}$	None	None
4	None	$\alpha\ [TA]_{org}^{n}$	Increases with increase in the speed of stirring	None

CHAPTER 4

MATERIALS AND METHODS

Experimental equilibrium and kinetic studies on reactive extraction of carboxylic acids with tertiary amines in 1-octanol and oleyl alcohol are explained in this chapter. In Section 4.1, the materials used in the study are described. In the next section, methods of equilibrium experiments are described, while kinetics experiments are explained in Section 4.3. Lastly, in Section 4.4, the analysis of the carboxylic acids with titration method and High Performance Liquid Chromatography are explained.

4.1. Materials

Pyruvic acid (Merck Co.) with analytical purity of 98% and acetic acid (Merck Co.) with analytical purity of 98%, were used as received without any pretreatment. Lactic acid (Merck Co.) with analytical purity of 88% was heat-treated before use. For concentrations over 20 wt% of lactic acid solutions, self-esterification and dimerization of the lactic acid molecules are observed. For the purpose of breaking these bonds, lactic acid was heated through with an attached a condenser for 12 hours as suggested in the literature (Holten, 1971). NaOH and HCl solutions were utilized to adjust the pH of the aqueous phase. The ultra high pure (UHP) water was produced by Millipore Milli-Q Water System.

The organic solvents oleyl alcohol (Merck Co.) and 1-octanol (Merck Co.) with analytical purities of 99% were used as received. The densities of the 1-octanol and oleyl alcohol are 0.83 and 0.85 respectively. The use of diluents is required in order to control the viscosity and concentration of the organic phase. Tertiary amines, trioctylamine (Fluka) and Alamine 336 (Cognis) were also used as received.

H_2SO_4 (Merck Co., 95-98%) was used so as to prepare the mobile phase for the analysis of carboxylic acids by HPLC.

In the experiments performed at Iowa State University, pyruvic acid (Acros) with analytical purity of 99%, 1-octanol (Acros) and trioctylamine (Acros) were utilized.

4.2. Methodology of the Equilibrium Experiments

The equilibrium between two phases is constrained by Gibbs' Phase Rule (Equation E4.1.1), where F is degrees of freedom, C is the number of the components and P is the number of the phases in the system (Geankoplis, 1983). The least number of intensive parameter that must be known in order to describe the system is calculated by the following equation:

$$F = C - P + 2 \qquad \text{(E4.1.1)}$$

For solvent extraction experiments, there are three degrees of freedom. Temperature and pressure were kept constant as 25^0C and 676 mm-Hg respectively for all experiments in the present study. In addition to these, concentration of the carboxylic acid in the aqueous phase was varied between 0.1 and 1.0 M for solvent extraction experiments.

In a reactive extraction system consisting of an extractant dissolved in a diluent and an aqueous solution of a carboxylic acid, number of the degrees of freedom is four. Temperature and pressure of the reactive extraction system were kept constant during the experiments. Initial concentrations of the acid and the extractant in the phases were set.

4.3. Equilibrium Studies

In the present study, most of the equilibrium experiments were performed at the Middle East Technical University (METU). Some of the experiments, which were selected to control the reproducibility of the data, were conducted at the Iowa State University (ISU) using different instruments. The results of the experiments are compared and the reproducibility of the data is presented in Section 5.1.1.7.

The aqueous phase was prepared by dissolving carboxylic acids in UHP water. The pH of the solution was adjusted by using either NaOH or HCl solutions. The organic phase for reactive extraction experiments was prepared by dissolving the extractants into the diluents depending on the type of the experiment.

Equal volumes (10 mL) of the organic and aqueous phases were mixed and equilibrated for 12 hours at 25^0C using a constant temperature

Figure 4.2.1 Constant temperature shaker bath, GFL 1083 (Middle East Technical University)

Figure 4.2.2 Incubator, New Brunswick (Iowa State University)

Table 4.2.1 Ranges of parameters used in equilibrium experiments

Parameter	**Range**
Type of acids	Pyruvic, Lactic, Acetic Acids
Type of extractant	Trioctylamine / Alamine 336
Type of diluent	1-Octanol / Oleyl Alcohol
Aqueous phase concentration of the carboxylic acid	0.10 – 1.0 M
Initial pH of the aqueous phase	1.35 – 6.0 (depending upon the content)
Organic phase concentration of the extractant	0.2 – 0.8 M

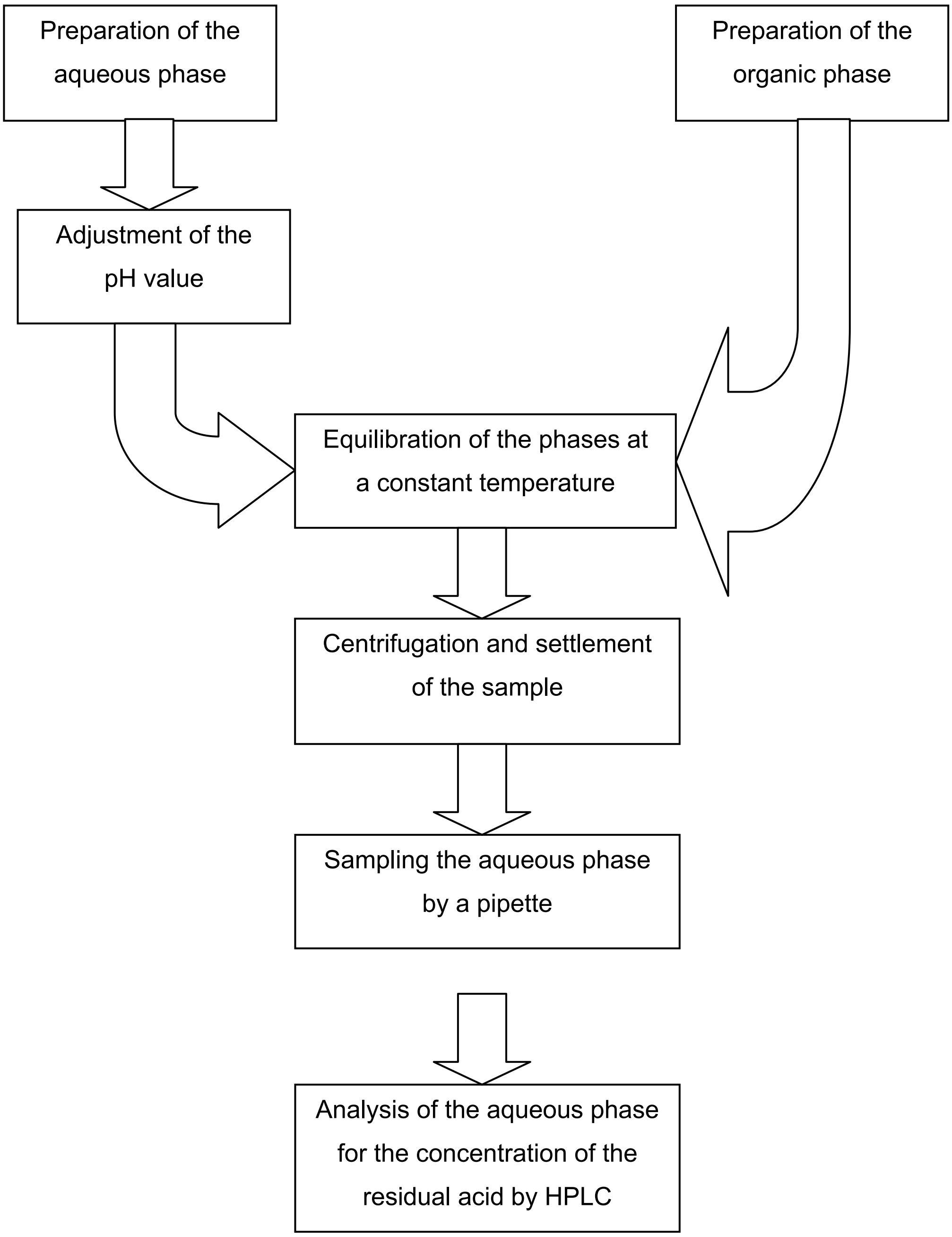

Figure 4.2.3 Flowchart of equilibrium experiments

shaker bath, GFL 1083 (Figure 4.2.1) at METU, and an incubator, New Brunswick (Figure 4.2.2) at ISU. Reaching the equilibrium was followed by separating the phases by centrifuging the samples at 10000 x g for 15 minutes before settling the samples for one hour to have a clear separation of the phases at the same temperature. After separation, aqueous phase was sampled by a pipette. Some amount of aqueous phase was left in the sample tube to eliminate the contamination of the sample. This was followed by analysis of the residual carboxylic acid in the aqueous phase using High Pressure Liquid Chromatography (HPLC), which is explained in Section 4.4.

Equilibrium experiments and the analysis were repeated twice. Distribution coefficients and loading ratios were calculated by using the average values of the experiments. The relative uncertainty of the data was 0.1%, and that of replicated experiments 2% (Appendix B). Concentration of pyruvic acid in the organic phase was determined by a mass balance about the aqueous phase, as stated in Section 3.1.

Table 4.2.1 shows the parameters and their ranges, which were selected for the equilibrium studies of the reactive extraction of carboxylic acids with tertiary amines in 1-octanol or oleyl alcohol. The experimental parameters and their ranges were chosen based on information given in the literature. Figure 4.2.3 is a flowchart of the equilibrium experiments conducted in this study.

4.4. Kinetic Studies

As mentioned in Section 3.2, use of a Lewis type stirred cell was suggested by Doraiswamy and Sharma (1984) to obtain the kinetic data. Therefore, in the present study, a stirred cell, 0.045 m in diameter (ID) and 0.1 m in height, was utilized in order to obtain the data to calculate and evaluate kinetic parameters (Figure 4.3.1).

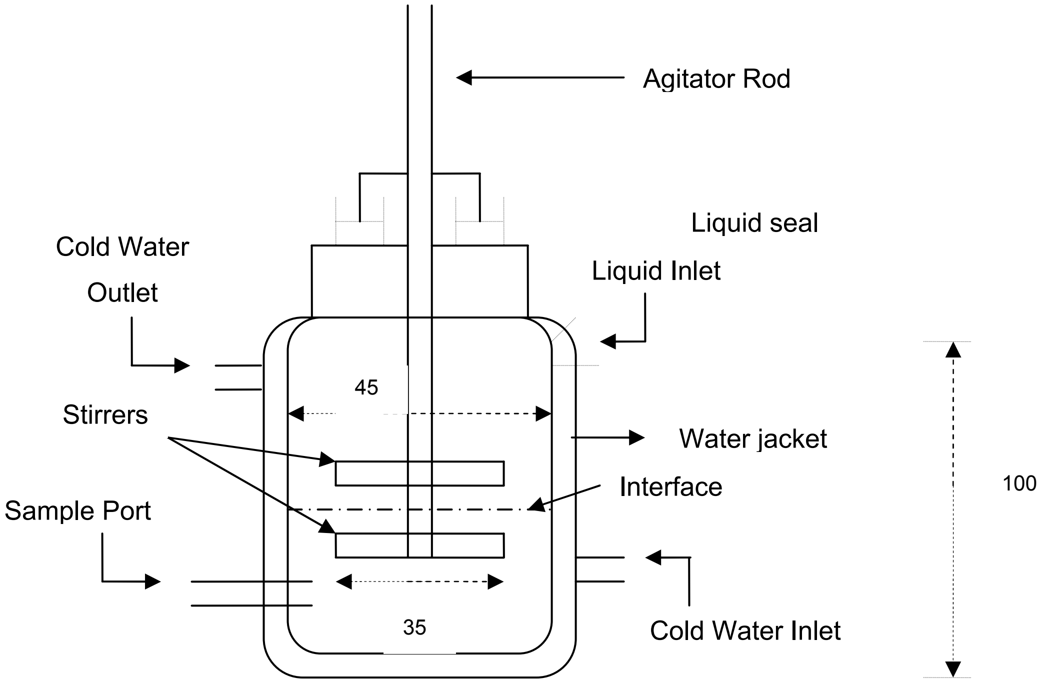

Figure 4.3.1 A schematic diagram of a Lewis type stirred cell used in the present study (All dimensions are in “mm.”)

The preparation of the system is very important in collecting kinetic data. First, the temperature of the system was maintained at 25^{0}C by a constant temperature water circulator bath and the jacket outside the stirred cell. Then, the aqueous phase was added to the cell. After adjusting the position of the four blade paddle, which is controlled by an overhead mechanic stirrer, IKA RW 20 DZM (Figure 4.3.2), the organic phase was added very slowly and carefully into the stirred cell. It is very critical not to damage the interface while pouring the solvent phase before the start of stirring. It was followed by setting the speed of agitation and the start of stirring. Proper selection of the speed of agitation is crucial to obtain dependable data. Thus the interface should not be disturbed and the interfacial area should be very close to the geometric area during stirring at the selected agitation rate (Figure 4.3.3). Subsequent to these, samples were taken from the aqueous phase periodically for analyzing the residual pyruvic acid in the aqueous phase with HPLC.

Kinetic experiments were repeated and all samples were assayed twice. The average values of experimental results were used to calculate the kinetic parameters. The concentration of pyruvic acid in the organic phase was determined by a mass balance.

Table 4.3.1 shows the parameters and their ranges, selected for the kinetic experiments. Experimental parameters and their ranges were chosen based on information cited in the related literature. Figure 4.3.4 is a flowchart of the kinetics experiments performed in this study.

Figure 4.3.2 Lewis type stirred-cell with overhead agitator


Figure 4.3.3 Lewis type stirred-cell

Table 4.3.1 Ranges of parameters used in kinetic experiments

Parameter	**Range**
Type of acid	Pyruvic acid
Type extractant and diluent	Trioctylamine and 1-Octanol
Speed of agitation	1.0 – 1.4 rev s^{-1}
Volume ratio (V_{org} / V_{aq})	0.8 – 1.25
Aqueous phase concentration	0.25 – 1.0 M
Organic phase concentration	0.2 – 0.6 M

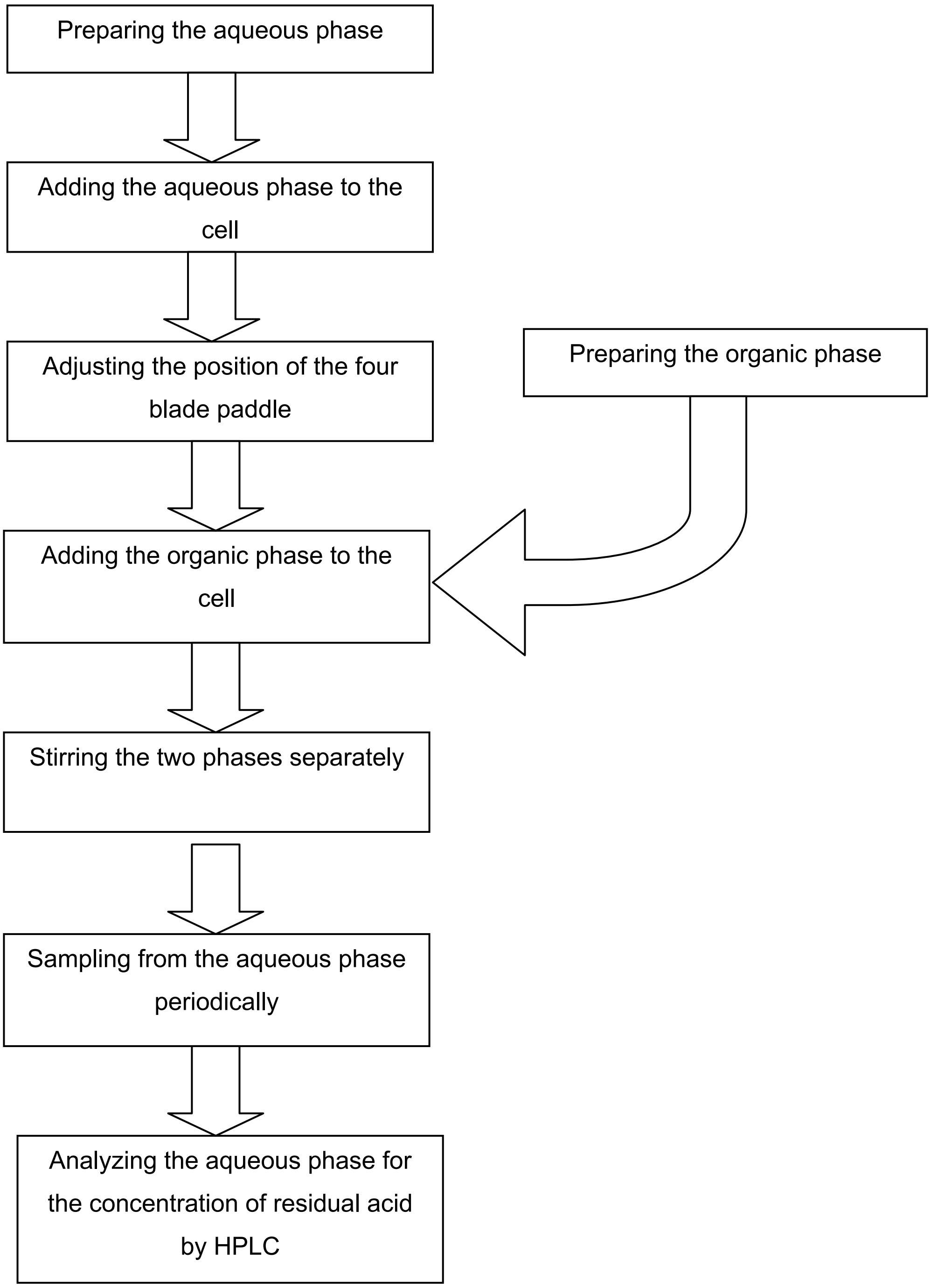

Figure 4.3.4 Flowchart of kinetic experiments

4.5. Experimental Analysis

As mentioned in Sections 4.2 and 4.3, High Performance Liquid Chromatography was used to determine the concentration of carboxylic acid concentration in the aqueous phase.

In some experiments, determination of the concentration of the carboxylic acid was carried out by the traditional method, titration with NaOH solution. Phenolphthalein was used as an indicator, while potassium hydrogen phthalate was used to calibrate the sodium hydroxide solution.

In the present study, several HPLC instruments and columns were used. The instruments and accessories used during the study are listed in Table 4.4.1. In Figures 4.4.1 and 4.4.2, the HPLC instruments used in METU, in Figure 4.4.3, the HPLC used in ISU are shown.

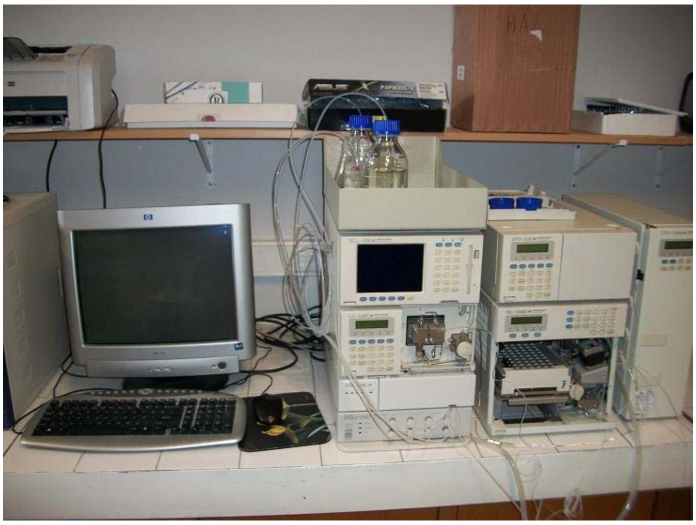

Figure 4.4.1 Shimadzu LC 10 AT

Table 4.4.1 HPLC instruments and columns used in METU and ISU

Name of the Study	Equilibrium Studies at METU	Kinetic Studies at METU	Equilibrium Studies at ISU
HPLC Pump	Shimadzu LC-20 Ad	Shimadzu LC-10 AT	Agilent 1100 Series
Mobile Phase	0.008 N H_2SO_4	0.008 N H_2SO_4	0.005 M H_2SO_4
Column	MetaCarb 87H (7,8 x 300 mm)	Alltech IOA-1000 (7,8 x 300 mm)	BioRad Aminex HPX-87H
Detector	UV detector at 210 nm	UV detector at 210 nm	UV detector at 210 nm
Flow Rate	0.6 ml min^{-1}	0.4 ml min^{-1}	0.6 ml min^{-1}

Figure 4.4.2 Shimadzu LC 20 VP

Figure 4.4.3 Agilent 1100 series

CHAPTER 5

RESULTS AND DISCUSSION

The results of experimental studies are presented and discussed in this chapter. In the first part of the chapter, the results of equilibrium studies on reactive extraction of pyruvic, acetic and lactic acids from their single and mixed acid solutions are described. This is followed by the section in which the results of kinetic studies are interpreted.

5.1. Results of Equilibrium Studies

The results of equilibrium studies are explained in two sub-sections. In the first, equilibrium studies on the extraction of carboxylic acids from their single acid solutions, in the second, the extraction of carboxylic acids from their mixed acid solutions are explained.

5.1.1. Results of Equilibrium Studies for Single Acid Solutions

The partitioning of undissociated pyruvic acid between the aqueous and organic phases can be shown as:

$$HP_{aq} \leftrightarrow HP_{org} \qquad \text{(R5.1.1)}$$

The equilibrium complexation reaction between trioctylamine and pyruvic acid can be written as

$$HP_{aq} + TOA_{org} \leftrightarrow (HP - TOA)_{org} \qquad \text{(R5.1.2)}$$

$$HP_{aq} + \left(HP_{n-1} - TOA\right)_{org} \leftrightarrow \left(HP_n - TOA\right)_{org} \qquad \text{(R5.1.3)}$$

where HP represents undissociated pyruvic acid and TOA denotes trioctylamine. These representations describe the mechanism of the reactive extraction of pyruvic acid with the tertiary amines. In the first representation, R5.1.1, the undissociated pyruvic acid is extracted from the aqueous phase to the organic phase. In the second and third, the extracted acid is bound to the amine present in the organic phase. The extracted acid and the amine present in the organic phase form the acid-amine complexes, which are stabilized by the diluent.

First, equilibrium experiments were performed to determine the equilibration time of the extraction of pyruvic acid with 1-octanol and oleyl alcohol. These were followed by solvent extraction experiments using organic phases comprised of only diluents, oleyl alcohol or 1-octanol in order to compare the diluents and calculate the distribution coefficients with the diluents. Tertiary amines, Alamine 336 and trioctylamine were compared before investigating the effects of concentration of the phases. Then, applicability of Mass Action Law was examined and the effect of the initial pH value of the aqueous solution on reactive extraction was investigated. Finally, the data obtained at Middle East Technical University and Iowa State University was compared and the reproducibility of the data was evaluated for the extraction of pyruvic acid with trioctylamine in 1-octanol.

5.1.1.1. Equilibration Time

In the literature, researchers state a variety of equilibration periods for different systems (Table 5.1). In the present study, equilibration time was determined by conducting extraction experiments for different end-times. The results of these kinetic experiments of solvent extraction of pyruvic acid with the diluents (oleyl alcohol and 1-octanol) are presented in Figure 5.1.1. It is observed that equilibration times for both of the diluents are between 5 and 6 hours. This is reasonable when compared with the literature. To be on the safe side, equilibrium experiments were carried out for 12 hours.

Table 5.1 Equilibration times in the literature for similar systems

Authors	**System**	**Equilibration Time** **Settling Time**
Wasewar *et al.* (2002)	Alamine 336 in MIBK-Lactic Acid	24 hrs 30 min
Ma *et al.* (2005)	TOA in n-Octanol - Pyruvic & Lactic Acid	60 min No information
Senol (2006)	Alamine 336 in Organic Solvents-Pyruvic Acid	2 hrs 18-20 hrs

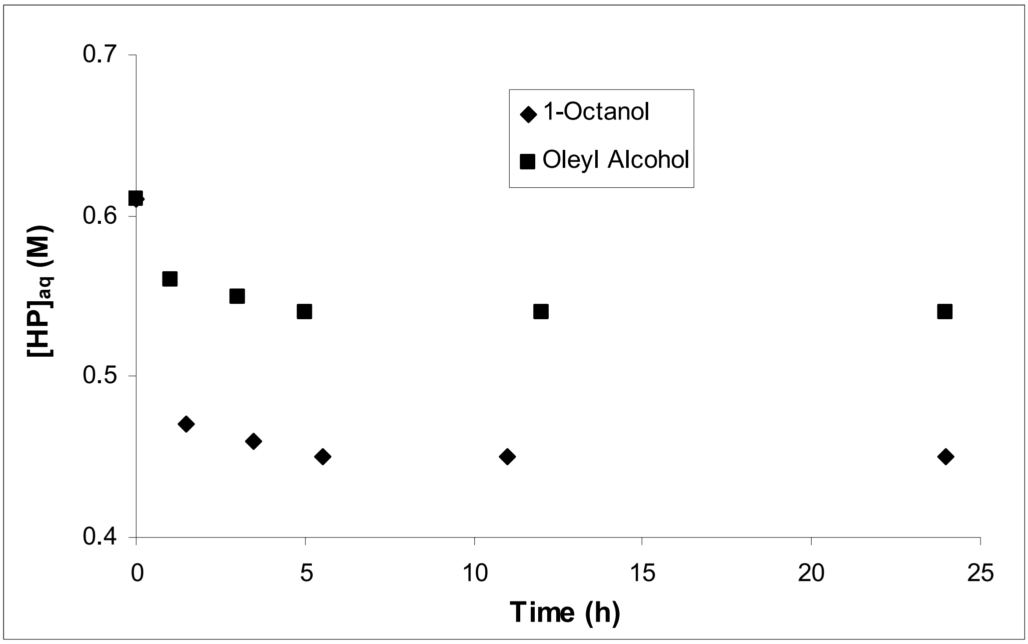

Figure 5.1.1 Equilibration time experiments ($[HP]_{aq,0}$ = 0.56 M)

5.1.1.2. Effect of Diluent

As stated in the literature, active diluents such as alcohols, ketones and ethers are proposed to enhance the solubility of acid-amine complex in the organic phase (Kertes and King, 1986). High polarity of these types of solvents causes an increase in the Lewis-basicity of tertiary amines and consequently, an increase in the distribution coefficient of the carboxylic acids. Molecular weight and structure play important roles in the polarity of a solvent. Therefore, the distribution of carboxylic acid is dependent on the chemical properties among the homologous solvents. Extraction level was observed to decrease with an increase in the molecular weight of the diluents. Thus, high molecular weight solvents are not favored in the extraction of carboxylic acids (Holten, 1971). On the other hand, the low molecular weight solvents have higher miscibility with aqueous solutions than the higher ones. Hence, diluents with intermediate molecular weights are usually chosen in the extraction of carboxylic acids (Holten, 1971).

1-octanol, which is an intermediate molecular weight alcohol, was selected in the present study due to its high polarity and extractability of the carboxylic acids among similar diluents. Cases of successful recovery of several carboxylic acids such as lactic, acetic and succinic acids using 1-octanol with the appropriate extractants were reported in the literature (Jaquet *et al.*, 1999, Hong and Hong, 2000, Wasewar *et al.*, 2004). Oleyl alcohol was chosen as the second diluent due to its non-toxicity to microorganisms producing carboxylic acids. Oleyl alcohol was recommended as the most appropriate diluent when compared with some organic diluents in the literature owing to its non-toxicity (Gu *et al.*, 1998; Tong *et al.*, 1998; Schugerl, 2000, Tik *et al.*, 2001). The results of these equilibrium experiments are presented in Tables A1.1 and A1.2. The equilibrium concentration of pyruvic acid in the organic phase was calculated by applying mass balance using the initial and final concentrations of pyruvic acid in the aqueous phase (Equation E5.1.1). Distribution coefficients were calculated by using Equation E5.1.2.

$$[HP_t]_{aq,0} = [HP_t]_{aq} + [HP]_{org} \qquad \text{(E5.1.1)}$$

$$K_D = \frac{[HP_t]_{org}}{[HP_t]_{aq}} \qquad \text{(E5.1.2)}$$

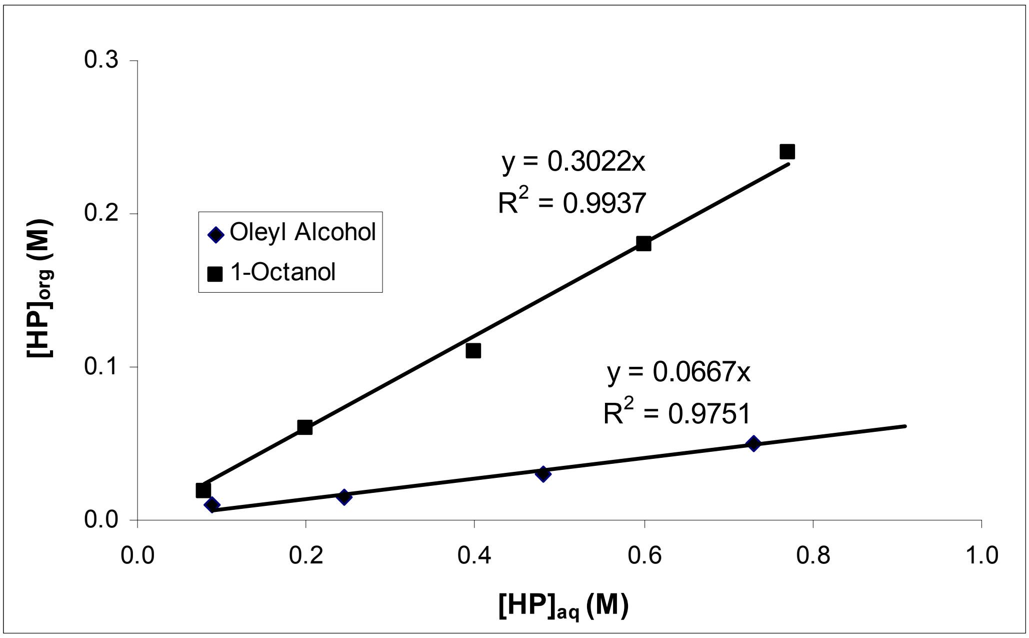

Figure 5.1.2 Solvent extraction of pyruvic acid with 1-octanol and oleyl alcohol

The results of the solvent extraction of pyruvic acid with 1-octanol and oleyl alcohol at various concentrations of the acid in the aqueous phase are plotted on Figure 5.1.2. It can be seen that the equilibrium concentration of pyruvic acid in the organic phase increased linearly with the equilibrium concentration of pyruvic acid in the aqueous phase. The slope of the linearity yields the distribution coefficient of pyruvic acid with the corresponding diluent. The distribution coefficient of pyruvic acid was found as 0.30 with 1-octanol and 0.07 with oleyl alcohol. The amount of acid extracted to the organic phase was higher with 1-octanol than oleyl alcohol. The differences in chemical properties such as polarity, dielectric constant and molecular weights of the diluents are proposed to be the main reasons of the difference in the distribution coefficients (Senol, 2006).

The distribution coefficient of pyruvic acid with the diluent ($K_{D,d}$) is also required for the calculation of the loading ratio (z). The concentration of carboxylic acid in the organic phase was calculated by a mass balance about the aqueous phase. As mentioned earlier, the amount of acid kept by the active diluent should be subtracted from the total acid concentration in the organic phase in order to calculate the concentration of the acid reacted with the extractant at the equilibrium, which is needed for the calculation of loading ratio (Kertes and King, 1986). The concentration of the acid kept by the active diluent can be calculated by Equation E5.1.3 using the volume fraction of the diluent in the solvent phase and the distribution coefficient of pyruvic acid with the corresponding diluent.

$$[HP_d]_{org} = \frac{K_{D,d}[HP_t]_{aq,0}}{(1+\nu K_{D,d})} \qquad \text{(E5.1.3)}$$

After conducting the experiments of solvent extraction of pyruvic acid with the diluents, those of acetic and lactic acids were performed with the same diluents. As expected, the trend obtained with pyruvic acid and similar extraction behaviors were obtained with acetic and lactic acids. 1-octanol extracted more carboxylic acid than oleyl alcohol from the aqueous solution. This superiority of octanol to oleyl alcohol is consistent with the results for propionic acid (Gu *et al.*, 1998) and lactic acid (Wasewar *et al.*, 2004) in the literature. The results of these experiments are presented in Tables A1.3, A1.4, A1.5 and A1.6. The distribution coefficients of the acids with the diluents were obtained by taking the slope of the plot of equilibrium concentration of carboxylic acid in the organic phase versus that of in the aqueous phase. The results of distribution coefficients are tabulated in Table 5.2. It is seen that acetic acid was extracted more easily than the other two acids. The distribution coefficients of the acids decreased in the order of acetic > pyruvic > lactic acids. One of the reasons of this order is related with the pK_A values of the acids and this is explained in Section 5.1.1.6. Lactic acid has the lowest distribution coefficients with the diluents, most probably due to its highest hydrophilicity among these acids. The additional hydroxyl group to the carboxylic group is the reason of its high hydrophilicity. The distribution coefficient of lactic acid with 1-octanol obtained in this study is consistent with the reported literature value of 0.15 (Han and Hong, 1998).

Table 5.2 Distribution coefficients of the carboxylic acids with the diluents

	Distribution Coefficient ($K_{D,d}$)	
Acids	**1-Octanol**	**Oleyl Alcohol**
Pyruvic Acid	0.30	0.07
Acetic Acid	0.50	0.22
Lactic Acid	0.15	0.07

5.1.1.3. Comparison of Extractants

It is mentioned in the literature that the recovery of carboxylic acids with the conventional diluents is not a satisfactory commercial technique. Hence, tertiary amines were suggested as effective extractants in order to increase the extraction power of the solvent phase. The effect of the presence of an extractant, trioctylamine or Alamine 336, in the organic phase with an appropriate diluent is shown in Figure 5.1.3. These results were compared with the results obtained from solvent extraction with diluents only. The initial concentration of pyruvic acid in the aqueous

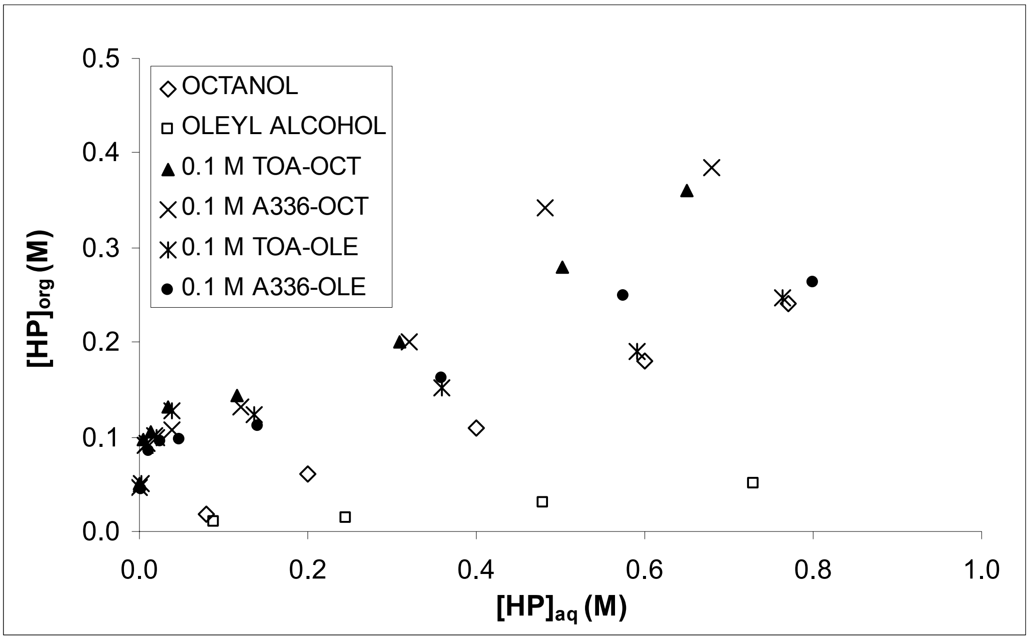

Figure 5.1.3 Equilibrium isotherms for reactive extraction of pyruvic acid with tertiary amines in 1-octanol and oleyl alcohol

phases was between 0.1 and 1.0 M, while that of tertiary amine in the organic phase was maintained as 0.1 M. It can be seen that extraction power of the organic phase was significantly affected by tertiary amines and they caused an important increase in the transfer of pyruvic acid to the organic phase. Even a relatively low concentration of the extractant was enough to observe the positive effect of tertiary amines. At higher concentrations of the acid in the aqueous phase, this behavior is more evident. These are consistent with the results in the literature. Wasewar *et al.* (2002) studied reactive extraction of lactic acid with Alamine 336 in organic diluents, MIBK and decanol, and observed the same trend, observed in the present study (Wasewar *et al.*, 2002a, 2002b). The results of these experiments are presented in Tables A2.1.1a-d. The effects of the concentration of the components in the organic and aqueous phases are discussed in Section 5.1.1.4.

The formation of an emulsion phase at the interface during the reactive extraction with tertiary amines in oleyl alcohol was observed. This third phase was observed especially at high initial concentration of the extractant in the organic phase and could not be completely eliminated by centrifuging the samples at 10000*g* for one hour. The polar acid-amine complexes tended to cluster together and formed an additional phase at the interface since the polarity of oleyl alcohol is limited for stabilization of the acid-amine complex. In literature, similar behavior was observed with inert diluents (Tamada *et al.*, 1990a; Yang *et al.*, 1991). Previous researchers mentioned that most of the acid extracted from the aqueous phase is kept in this emulsion layer (Tamada *et al.*, 1990a). It was observed in the experiments in the present study that the extent of the formation of a dense and opaque emulsion phase increased with an increase in concentration of components in the phases. This implies that the amount of acid in this emulsion layer is not negligible and the acid could not be completely transferred to the organic phase. In order to obtain consistent data, 1-octanol was chosen as the solvent and solely used in the rest of the study.

The results obtained by Uslu and Kirbaslar (2010) showed that among the alcohols which are immiscible with water, the lower the molecular weight and the higher the polarity of alcohol the higher was the distribution coefficient with aliphatic amines. They tested five alcohols (isoamyl alcohol (C5), hexan-1-ol (C6), octan-1-ol (C8), nonan-1-ol (C9) and decan-1-ol (C10)) in the extraction of malic acid. They achieved the highest distribution coefficients with isoamyl alcohol, which has the lowest molecular weight among the alcohols studied by the authors. Similar to these results, in the present study, the distribution coefficients with 1-octanol (C8) was higher than the ones with oleyl alcohol (C18).

It can be seen in Figure 5.1.3 that distribution coefficients of pyruvic acid do not differ considerably with the different tertiary amines used in this study. Alamine 336, a mixture of C_8-C_{10} straight-chain tertiary amine could extract undissociated portion of the acid like trioctylamine. Instead of using a mixture of tertiary amines with different number of carbon atoms and molecular weights; trioctylamine was utilized as the extractant in rest of the study.

5.1.1.4. Effect of Concentrations in the Phases

In Figure 5.1.4 the effects of concentration of components in the aqueous and organic phases on the distribution coefficients of pyruvic acid are presented. With an increase in the initial concentration of the extractant in the organic phase, the distribution coefficient of pyruvic acid increases, which is consistent with the literature (Wasewar *et al.*, 2002a, Poposka *et al.*, 1997, Matsumoto *et al.*, 2004). An increase in the concentration of the extractant increases the probability of the reaction in the organic phase. In the same figure, a decrease in the distribution coefficient of pyruvic acid is observed with an increase of its equilibrium concentration in the aqueous phase. This is consistent with the results in the literature (Kertes and King, 1986, Juang and Lin, 1996, Poposka *et al.*, 1998, Tong *et al.*, 1998). At the low concentration range of pyruvic acid, the ratio of the concentration of pyruvic acid bound to the amine to the concentration of pyruvic acid remaining in the aqueous phase is expected to be higher than the ratio at the higher concentration range of pyruvic acid in the aqueous phase.

These two trends of the variation of the distribution coefficient with the concentration of the phases indicate that the limiting reagent for the reversible acid-amine complex formation reaction is the extractant in the diluent (Poposka *et al.*, 1998). Accordingly, at low equilibrium acid concentrations in the aqueous phase, the distribution coefficient of pyruvic acid is extremely high; however it decreases very sharply with an increase in equilibrium acid concentration in the aqueous phase, to a value of 0.2 mol L^{-1}. As can be seen in Figure 5.1.4, the same trend repeated itself showing a slight increase in the limiting equilibrium distribution coefficient with an increase of the extractant.

The results of these experiments are presented in Tables A2.1.1-5. Equilibrium isotherms of pyruvic acid with trioctylamine in 1-octanol are shown in Figure 5.1.5. As expected, equilibrium concentration of pyruvic acid in the organic phase increased with the increase in the initial concentration of the extractant as well as the equilibrium concentration of the aqueous phase. This is consistent with the results in the literature (Poposka *et al.*, 1998, Wasewar *et al.*, 2002b). The effect is more evident at higher amine concentration levels.

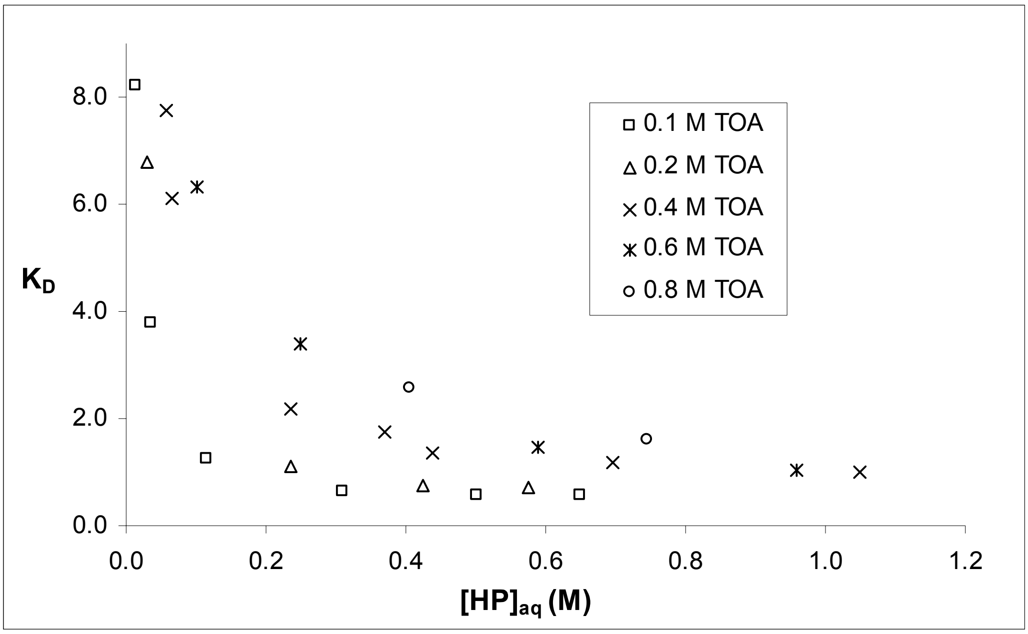

Figure 5.1.4 Variation of distribution coefficient with equilibrium concentration of pyruvic acid in the aqueous phase for various concentration levels of trioctylamine in 1-octanol

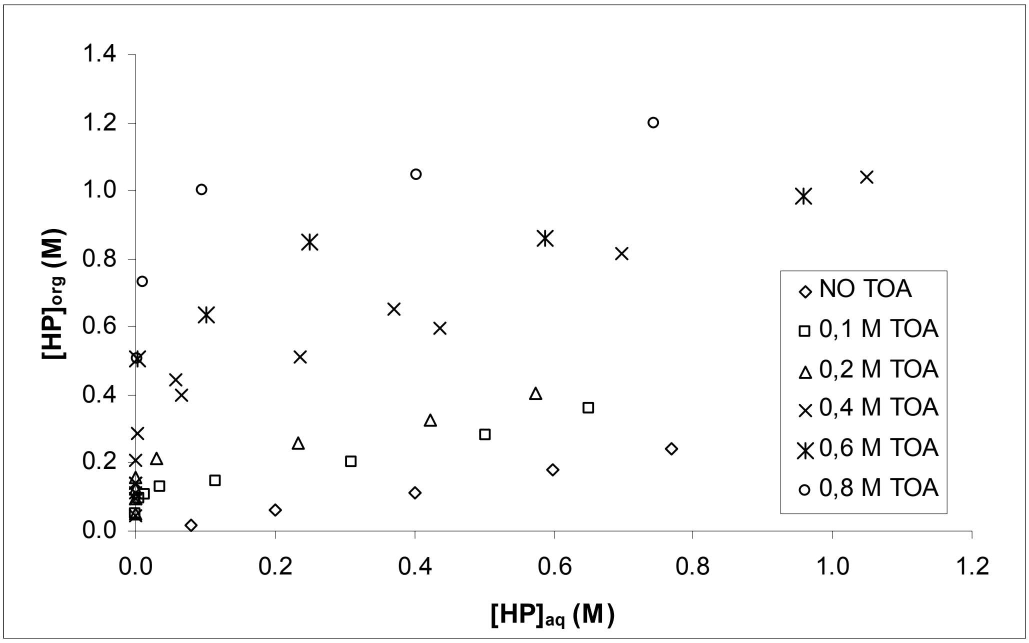

Figure 5.1.5 Equilibrium isotherms for reactive extraction of pyruvic acid with trioctylamne in 1-octanol

5.1.1.5. Stoichiometric Results of Equilibrium Experiments

As described earlier, the loading ratio is an expression that defines the extent of undissociated acid to which the organic phase can be loaded with the extracted carboxylic acid. It is the ratio of the concentration of undissociated acid that reacts with the extractant to the initial amine concentration in the organic phase (Equation E5.1.4)

$$z = \frac{[HP_{ex}]_{org}}{[TOA]_{org,0}} \tag{E5.1.4}$$

where *z* denotes the loading ratio of the organic phase for the extraction of pyruvic acid, whereas HP_{ex} is the total amount of acid that reacts with the extractant in the organic phase.

If the diluent, which is used to dissolve the extractant is an inert diluent such as xylene or hexane, the total amount of acid extracted to the organic phase can be set equal to the concentration of the acid reacted with the amine. However, if it is an active diluent such as alcohols, ketones or ethers, the concentration of carboxylic acid reacting with the extractant should be calculated by considering the amount of acid kept by the diluent using Equations E5.1.3, E5.1.5 and E5.1.6.

$$[HP_t]_{aq,0} = [HP_t]_{aq} + [HP]_{org} \qquad \text{(E5.1.5)}$$

$$[HP]_{org} = [HP_d]_{org} + [HP_{ex}]_{org} \qquad \text{(E5.1.6)}$$

where HP represents the undissociated pyruvic acid, HP_t denotes the total pyruvic acid, v is the volume fraction of diluent in the organic phase, and $K_{D,d}$ is the diluent/water distribution coefficient of the corresponding acid.

Besides providing information on the level of the loading in the organic phase, loading ratio is also used for the estimation the stoichiometry of the reaction occurring in the organic phase. Consequently, the nature of complexes formed by acid and extractant in the organic phase can be determined. The loading ratio and the distribution coefficient of the reactive extraction of pyruvic acid are presented in Table A2.1.1-5. In Figure 5.1.6, the variation of the loading ratio with the logarithm of the equilibrium concentration of the acid in the aqueous phase is shown. The concentration range of the extractant in organic phase studied is between 0.1 and 0.8 mol L^{-1}, while that of the acid concentration in the aqueous phase is between 0.1 Mol L^{-1} and 1.0 mol L^{-1}. It can be seen that the loading ratio increases with the equilibrium concentration of the acid in the aqueous phase and is independent of the initial concentration of the extractant. Both of these observations are in consistent with the results in the literature and as were reported by previous investigators for monocarboxylic acids such as acetic, formic and lactic acids (Tamada *et al.*, 1990a; Wasewar *et al.*, 2002, Nikhade *et al.*, 2008). With respect to the figure, the formation of (1-1) acid-amine complex was expected in the equilibrium concentration range of the aqueous phase between 10^{-4} and

10^{-3} M, because loading ratio was very low (Z<0.5). In the second part of the curve, (1-1) acid-amine complex formation was expected with higher loading ratios to a value of $10^{-0.5}$ of equilibrium concentration of pyruvic acid. Beyond this concentration value, overloading was observed and the loading ratio was higher than the unity (Z>1) but less than 1.5. Therefore, it is probable that (2-1) acid-amine complex is formed in this range of equilibrium acid concentration and for the loading ratio values greater than unity (Z>1).

As explained in Section 3.1, Mass Action Law provides a means for the determination of the complexation constant of the acid-amine reaction. If only (1-1) acid-amine complex occurs in the organic phase, a plot of Equation E5.1.8 yields the complexation constant with a slope of unity. If the organic phase extracts more acid than is needed for the neutralization

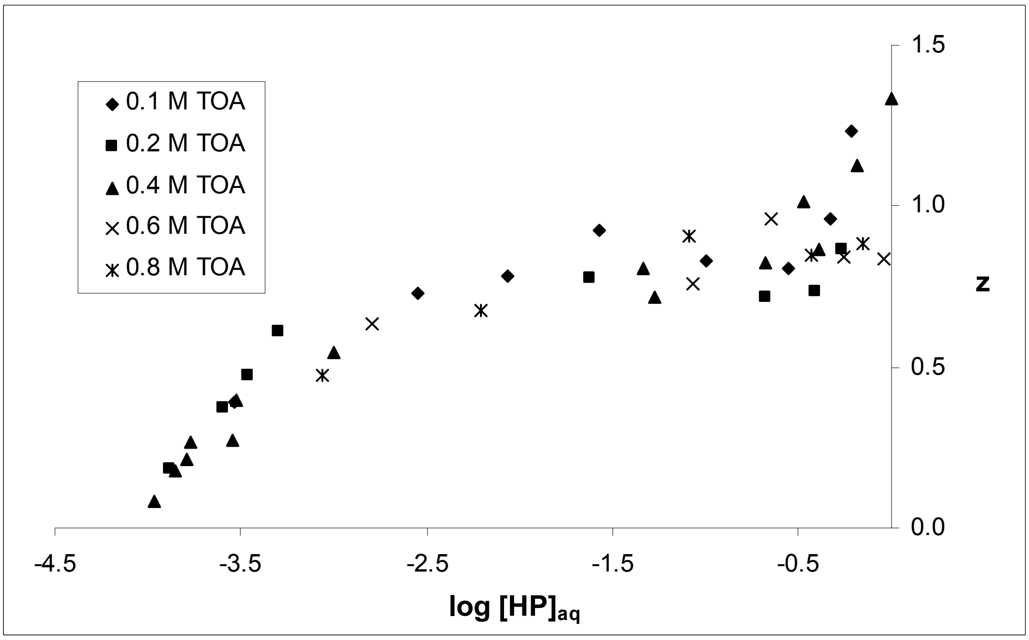

Figure 5.1.6 Variation of the loading ratio with the equilibrium concentration of pyruvic acid in the aqueous phase for trioctylamine in 1-octanol

of the carboxylic acid, the slope is expected to be less than unity (Wasewar *et al.*, 2002a, 2002b, Nikhade *et al.*, 2004). In Figure 5.1.7, the data was plotted and the slope is obtained as 1.59 which is greater than unity. In other words trioctylamine in 1-octanol does not extract pyruvic acid more than required for the neutralization of the tertiary amine and (1-1) type of complex is expected in the organic phase. With respect to the Equation E5.1.8, complexation constant is found to be 315.35 L mol^{-1}.

$$\log K_D = \log K_E + \log\left([TOA]_{org}\right) \qquad \text{(E5.1.8)}$$

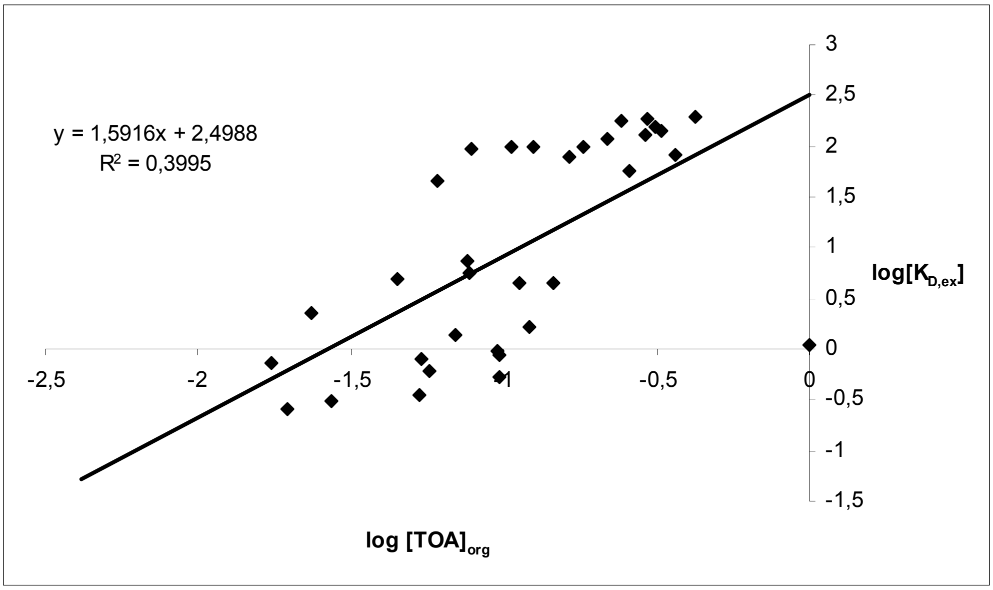

Figure 5.1.7 Variation of the distribution coefficient of pyruvic acid with TOA in 1-octanol as a function of free amine concentration in the organic phase

King and co-workers proposed an equation by combining Equations E5.1.2 and E5.1.4 for systems extracting more acid than needed for the neutralization of the amine (Kertes and King, 1986). According to Equation E5.1.9, the slope of a graph of z/(1-z) vs. $[HP]_{aq}$ gives the complexation constant for the (1-1) acid-amine complex. For the formation of (n-1) acid-amine complex, equation E5.1.10 is obtained and a plot of z/(n-z) versus $[HP]^n_{aq}$ is required. The slope yields the complexation constant of the corresponding reaction

$$\frac{z}{1-z} = K_{E1}[HP]_{aq} \quad \text{(E5.1.9)}$$

$$\frac{z}{n-z} = K_{En}[HP]^{n}{}_{aq} \quad \text{(E5.1.10)}$$

The complexation constant of (1-1) pyruvic acid-trioctylamine complex was obtained for extraction of pyruvic acid with trioctylamine in 1-octanol (Figure 5.1.8). For values of loading ratios less than 0.5 and for equilibrium concentration of pyruvic acid in the aqueous phase less than 4×10^{-3} M, the complexation constant for (1-1) acid-amine complex was

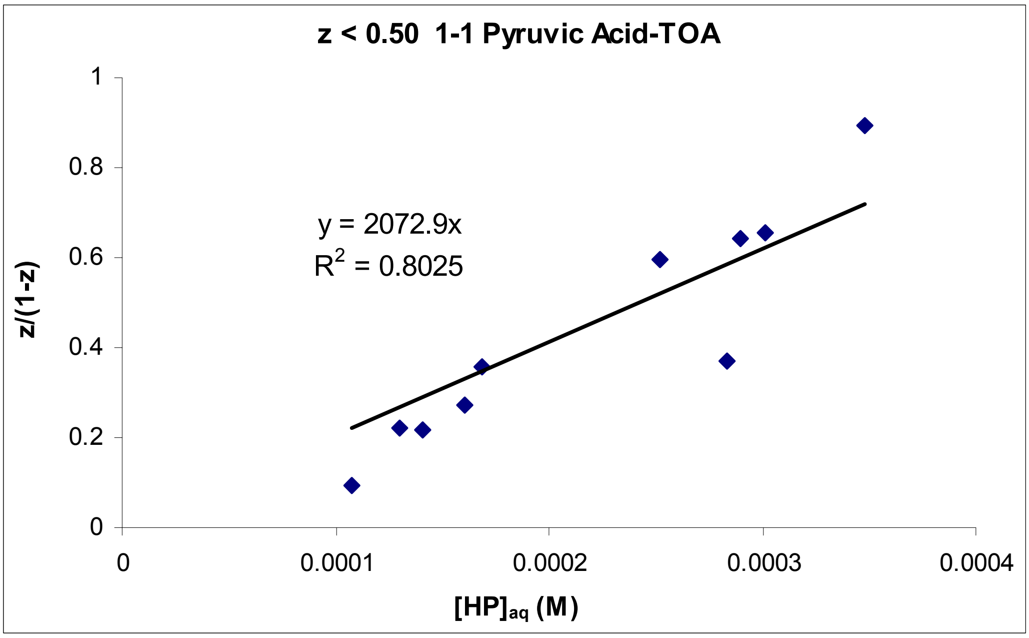

Figure 5.1.8 Plot of z/(1-z) vs $[HP]_{aq}$ for estimation of (1-1) pyruvic acid-trioctylamine equilibrium complexation constant (z<0.5)

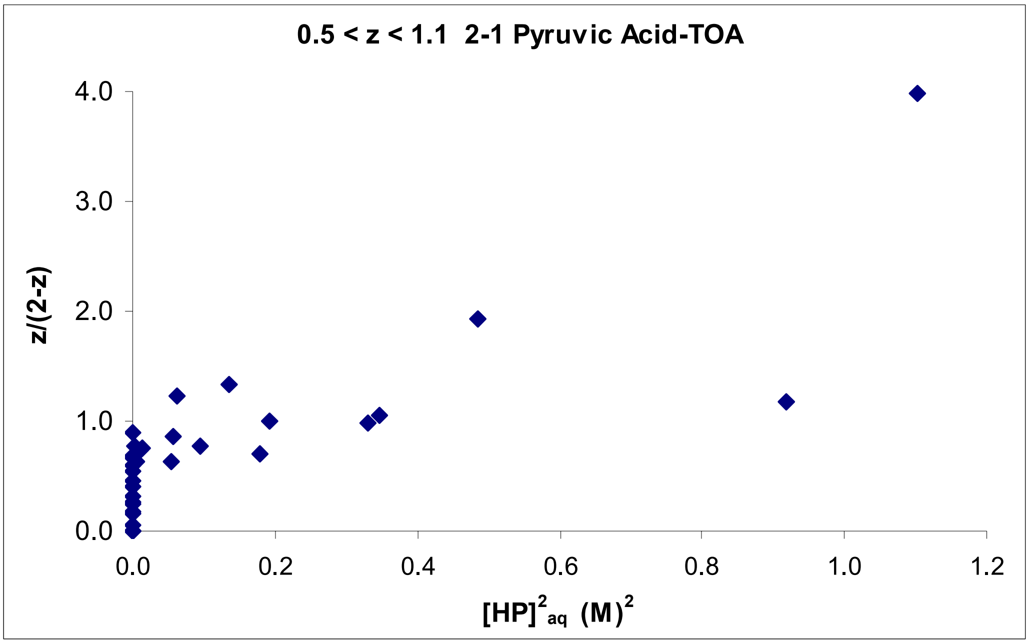

Figure 5.1.9 Plot of z/(2-z) vs $[HP]^2_{aq}$ for estimation of (2-1) pyruvic acid-trioctylamine equilibrium complexation constant

calculated as 2072.9 L mol^{-1}. It is seen that complexation constant calculated with the use of Equation 5.1.8 and Equation 5.1.9 are not equal. Because at the second calculation only the data with loading ratios less than 0.5 are considered, while nearly all data are taken into consideration at the first one.

In Figure 5.1.9, the complexation constant of the (2-1) acid-amine complex was attempted to be calculated by using the data and the plot of z/(2-z) vs. $[HP]^2_{aq}$. Since a straight line through the origin could not be obtained, the complexation constant of the pyruvic acid - trioctylamine reaction could not be estimated with respect to Equation E5.1.10 with the available data. It can be seen that the equation, which is derived from Mass Action Law is not valid for the values of loading ratios greater than 0.5 (z>0.5) for this system.

The procedure for the estimation of equilibrium complexation constant of the reaction between pyruvic acid and trioctylamine in 1-octanol was repeated for other two acids. On Figures 5.1.10 and 5.1.11, the plots for lactic acid and on Figures 5.1.12 and 5.1.13, those for acetic acid are given. The complexation constant for the formation of (1-1) lactic acid-trioctylamine complex was estimated as 139.32 26.12 L mol^{-1} for equilibrium concentrations in the aqueous phase less than 0.008 M and loading values less than 0.5, while it was estimated as 26.12 L mol^{-1} for similar loading values and equilibrium concentrations in the aqueous phase less than 0.04 M for acetic acid-trioctylamine complex. As in the case of pyruvic acid, the plots of z/(2-z) vs. $[HA]^2_{aq}$ did not yield linear relations for both acids.

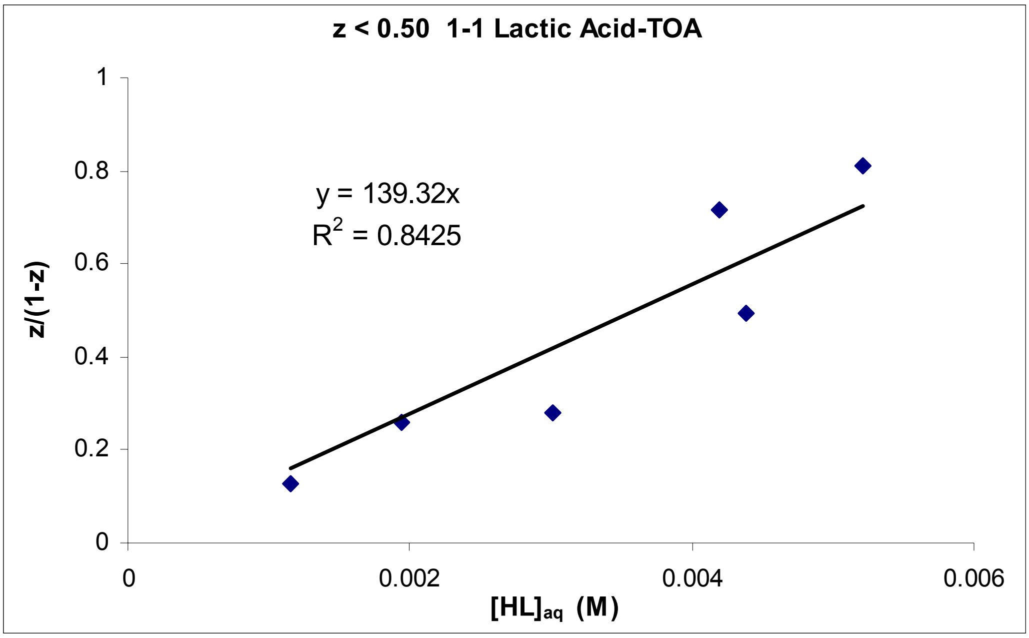

Figure 5.1.10 Plot of z/(1-z) vs $[HAc]_{aq}$ for estimation of (1-1) acetic acid-trioctylamine equilibrium complexation constant (z<0.5)

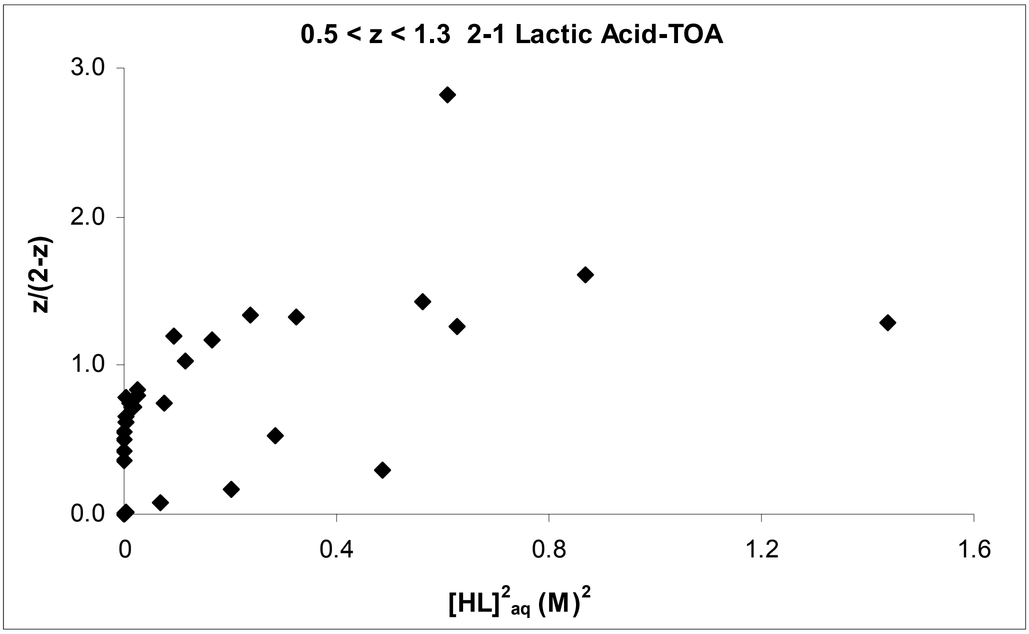

Figure 5.1.11 Plot of z/(2-z) vs $[HAc]^2_{aq}$ for estimation of (2-1) lactic acid-trioctylamine equilibrium complexation constant

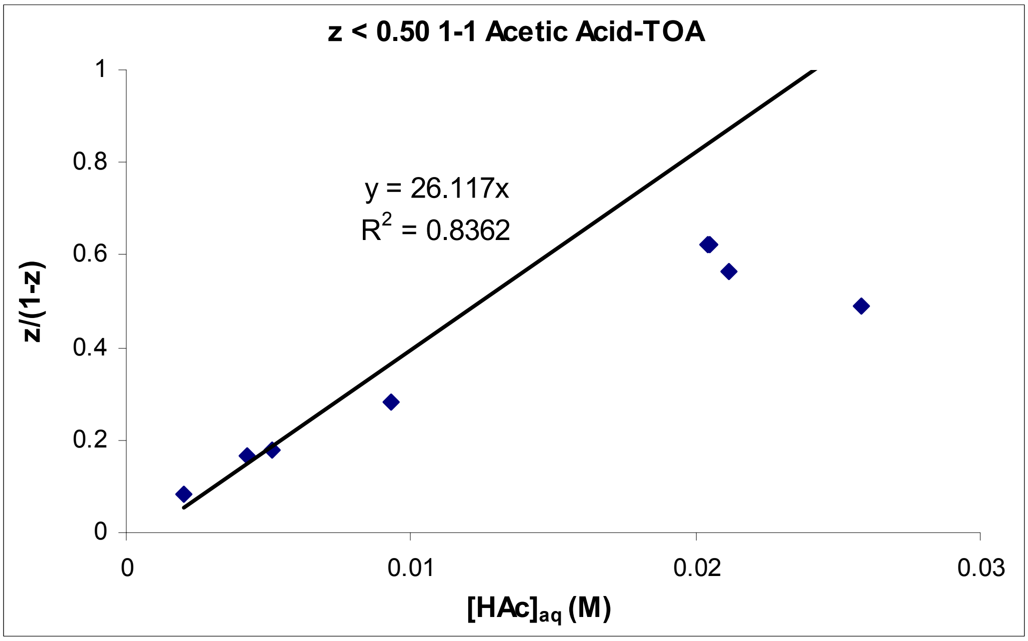

Figure 5.1.12 Plot of z/(1-z) vs $[HL]_{aq}$ for estimation of (1-1) acetic acid-trioctylamine equilibrium complexation constant (z<0.5)

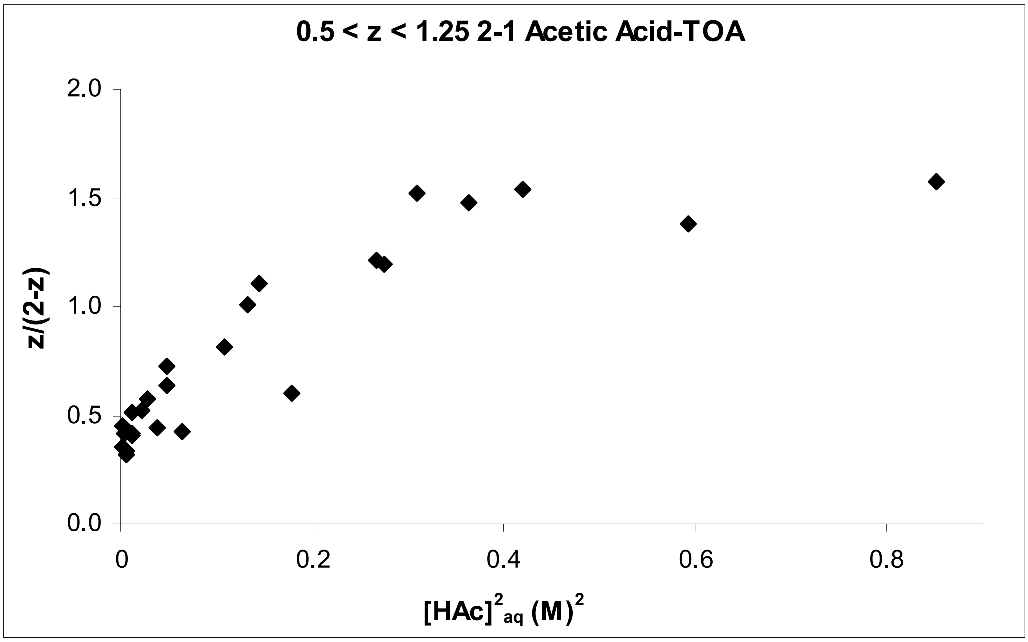

Figure 5.1.13 Plot of z/(2-z) vs $[HL]^2_{aq}$ for estimation of (2-1) acetic acid-trioctylamine equilibrium complexation constant

5.1.1.6. Effect of Initial pH of the Aqueous Phase

The pH dependency of reactive extraction of carboxylic acids is more noticeable when tertiary amines are used as extractants. Compared to quaternary amine salts, they have the disadvantage of not being able to react with the dissociated portion of the acid. This means that, the transfer of the acids with tertiary amines is affected by the pH value of the aqueous solution and the pK_A of the corresponding acid. These two dependencies were used in the recovery of carboxylic acids from their mixed acid solutions. As stated earlier, in the present study trioctylamine was utilized to achieve a selective separation of pyruvic acid from an aqueous solution that has an equivalent carboxylic acid composition of a biological production medium. Accordingly, reactive extraction of pyruvic acid from its single acid solution with trioctylamine in 1-octanol at various pH values was studied before investigating the behavior of the mixed acid solution of pyruvic, acetic and lactic acids. The initial amount of undissociated acid in the aqueous phase can be estimated using Henderson-Hasselbach Equation:

$$pH = pK_A + \log\left(\frac{\left[P^-\right]_{aq}}{\left[HP\right]_{aq}}\right) \tag{E5.1.11}$$

With respect to this equation, the amount of undissociated acid in the aqueous phase depends on the pK_A of the acid and the pH of the aqueous solution. In order to achieve a high recovery, pH of the solution should be lower than the pK_A of the carboxylic acid, which is 2.49 for the pyruvic acid. Nonetheless, the pH of the production medium was reported higher than this value (Eisenberg *et al.*, 1997, Zelic *et al.*, 2003). Eisenberg *et al.* (1997) produced pyruvic acid using methylotrophic yeast transformants by enzymatic methods and stated that the final pH of their production media was between 6 and 7. In another study Zelic *et al.* (2003) reported that the final pH of the production media was 3.0 or higher with the direct fermentation method. As known, acidic conditions are harmful for the microorganisms in the production media. It can be concluded that the pH of the production media can be varied depending on the substrate, microorganism, components in the production media and the production method. Therefore, forward extraction experiments with aqueous solution of pyruvic acid and 1-octanol solution of trioctylamine were conducted to observe the effect of initial pH value of the aqueous phase on the distribution coefficient. In these experiments, the concentration of pyruvic acid in the aqueous phase was kept constant at 0.5 M, and the effects of concentration of the extractant and the initial pH of the aqueous phase were observed. The effect of concentration of the extractant is clearly seen at a low pH value such as 1.35, which is the pH value of the 0.5 M aqueous solution of pyruvic acid without pH adjustment. A sharp decrease is observed in the distribution coefficient of pyruvic acid with an increase in the initial pH value of the aqueous solution. As expected, decrease in the distribution coefficient is more dramatic for higher concentrations of the extractant. The results are consistent with those in the literature for lactic, acetic and succinic acids (Kyuchoukov *et al.*, 2001, Hong and Hong, 2005, Huh *et al.*, 2006).

Similar to the results obtained by Ma *et al.* (2006), the distribution coefficient of pyruvic acid decreases sharply with an increase in the pH from about 1.35 to about 2.0. The effect of the extractant concentration in the organic phase disappears at higher initial pH values of the aqueous phase. When the initial pH of the aqueous phase is about 4.0; most of the tertiary amine molecules can not react because most of the acid in the aqueous is in dissociated form. The distribution coefficient is nearly 0.10 at those conditions, which is consistent with the literature values. Ma *et al.* (2006) obtained a distribution coefficient value of 0.20 for a 0.37 M pyruvic acid solution at a pH value of 3.70. As calculated with the Henderson-Hasselbach equation, at an initial pH value of 4, only three percent of the acid is undissociated. Results of experiments on the reactive extraction of pyruvic acid with trioctylamine in 1-octanol for parametric values of concentrations and the pH are presented in Tables A3.1.1-4 and are plotted on Figure 5.1.14.

The effect of the initial pH of the aqueous solution was studied with lactic and acetic acids. The trend observed in the case of pyruvic acid was observed with these acids as well. As can be seen in Figures 5.1.15 and 5.1.16, the distribution coefficients decreases with an increase in the initial pH value of the aqueous phase and for pH values about 1.0-1.5 pH units greater than the pK_A value of the corresponding acid, there is practically no transfer of any carboxylic acid to the organic phase. Results for acetic and lactic acids are presented in Tables A3.2.1-4 and A3.3.1-4.

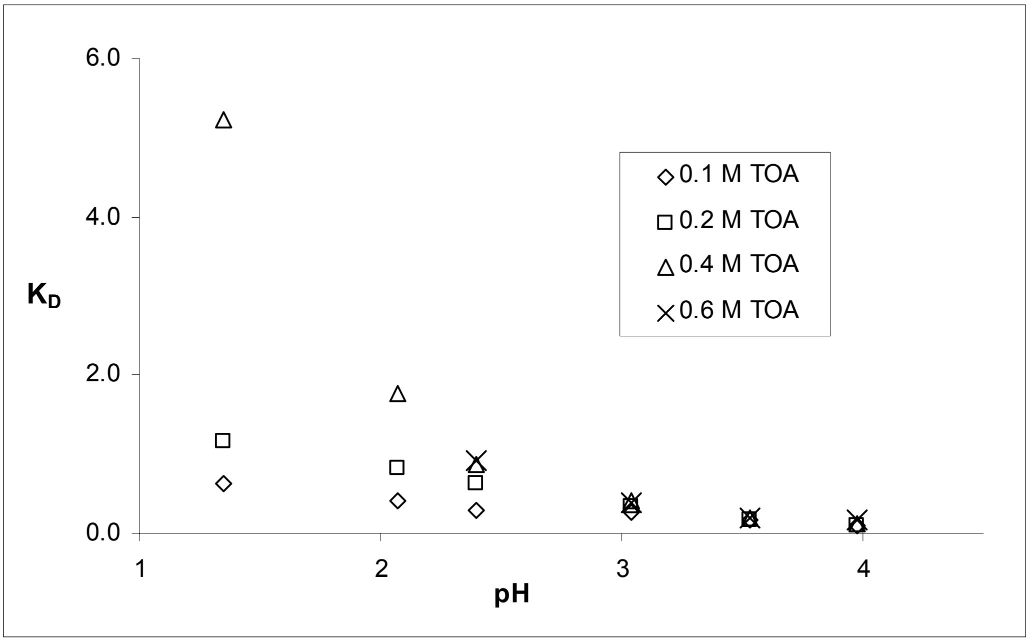

Figure 5.1.14 Variation of the distribution coefficient of pyruvic acid with the initial pH of the aqueous phase and the concentration of extractant in the organic phase

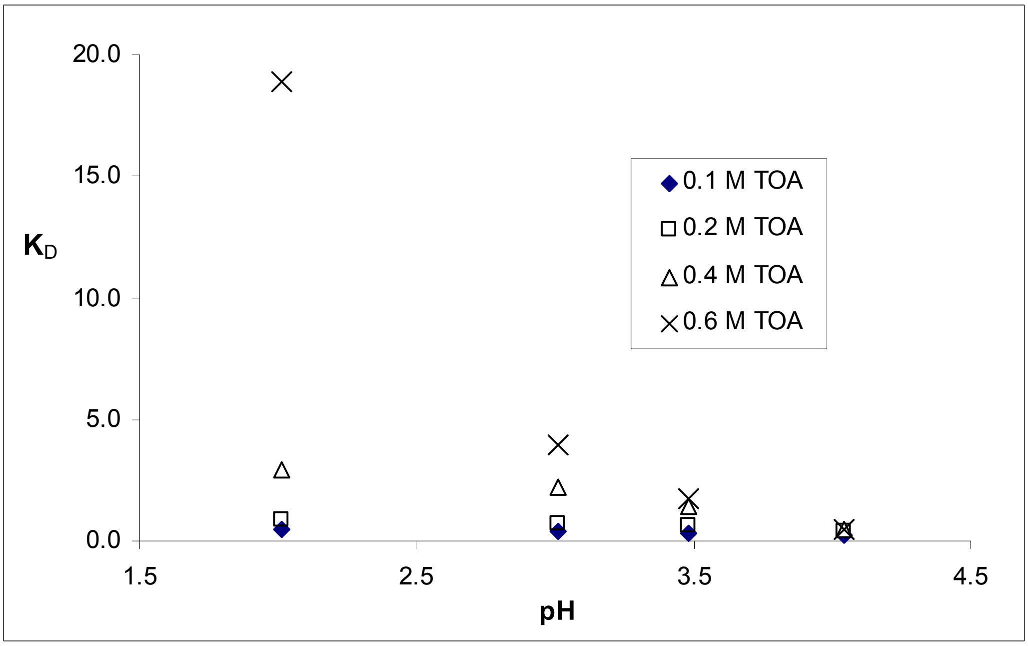

Figure 5.1.15 Variation of the distribution coefficient of lactic acid with the initial pH value of the aqueous phase and the concentration of the extractant in the organic phase

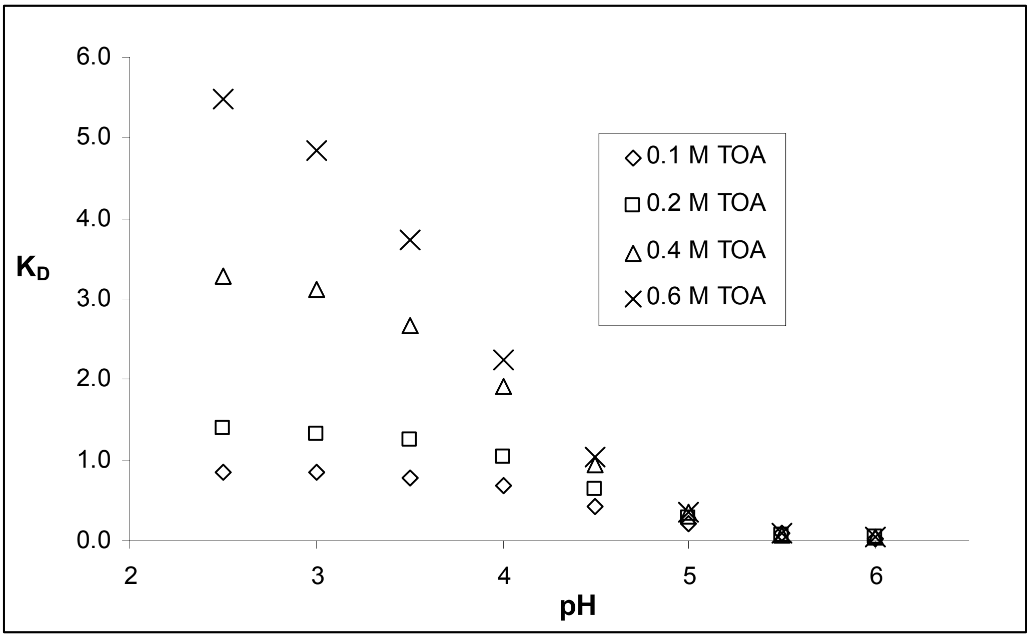

Figure 5.1.16 Variation of the distribution coefficient of acetic acid with the initial pH value of the aqueous phase and the concentration of the extractant in the organic phase

Table 5.3 Dissociation constants of the carboxylic acids investigated in the present study

Acid Name	**K_A Value**	**pK_A Value**
Acetic Acid	1.78×10^{-5}	4.75
Lactic Acid	1.38×10^{-4}	3.86
Pyruvic Acid	3.24×10^{-3}	2.49

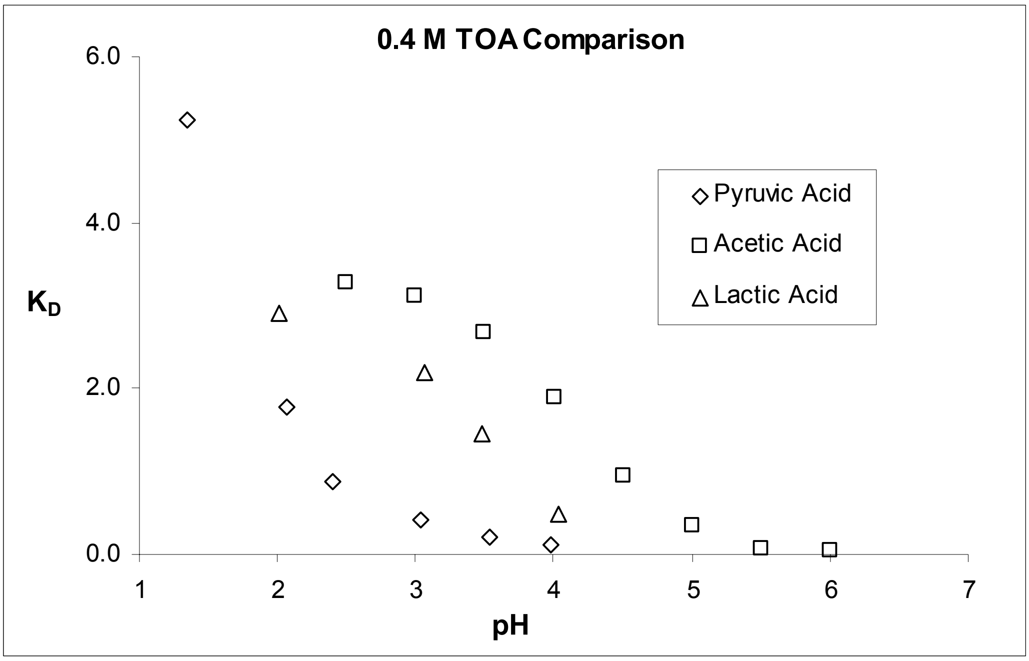

Figure 5.1.17 Comparison of distribution coefficient of the three acids as a function of the initial pH of the aqueous phase

In Figure 5.1.17, the distribution coefficients of the three acids were compared at various initial pH values while maintaining the initial concentrations in the aqueous and organic phases constant. The initial pH values of the 0.5 M aqueous solutions of the acids are, 1.35 for pyruvic acid, 2.00 for lactic acid and 2.50 for acetic acid. It is observed that in the pH range of pH 2 to 4, acetic acid has the highest distribution coefficient due to its highest pK_A whereas the pyruvic acid has the lowest because of its lowest pK_A (Table 5.3).

As stated earlier, initial amount of undissociated acid in the aqueous phase is dependent on the pK_A of the corresponding acid and the pH of the solution, which directly affect the distribution coefficient. It is seen in the figure that the trends of the distribution coefficients with the initial pH value of the aqueous phase are similar for the three acids. The lack of transfer of the acids is observed to be close to a pH 4 value for pyruvic, a pH 5 value for lactic and a pH 6 value for acetic acid. Between pH 1 and pH 2, a dramatic decrease in the distribution coefficient of pyruvic acid is observed. In this range, data for the distribution coefficient of acetic and lactic acid do not exist. However, previous investigators found that in this range it is nearly constant for both acids (Yang *et al.*, 1991).

In the next range, between pH 2 and pH 3, decrease is seen in the distribution coefficient of all three acids, although in different ratios. The ratio of decrease in the distribution coefficient of pyruvic acid is higher than the others. Lactic acid has the second and the acetic acid has the third when we compare the ratio of decreases in this range. The highest rate of decrease for pyruvic acid is related with its pK_A value, 2.49, which is in this range. This behavior for the distribution coefficient of pyruvic acid is consistent with our expectations and the literature data. In the pH range of pH 3 and pH 4, distribution coefficients continued to decrease at different ratios. In this range, the ratio of decrease is highest for lactic acid due to its pK_A value, 3.86. The smallest ratio of decrease is observed for pyruvic acid because the pH of the solution is 0.5-1.5 units higher than the pK_A of the corresponding acid. Hence, there is an insufficient proportion of undissociated acid in the aqueous phase for the acid-amine reaction. Among all the pH values, the highest rate of decrease for lactic acid is obtained in this range, between pH 3 and pH 4. This behavior is also consistent with the results in the literature reported by earlier investigators (Yang *et al.*, 1991, Tung and King, 1994). As is expected, in the next pH range, the ratio of decrease is highest for acetic acid among the acids studied owing to its pK_A value of 4.75. Similar to lactic acid, the highest ratio of decrease of acetic acid is seen in this range due to its pK_A value. This is consistent with the literature (Yang *et al.*, 1991, Siebold *et al.*, 1995).

5.1.1.7. Reproducibility of Equilibrium Data: The Comparison of Data Obtained at the Middle East Technical University and Iowa State University

In this sub-section, reproducibility of equilibrium data was checked by the results obtained in two different laboratories. The results of some equilibrium experiments for reactive extraction of pyruvic acid with trioctylamine in 1-octanol obtained at the Middle East Technical University are compared with the data obtained at the Iowa State University. The materials and instruments used in each laboratory were explained in the Section 4.1.

First, the distribution coefficient of pyruvic acid with 1-octanol alone was checked. As mentioned earlier, this constant is very important in the calculation of loading ratio, and consequently in the determination of stoichiometry of the acid-amine complex. For this purpose, solvent extraction experiments were conducted for the distribution coefficient of pyruvic acid with 1-octanol at the Iowa State University. The data obtained at the Iowa State University is presented in Table A4.1. It can be seen in Figure 5.1.18 that the distribution coefficient obtained at the Iowa State University is 0.3021, which is almost equal to the distribution coefficient (0.3022) obtained at METU (Figure 5.1.2).

In the second part of reproducibility studies, variation of distribution coefficient with the equilibrium concentration of pyruvic acid in the aqueous phase was checked. The organic phase consisted of 0.3 M trioctylamine dissolved in 1-octanol. It can be seen in Figure 5.1.19 that the distribution values obtained with 0.3 M TOA in 1-octanol are in between the distribution values previously obtained at Middle East Technical University with 0.2 and 0.4 M TOA in 1-octanol. The trend of the change is similar to the ones obtained for other concentration levels of extractant in the organic phase: showing a distribution ratio increase with a decrease in the equilibrium concentration of pyruvic acid in the

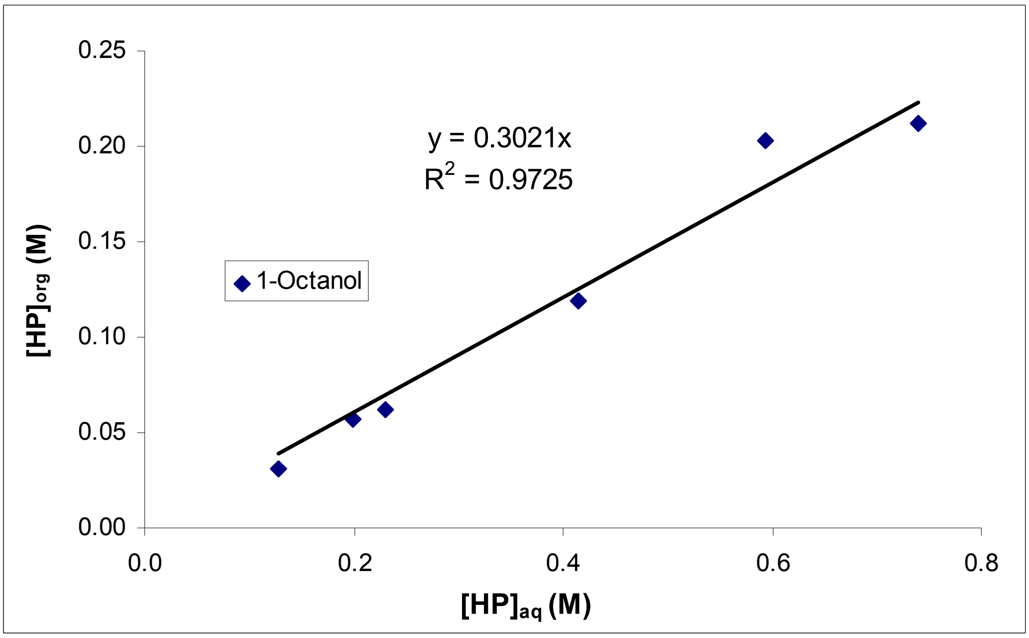

Figure 5.1.18 Solvent extraction of pyruvic acid with 1-octanol (results obtained at the Iowa State University)

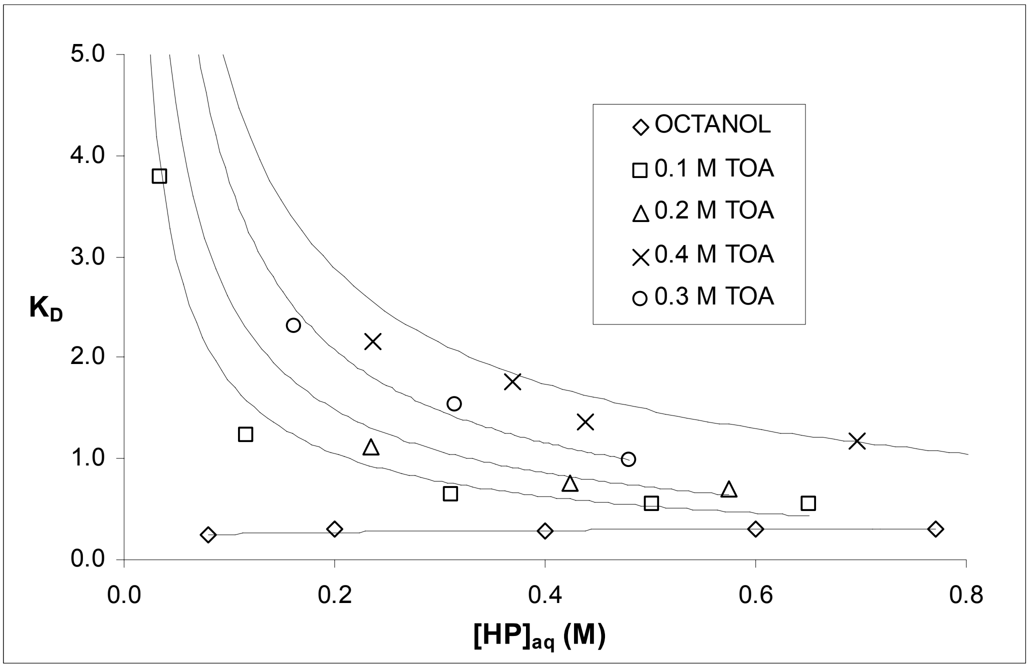

Figure 5.1.19 Variation of the distribution coefficient with equilibrium concentration of pyruvic acid in the aqueous phase for various concentration levels of trioctylamine in 1-octanol

aqueous phase and an increase in initial concentration of extractant in the organic phase. At very low acid concentration levels in the aqueous phase, the distribution coefficient values are extremely high and it decreases sharply with an increase in the equilibrium acid concentration in the aqueous phase, to a value of 0.2 mol L^{-1}. Beyond this value, distribution coefficient continues to decrease slightly, as can be observed in Figure 5.1.4. These results confirmed that data obtained with the use of different instruments and brands of chemicals in the two different laboratories are consistent with each other. The results of these experiments are given in detail in Table A4.2

In the last part of reproducibility studies, variation of the distribution coefficient with the initial concentration in the organic phase was investigated. Initial concentration of the aqueous phase was maintained at 0.5 M. The concentration of trioctylamine in 1-octanol was varied from 0.1 to 0.6 M. It can be seen in Figure 5.1.20 that the distribution coefficient values obtained at the two laboratories using different equipment are nearly the same for similar conditions. The difference in the distribution coefficient values obtained at the two laboratories using an organic phase with 0.6 Mol L^{-1} trioctylamine is probably due to extremely low concentrations of carboxylic acid left in the aqueous phase at equilibrium and the difficulty in its accurate measurement. Thus when equilibrium acid concentration values in the aqueous phase is lower than about 0.05 M, the distribution coefficient values are extremely high and may differ due to very low concentrations in the aqueous phase. The results of these experiments are given in detail in Table A4.3

The results obtained with the different brand of chemicals and different instruments at the Middle East Technical University and the Iowa State University showed that similar distribution coefficient values are obtained under similar conditions.

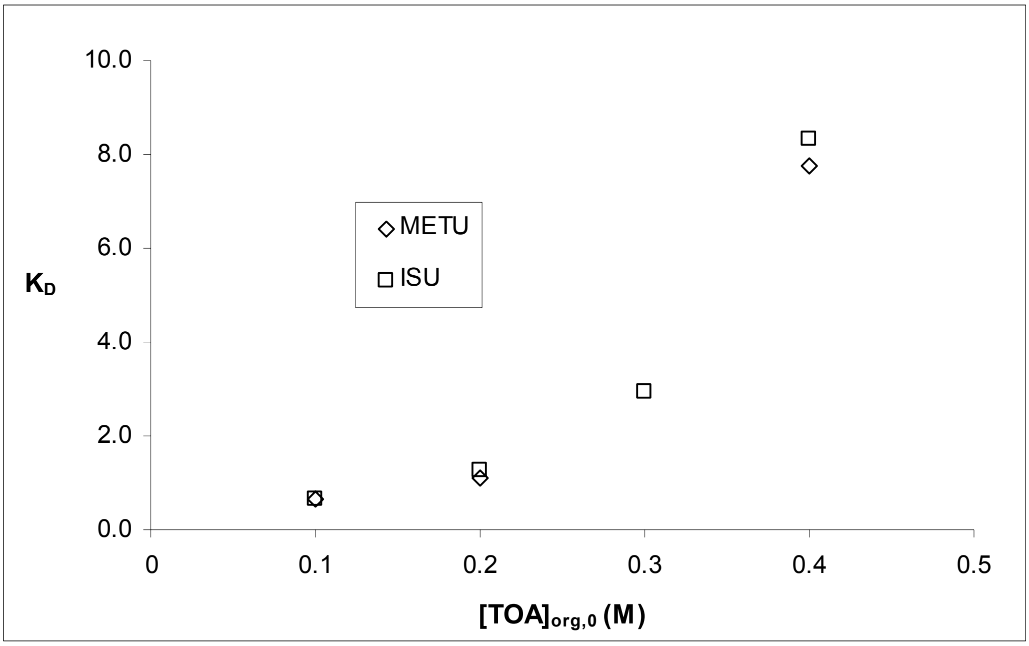

Figure 5.1.20 Comparison of the data obtained at different laboratories for the reactive extraction of pyruvic acid from its aqueous solution of 0.5 M with organic phases with several concentrations of TOA

5.1.2. Results of Equilibrium Studies for Mixed Acid Solutions

As stated earlier, one of the purposes of the present study is the recovery of carboxylic acids with reactive extraction from a mixed acid solution containing pyruvic, lactic and acetic acids which has an equivalent carboxylic acid composition of a biotechnological production medium. An organic phase consisting of trioctylamine as the extractant and 1-octanol as the diluent was used for this purpose. The effects of concentration of the extractant in the organic phase and the initial pH value of the mixed acid solution on the distribution coefficients of the three acids were examined. The results of these experiments were compared with the results obtained in the extraction of carboxylic acids from their single acid solutions to determine the effect of the presence of other acids on extraction.

Eisenberg *et al.* (1997) stated that while pyruvic acid is produced by enzymatic methods using as biocatalysts, methylotrophic yeast transformants, besides the main product, acetic acid is formed as a by-product and there remains unreacted lactic acid in the production medium. In order to optimize the process conditions for the recovery of pyruvic acid from a solution with an equivalent carboxylic acid composition of a biological production medium, a mixed acid solution was prepared containing 0,75 M pyruvic, 0,08 M acetic, and 0,05 M lactic acids and forward extraction experiments were performed with this mixed acid solution. These studies were followed by equilibrium experiments for the recovery of pyruvic and lactic acids from their binary solutions with trioctylamine dissolved in 1-octanol.

5.1.2.1. Reactive Extraction of Carboxylic Acids from Ternary Acid Solutions

In many biological production systems, carboxylic acids are produced as a target or as a by-product and their recovery is not a straightforward task. Thus, recovery of carboxylic acids from their biological production medium consisting of mixed acid solutions continues to be a challenging task. Tertiary amines were used by several investigators to selectively recover carboxylic acids from their mixed acid solutions (Juang and Huang, 1997, Hong and Hong, 2005, Huh *et al.*, 2006, Ma *et al.*, 2006). Extraction power of tertiary amines differ depending on the pK_A value of the weak acids and the pH of the aqueous solution, since they can only react with the undissociated part of the acid. As stated earlier, the concentration of the initial undissociated acid in the aqueous phase can be estimated by the Henderson-Hasselbach Equation (E5.1.11). According to this equation, it is expected that a carboxylic acid with a higher pK_A value is expected to be extracted preferably than an acid with a lower pK_A value. This difference in the extraction efficiencies of the acids have been utilized for the recovery of the carboxylic acids from their mixed acid solutions (Hong and Hong, 2005, Huh *et al.*, 2006). Extraction efficiency of the acids studied in the present investigation is compared in Figure 5.1.17. Reactive extraction of the three acids were conducted from their 0.5 M single acid solutions, with trioctylamine in 1-octanol, at different pH values and extractant concentrations. As expected, with an increase in the concentration of the extractant, the distribution coefficient of the acids increase. When the distribution coefficient of the acids are compared, decreasing order of the distribution coefficient values is in the order of the decreasing order of the pK_A values of the three acids (see Section 5.1.1.6).

$$pH = pK_A + \log\left(\frac{[P^-]_{aq}}{[HP]_{aq}}\right) \tag{E5.1.11}$$

Equilibrium experiments for the reactive extraction of the acids from their ternary acid solutions were started with the solvent extraction experiments with 1-octanol alone. Since 1-octanol is an active diluent, it extracts an amount of solute from the aqueous phase depending on the distribution coefficient of the acid with the corresponding diluent. The results of equilibrium experiments showed that the distribution coefficient values of the acids with 1-octanol alone from their single acid solutions are same as the ones with the same diluent from their mixed acid solution. Hence, the amount of the acids kept by 1-octanol is subtracted in the calculation in order to find the amount of acid bound to the extractant.

In Figures 5.1.21, 5.1.22, 5.1.23, and 5.1.24, the effect of the initial pH of the aqueous solution on the distribution coefficients of the three acids for a constant composition of the organic phase is shown. It can be seen in the figures that, with an increase in pH of the aqueous phase, distribution coefficient values of the acids decrease in a similar manner obtained with the single acid solutions. In Figure 5.1.21, a plot of the results of the reactive extraction of the acids with 0.1 M trioctylamine in 1-octanol are shown. Acetic acid has the highest pK_A value among the acids in the aqueous solution and due to this, it is preferentially extracted. A second reason for the higher value of the distribution coefficient value of the acetic acid compared to that of the pyruvic acid is its lower initial concentration.

According to the results obtained on the effect of the concentration of carboxylic acid and the initial pH value of the aqueous phase, lactic acid was expected to be extracted with the second highest efficiency from the aqueous solution; however, the distribution coefficient values of the pyruvic and lactic acids were found to be similar. It can be seen that at a pH value of 2.0, the distribution coefficient of acetic acid reaches a maximum and more than one third of the acetic acid is extracted in only one stage with impurity level concentrations of the two other acids. At a pH value of 4.0, the concentration of the other two acids become even smaller, however, the amount of acetic acid extracted decreases to one fourth of its initial amount.

In order to observe the effect of concentration of the extractant on the distribution coefficient, reactive extraction of the acids with 0.2 M trioctylamine in 1-octanol was conducted under various initial pH values. The results are plotted on Figure 5.1.22. A relatively small change is observed in the distribution coefficient of the acetic and lactic acids, while that of the pyruvic acid decreases from a pH value of 1.5 to about 4.0. Beyond this pH value, distribution coefficient of the pyruvic acid is nearly identical to the value obtained with the 0.1 M trioctylamine.

Unexpectedly, with an increase in the concentration of the extractant, the distribution coefficient of lactic acid remains relatively constant while that of the pyruvic acid increases. It may be argued that due to its additional hydroxyl group and the resulting higher hydrophilicity, lactic acid has a higher tendency to remain in the aqueous phase. The same behaviour was observed by Ma *et al.* (2006) during reactive extraction of pyruvic and lactic acids from their binary aqueous solutions. Yang *et al.* (1991)

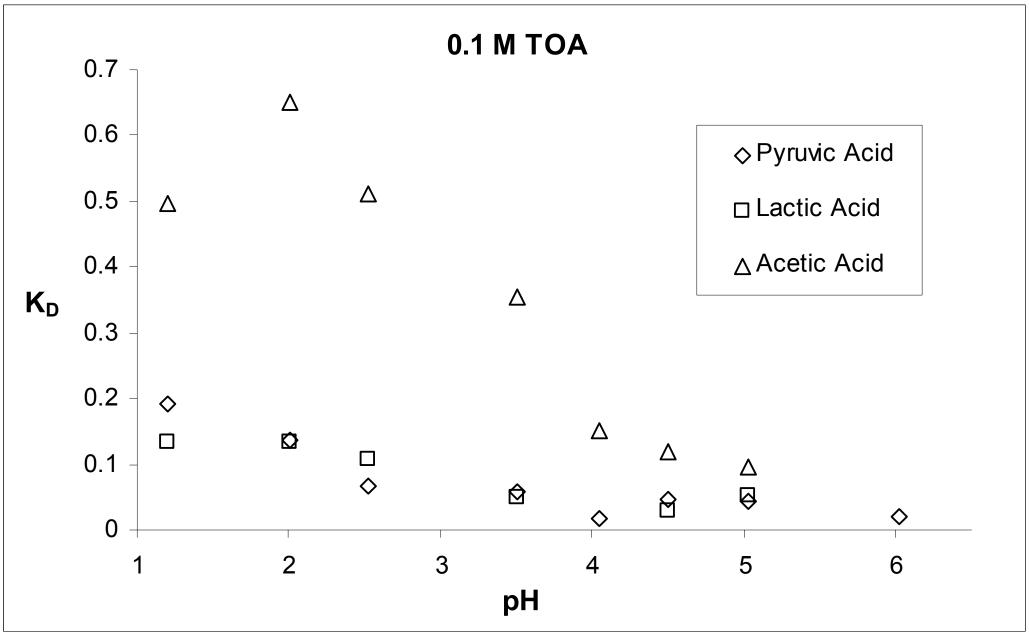

Figure 5.1.21 Variation of the distribution coefficient of the acids extracted from their mixed acid solution with 0.1 M trioctylamine in 1-octanol

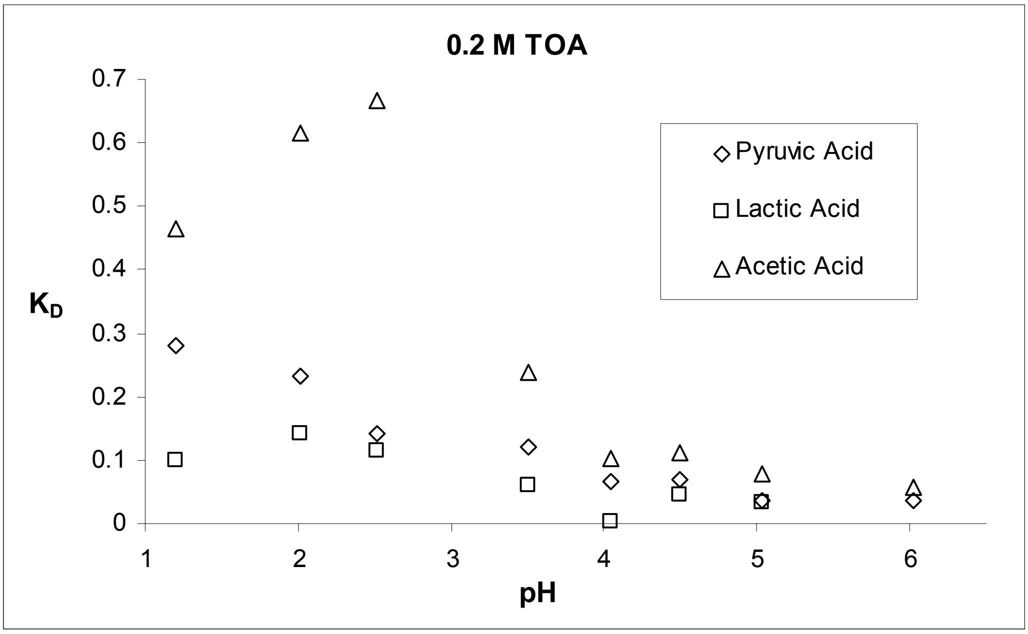

Figure 5.1.22 Variation of the distribution coefficient of the acids extracted from their mixed acid solution with 0.2 M trioctylamine in 1-octanol

compared the extraction of propionic and lactic acids and reasoned that lactic acid has a lower distribution coefficient because of its higher hydrophilicity. It is observed in Figure 5.1.23 that, when the initial concentration of the extractant is increased to 0.4 M, the distribution coefficients of acetic acid is relatively unaffected while that of pyruvic acid increases and reach nearly the ones of acetic acid. A very small increase in the distribution coefficient of lactic acid in the pH range of 3.0 to 4.0 is observed. However, still it is unexceptionally low. It is concluded that hydrophilicity of the lactic acid prevents its extraction from mixed acid solution.

Finally, concentration of the extractant was increased to 0.6 M. It can be seen in Figure 5.1.24 that there is a small increase in the distribution coefficient of acetic acid with an increase in the extractant concentration (note the change of scale) and the distribution coefficient of lactic acid is affected up to a pH value of 2.5. At a pH value of 1.2, pyruvic acid is extracted preferably with 0.6 M trioctylamine in 1-octanol, in spite of its highest initial aqueous phase concentration and its lowest pK_A value among the acids present in the mixed acid solution. However, similar to extraction with 0.4 M trioctylamine, the level of other acids co-extracted will have to be addressed. Results of the experiments, obtained with the ternary acid solutions are presented in Tables A5.1.1-4, A5.2.1-4 A5.3.1-4.

It is concluded that the distribution coefficient of pyruvic acid is negatively affected by the presence of other acids in the media. Especially acetic acid prevents extraction of pyruvic acid. In Figure 5.1.25, the antagonistic effects of the presence of acetic and lactic acids on the distribution coefficient of pyruvic acid can be seen. At every concentration level of the extractant, distribution coefficient of pyruvic acid is negatively affected by the simultaneous presence of the other acids. Acetic acid is preferentially extracted to the organic phase because of its highest pK_A value and its lower, compared to pyruvic acid, initial aqueous concentration level.

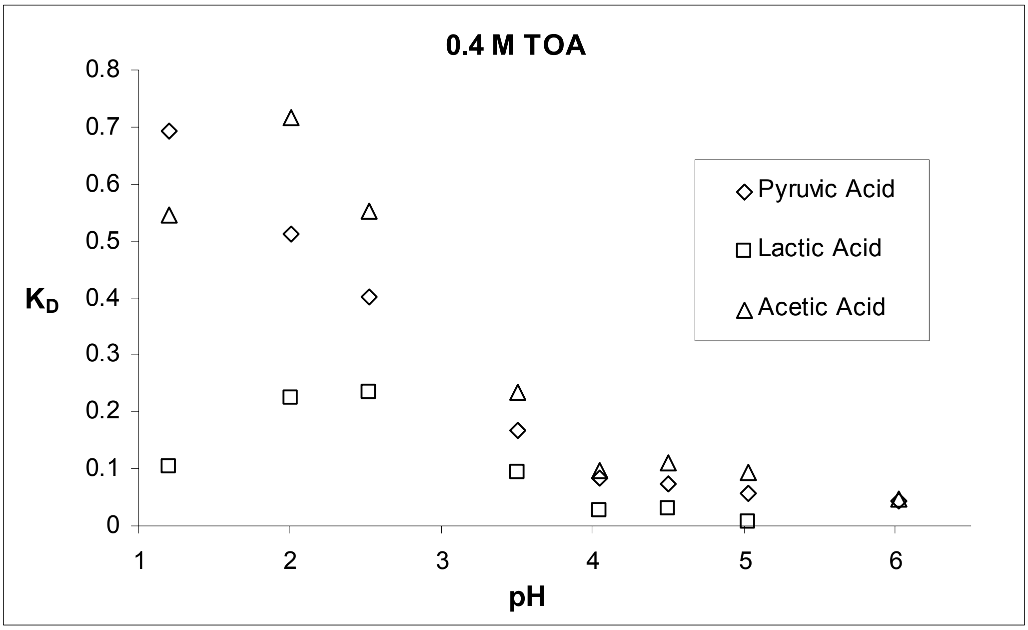

Figure 5.1.23 Variation of the distribution coefficient of the acids extracted from their mixed acid solution with 0.4 M trioctylamine in 1-octanol

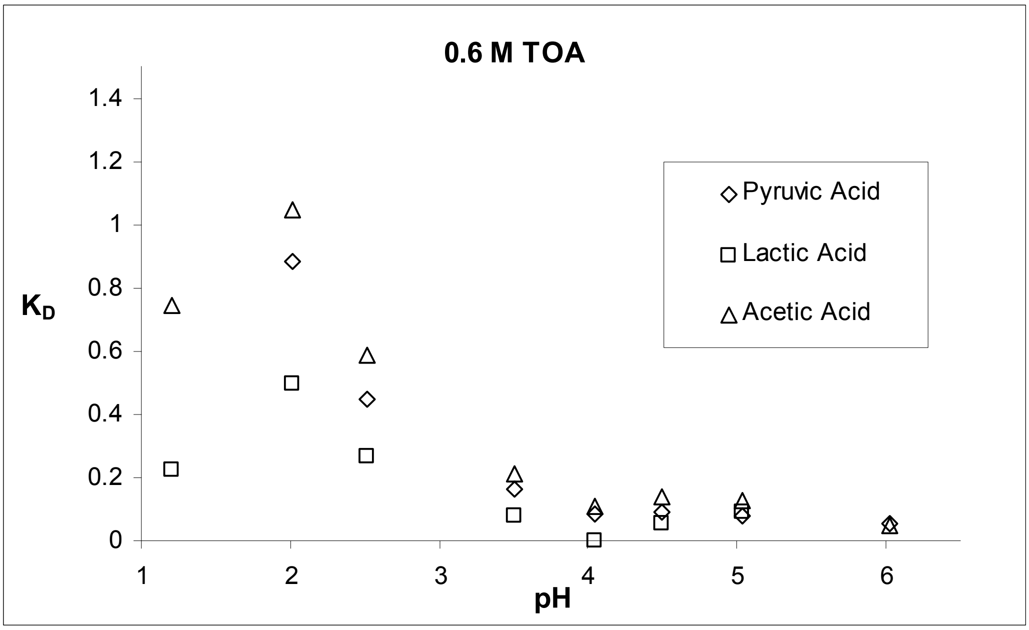

Figure 5.1.24 Variation of the distribution coefficient of the acids extracted from their mixed acid solution with 0.6 M trioctylamine in 1-octanol

These results are consistent with literature ones for various mixed acid solutions. Kirsch *et al.* (1998) indicated that acetic acid is extracted preferentially from binary solutions of citric-acetic and oxalic-acetic acids. Similarly, Hong and Hong (2005) observed the preferential removal of acetic acid from binary solutions of succinic and acetic acids.

Another important result is the hindrance caused by the presence of other acids on the transfer of lactic acid. Most probably, higher hydrophilicity of lactic acid prevents the transfer of lactic acid in the presence of other acids in the aqueous media. This is consistent with the literature results (Yang *et al.*, 1991). Although, it has the second highest pK_A value and the lowest initial aqueous phase concentration, lactic acid can not be extracted, at every level of extractant concentration in significant amount to the organic phase from a mixed acid solution. It is observed that acetic acid is preferentially extracted even at the lowest concentration level of trioctylamine, 0.1 M, at a pH value between 2.0 and 3.5. Thus, after an acetic acid removal step, the remaining solution is still predominantly composed of lactic and pyruvic acids.

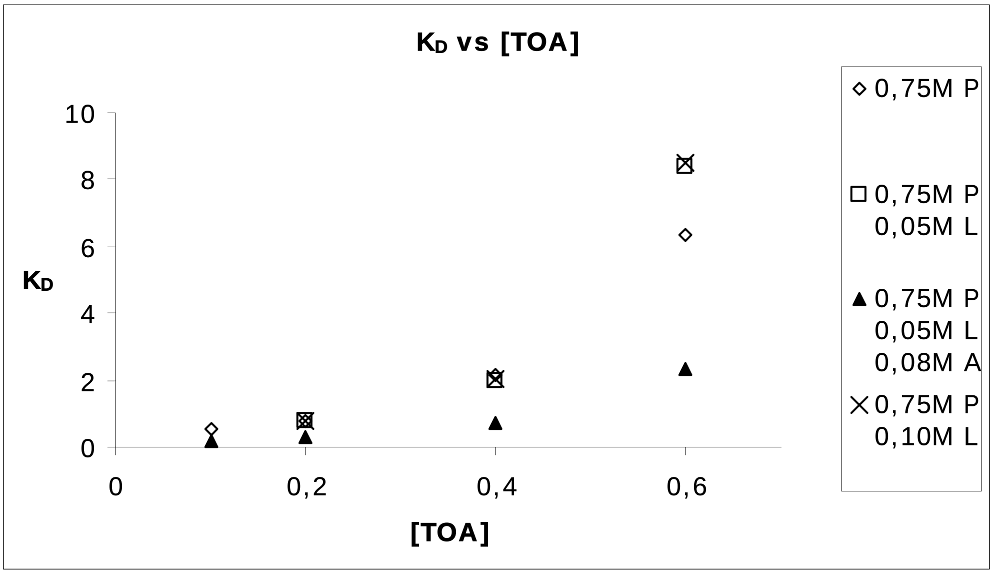

Figure 5.1.25 Effect of the presence of other acids on the distribution coefficient of pyruvic acid for various concentrations of extractant in the organic phase

Based on these results, a multi-stage reactive extraction system is proposed. Initially acetic acid is removed from the ternary acid solution with 0.1 M trioctylamine in 1-octanol. Following the removal of acetic acid, separation of lactic and pyruvic acids from their binary acid solutions can be considered. For this purpose, reactive extraction of pyruvic and lactic acids from their binary acid solutions was studied. The results are presented and discussed in the next section.

5.1.2.2. Stoichiometric Results of Equilibrium Experiments with Ternary Acid Solutions

In Figure 5.1.26, the variation of the loading ratio of pyruvic acid with the initial pH of the aqueous solution is shown for reactive extraction of pyruvic acid from a ternary acid solution containing acetic, lactic and pyruvic acids. A close examination of data indicates that for different concentration levels of the extractant, the trend is similar. That is the loading ratio increases to a maximum loading value and then decreases with an increase in the initial pH value of the aqueous phase. This trend is clearly observed for 0.2 M and 0.4 M trioctylamine concentration. The trend obtained at the 0.6 M trioctylamine concentration is similar for the decreasing part of the curve: loading ratio decreases with an increase in the initial pH value of the solution. Most probably an increasing part and a maximum would have been obtained if the pH could be further lowered. The data indicates that the loading ratio decrease with an increase in the pH value for all concentration levels of trioctylamine for the pH value range 3.5 to 5.5. This is consistent with the literature results for acetic and succinic acids (Hong and Hong, 2005) It is observed from the data presented in Figure 5.1.26 that for all values of the initial pH of the aqueous solution and for all extractant concentrations, the loading value is less than unity. Thus for the ranges of parameters investigated in the study, during pyruvic acid extraction from ternary aqueous solution, most probably the reaction yields a (1-1) type of acid-amine complex between pyruvic acid and trioctylamine in 1-octanol.

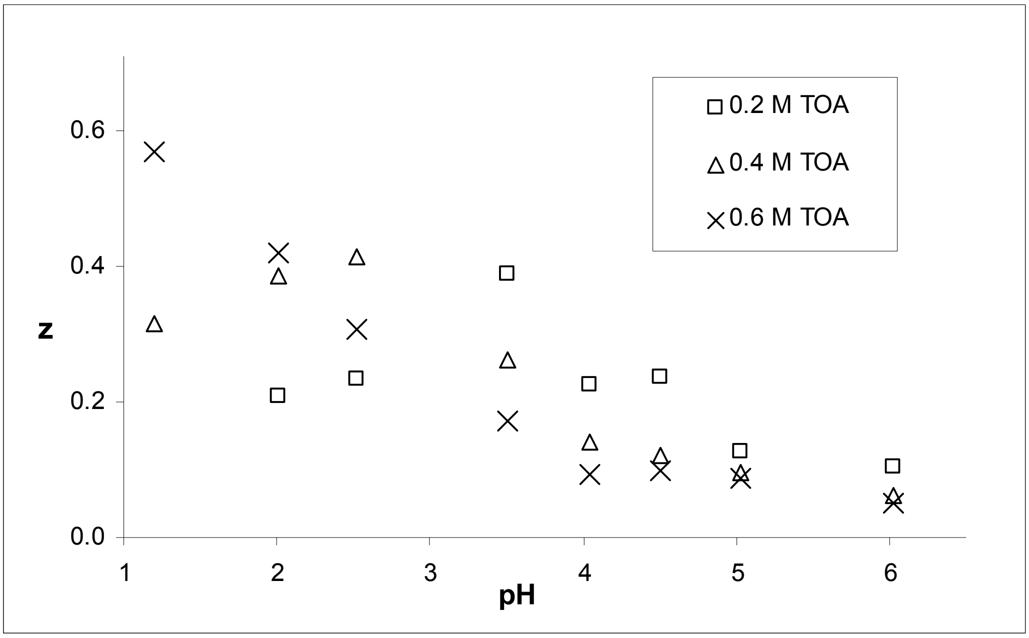

Figure 5.1.26 Variation of the loading ratio with the initial pH of aqueous phase

Table 5.4 The distribution of pyruvic and lactic acids in reactive extraction from their binary solutions

	Initial Conc. (M)	**Final Aqueous Phase Conc. with TOA in 1-octanol (M)**		
		0.2 M TOA	**0.4 M TOA**	**0.6 M TOA**
$[HP]_0$	0.85	0.50	0.31	0.14
$[HL]_0$	0.06	0.06	0.05	0.05
$[HP]_0$	0.75	0.42	0.25	0.08
$[HL]_0$	0.05	0.05	0.05	0.05
$[HP]_0$	0.75	0.41	0.20	0.06
$[HL]_0$	0.16	0.15	0.14	0.14

5.1.2.3. Reactive Extraction of Carboxylic Acids from Aqueous Binary Acid Solutions

In this part of the study, the results of reactive extraction of pyruvic and lactic acids from their aqueous binary solutions are discussed. Effect of the presence of a second acid on the distribution coefficient is investigated. While the total acid concentration in the aqueous phase was maintained constant, concentration of pyruvic and lactic acids were varied from 0.65 to 0.85 M and from 0.05 to 0.15 M respectively (Table 5.4). Lactic acid was expected to be extracted preferably from the binary solution since it has a greater pK_A value and a lower initial aqueous phase concentration. As can be seen in Table 5.4, contrary to this expectation extraction of lactic acid does not proceed to a significant extent. Most of the lactic acid is left in the aqueous phase. As explained in the previous section, this is most probably due to the higher hydrophilicity of lactic acid compared to that of pyruvic acid. On the other hand, the distribution coefficient of pyruvic acid from the binary solutions of pyruvic and lactic acids is nearly identical to the value obtained with the single acid. However a small synergistic effect is observed at concentration levels of extractant at 0.4 and 0.6 M. Thus, it can be concluded that the distribution coefficient of pyruvic acid is not

Table 5.5 Initial concentration of pyruvic and lactic acids in aqueous binary acid solutions

$[HP]_0$	$[HL]_0$	$[HP]_0 + [HL]_0$	$[HP]_0 / [HL]_0$
0.67 M	0.13 M	0.80 M	5.17
0.51 M	0.26 M	0.77 M	1.95
0.38 M	0.41 M	0.79 M	0.94
0.25 M	0.55 M	0.80 M	0.46
0.13 M	0.69 M	0.82 M	0.19

significantly affected in the concentration ranges of lactic and pyruvic acids studied by the presence of the lactic acid (Figure 5.1.25).

Effect of the concentration of the acids on the distribution coefficient of the lactic and pyruvic acids was investigated by maintaining the initial total acid concentration constant at 0.80 M and changing the initial concentration ratio of the acids. The initial concentration values of the

Table 5.6 The distributions and distribution coefficients of pyruvic and lactic acids in reactive extraction from their binary solutions

		Final Aqueous Phase Conc. (M)					
Initial Acid Conc. $[HA]_{aq,0}$ (M)		**0.2 M TOA**		**0.4 M TOA**		**0.6 M TOA**	
		$[HA]_{aq}$	**K_D**	**$[HA]_{aq}$**	**K_D**	**$[HA]_{aq}$**	**K_D**
PYR	0.67	0.36	0.86	0.13	4.15	0.05	13.03
LAC	0.13	0.12	0.07	0.12	0.10	0.10	0.24
PYR	0.51	0.25	1.07	0.09	4.69	0.02	28.44
LAC	0.26	0.24	0.10	0.23	0.16	0.16	0.69
PYR	0.38	0.16	1.47	0.03	10.06	0.01	53.43
LAC	0.41	0.37	0.11	0.32	0.28	0.17	1.34
PYR	0.25	0.07	2.63	0.01	20.91	0.00	58.01
LAC	0.55	0.49	0.12	0.36	0.54	0.21	1.59
PYR	0.13	0.02	6.59	0.01	24.62	0.00	76.77
LAC	0.69	0.56	0.23	0.40	0.73	0.23	2.01

aqueous phase are given in Table 5.5. The distributions and distribution coefficients obtained with trioctylamine in 1-octanol are presented in Table 5.6 and Table 5.7. It can be seen that reactive extraction of lactic acid is hindered by the presence of the pyruvic acid and comparatively higher but still low extent of extraction of lactic acid occurs at concentration ratio values lower than 1.95:1 ($[HP]_0 / [HL]_0$). The transfer of lactic acid in the presence of pyruvic acid is extremely low when compared to extraction from its single acid solution.

Table 5.7 Comparison of the distribution coefficients of pyruvic and lactic acids from their single and binary acid solutions

	Distribution Coefficient of Pyruvic Acid	
$[HP]_0$~0.51 M	**Zero Lactic**	**0.26 M Lactic Acid**
0.2 M TOA	1.11	1.07
0.4 M TOA	7.74	4.69
0.6 M TOA	158.88	6.30
$[HP]_0$~0.26 M	**Zero Lactic**	**0.55 M Lactic Acid**
0.2 M TOA	6.78	2.63
0.4 M TOA	180	20.91
0.6 M TOA	∞	58.01
	Distribution Coefficient of Lactic Acid	
$[HP]_0$~0.55 M	**Zero Pyruvic**	**0.26 M Pyruvic Acid**
0.2 M TOA	0.90	0.12
0.4 M TOA	3.78	0.54
0.6 M TOA	19.53	1.59

It can be also seen in Table 5.7 that the effect of lactic acid concentration on the distribution coefficient of pyruvic acid depends on the initial concentration of lactic acid in the solution. An antagonistic effect on the distribution coefficient of the acids owing to the other acid is observed for both acids.

Separation factor, another useful parameter, is used to specify the ratio of the distribution coefficients of two extractable solutes and is used when there is more than one solute (Equation 5.1.12). As can be observed in Figure 5.1.27, when the initial concentration of trioctylamine is 0.2 M, with an increase in the initial lactic acid concentration, the separation factor decreases because of a decrease in the distribution coefficient of pyruvic acid. The decrease is due to a limitation with respect to the amount of extractant in the organic phase. This is verified by observing the separation factor values for higher initial amine concentrations. Nearly no difference is observed for the separation factor with 0.4 M trioctylamine and an increase is noticed when the initial extractant concentration in the organic phase is 0.6 M.

$$\alpha_{HP/HL} = \frac{K_{D,HP}}{K_{D,HL}} \qquad \text{(E5.1.12)}$$

It is concluded that in order to achieve a selective recovery with a high distribution coefficient of pyruvic acid, recovery of pyruvic acid from a binary solution of pyruvic and lactic acids should be conducted at a low concentration of lactic acid. Otherwise, negative mutual effects may take place and a high selectivity will not be achieved. After a successful separation step for the pyruvic acid, remaining lactic acid in the aqueous phase can be recycled to the production medium to produce more pyruvic acid.

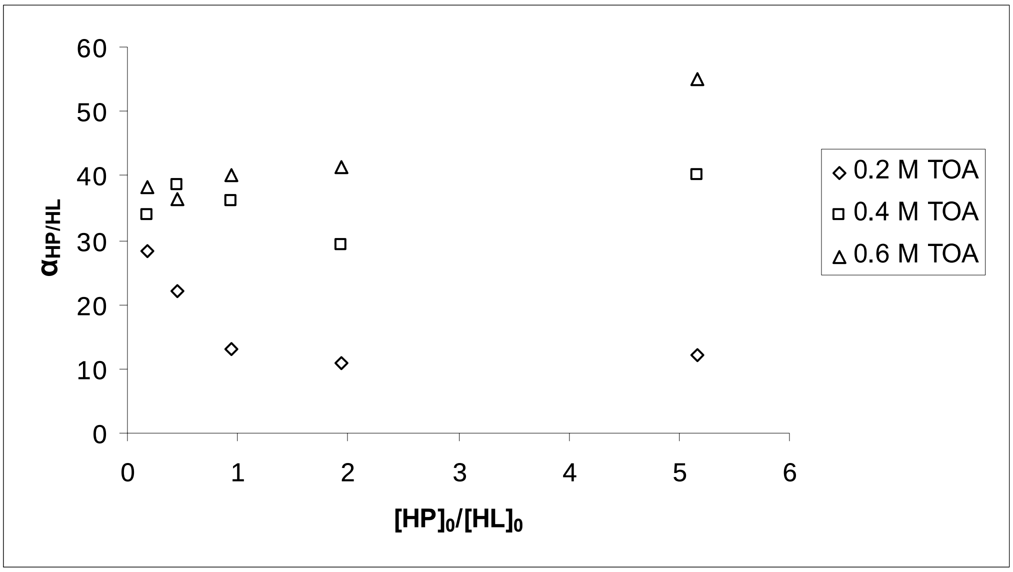

Figure 5.1.27 Variation of the separation factor with the ratio of initial aqueous phase concentration of pyruvic and lactic acids for reactive extraction of the acids from their aqueous binary acid solutions with trioctylamine in 1-octanol

5.2. Results of Kinetic Studies

As Bart (2001) has indicated kinetic data is needed in addition to equilibrium data for successful design of a recovery system. In this study a comprehensive approach proposed by Doraiswamy and Sharma (1984) is used to treat the kinetic data. Wasewar *et al.* (2002a, 2002b) and Nikhade *et al.* (2004) used the same type of equipment and model to assess kinetic parameters of the extraction of carboxylic acids with amines dissolved in various diluents.

A Lewis type stirred cell was utilized to obtain kinetic data as suggested in the literature (Wasewar *et al.*, 2002a). Temperature of the cell was maintained constant by circulating water at a constant temperature through the jacket outside of the cell. Both phases were mixed separately with a four-blade paddle with a double stirrer. Samples were periodically taken from the aqueous phase without stopping stirring by using a syringe with a long needle through the arrangement at the bottom of the stirred cell (Figure 4.3.1).

The specific rate of extraction was calculated using the rate of change of the concentration of pyruvic acid in the organic phase. As previously indicated the concentration of the acids in the aqueous phase was periodically monitored by taking samples and analysis with the use of liquid chromatography. With a mass balance about the aqueous phase, the concentration of pyruvic acid in the organic phase and its change with time was obtained (Figure 5.2.1). The volume change in the aqueous phase was assumed to be negligible during the experiment. Only initial rates were considered to standardize the results. The derivative of the concentration change at time zero yields the specific rate of extraction.

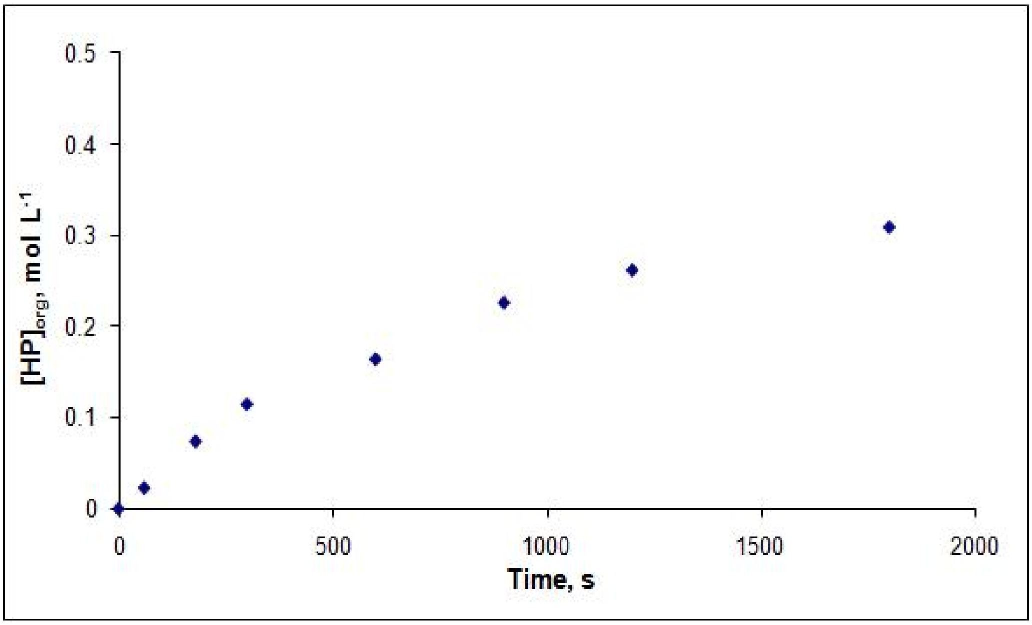

Figure 5.2.1 The variation of the concentration of pyruvic acid in the organic phase with time for reactive extraction of pyruvic acid with trioctylamine in 1-octanol

5.2.1. Physical Mass Transfer Coefficient

The physical mass transfer coefficient for the transfer of pyruvic acid from the aqueous phase to 1-octanol, k_L was measured as a function of the speed of agitation. This is needed to verify the regime of extraction. Kinetic experiments were performed by contacting the aqueous solution of pyruvic acid with the diluent, 1-octanol at various speeds of agitation. Differential mass balance of the batch system for the organic phase can be presented as follows as in Equation (E5.2.1):

$$V_{aq} \frac{d[HP]_{org}}{dt} = k_L A_c ([HP^*]_{org} - [HP]_{org}) \qquad \text{(E5.2.1)}$$

In the range of the variables, the relationship between the mass transfer coefficient and the speed of agitation is represented in Table 5.8 and Figure 5.2.2 and correlated by Equation 5.2.2.

Table 5.8 The physical mass transfer coefficient values obtained at different speed of agitation

Temperature T (^{0}C)	Speed of Agitation N (rev s^{-1})	Physical Mass Transfer Coefficient k_L (m s^{-1})
25	1.00	3.14 E-06
25	1.33	3.39 E-06
25	1.66	4.52 E-06

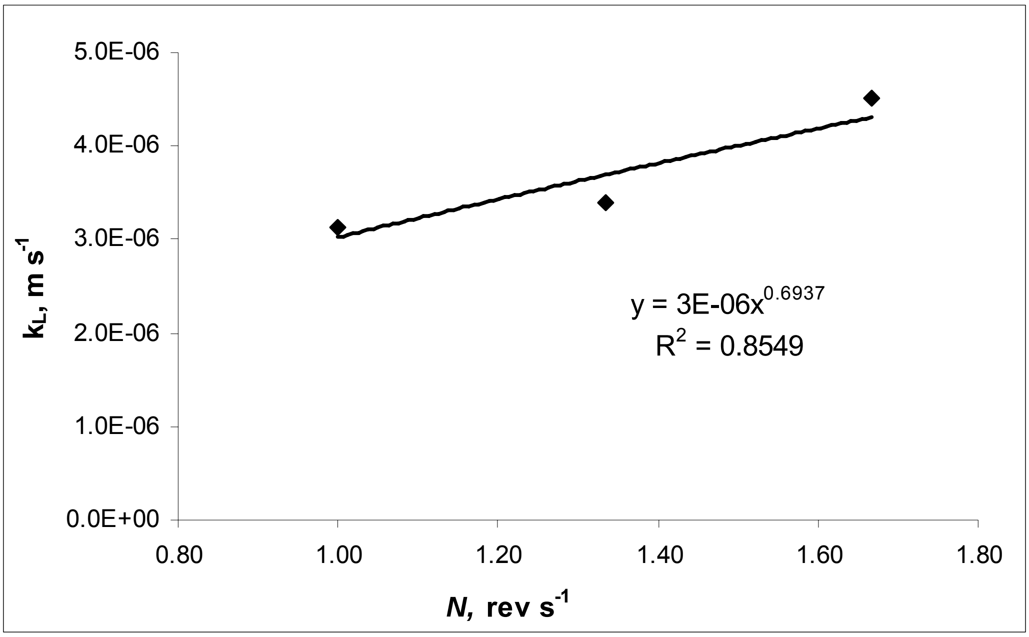

Figure 5.2.2 Variation of the physical mass transfer coefficient with the speed of agitation ($T=25^0C$, $[HP]_{aq,0}=0.5$ M)

$$k_L = 3.02 \times 10^{-6} N^{0.69} \qquad \text{(E5.2.2)}$$

5.2.2. Reaction Regimes

According to Doraiswamy and Sharma (1984), first step of the kinetic studies for reactive extraction of carboxylic acids is the determination of the reaction regime. As indicated earlier in Section 3.2, four regimes were proposed for the reaction of a solute and an extractant: very slow, slow, fast and instantaneous reaction regimes. A guide for the determination of the appropriate regime is presented in Table 3.1. Following the kinetic experiments used to obtain the physical mass transfer coefficient, experiments were conducted to observe the effects of the volume ratio of the phases and the speed of agitation on the specific rate of extraction. A 0.5 M aqueous solution of pyruvic acid and a 0.4 M trioctylamine solution of 1-octanol were utilized in the second part of the kinetic experiments to determine the order of the reaction.

5.2.3. Effect of Volume Ratio of the Phases

In order to evaluate the effect of the volume ratio of the phases on the specific rate of extraction experiments were conducted by keeping the concentration of the phases and the speed of agitation constant and varying the volume ratio of the organic to aqueous phase from 0.8 to 1.25. It can be seen in Figure 5.2.3 that the volume ratio of the phases has no effect on the specific rate of extraction for the reactive extraction of pyruvic acid with trioctylamine in1-octanol.

5.2.4. Effect of Speed of Agitation

Effect of the speed of agitation was observed by conducting kinetic measurements by keeping the concentration and the volume ratio of the phases constant. As noted in Section 4.3, in order to have an interfacial area equal to that of the geometric area during extraction, the interface should be, as much as possible, kept flat. Hence, the speed of agitation was varied between 0.66 and 1.33 rev s^{-1}. At higher mixing rates, the interface was disturbed and had an interfacial area greater than the geometric area. Figure 5.2.4 shows that the specific rate of extraction increases slightly with the speed of agitation up to a value of 1.00 rev s^{-1}. Beyond this, it remains constant. It is known that, at low agitation rates, the surface is not completely renewed during stirring. Thus, under these conditions the transfer of the acid is controlled by mass transfer resistances. The specific rate of extraction value was set over 1.00 rev s^{-1} in subsequent experiments.

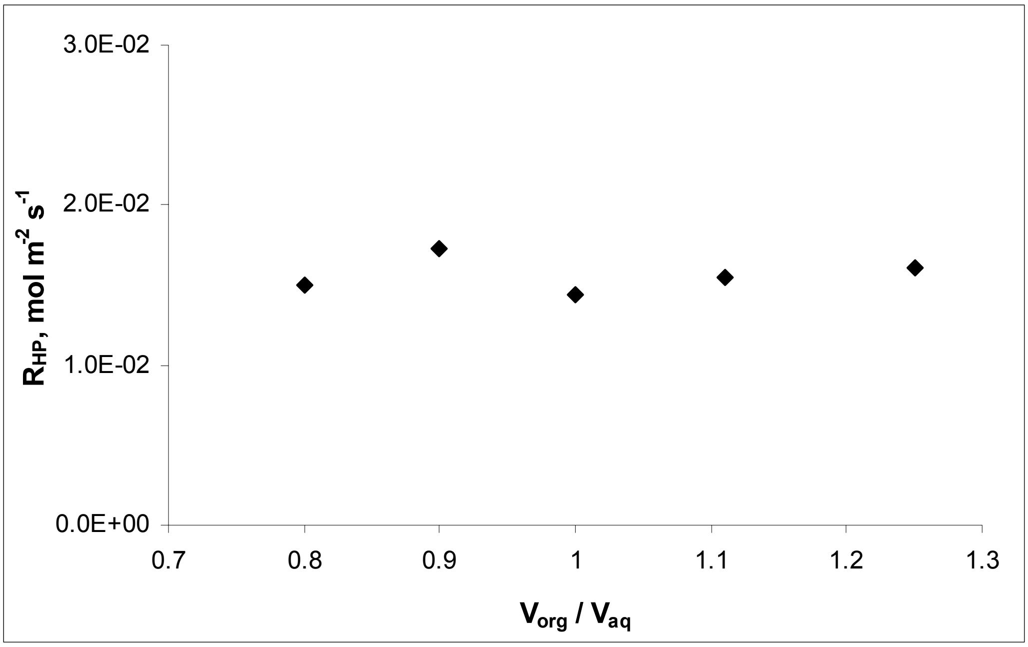

Figure 5.2.3 Variation of the specific rate of extraction with the volume ratio of the phases for reactive extraction of pyruvic acid with trioctylamine in 1-octanol (T=25^{0}C, N=1.00 rev s^{-1})

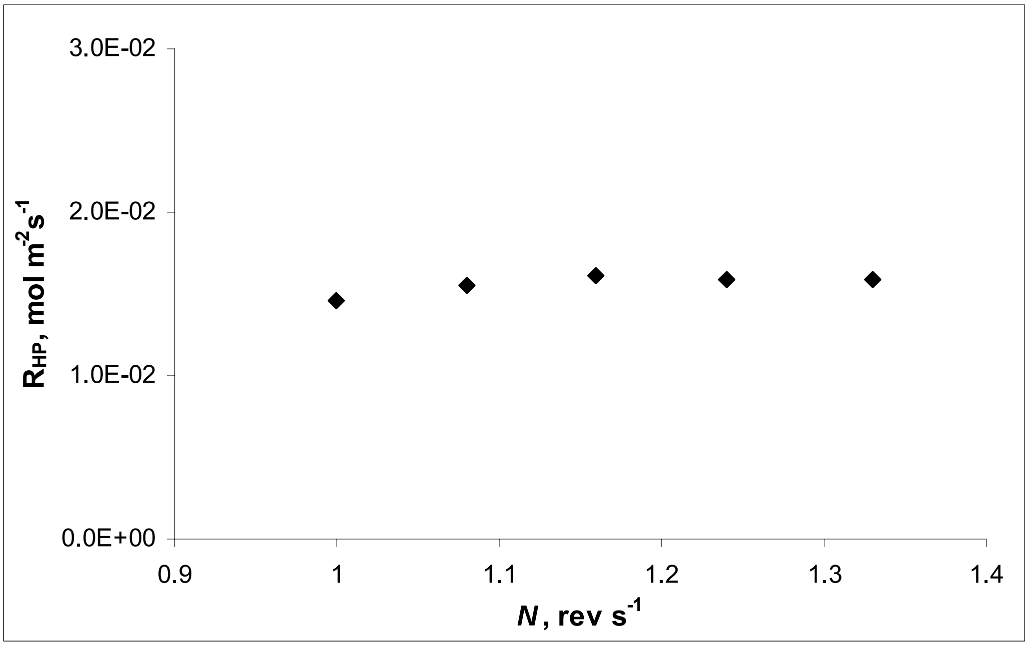

Figure 5.2.4 Variation of the specific rate of extraction with the speed of agitation for reactive extraction of pyruvic acid with trioctylamine in 1-octanol (T=25^{0}C, V_{org}/V_{aq}=1.00, $[HP]_{aq,0}$=0.5 M, $[TOA]_{org,0}$=0.4 M)

5.2.5. Regime of Mass Transfer Accompanied with Chemical Reaction

Results of preliminary studies conducted in order to determine the regime of reactive extraction of pyruvic acid with trioctylamine in 1-octanol are already shown in Figures 5.2.3 and 5.2.4. With the use of Table 3.1, it can be concluded that the reaction between pyruvic acid and trioctylamine in 1-octanol falls in Regime 3 that is extraction accompanied by a fast chemical reaction occurring in the diffusion film. The governing equation for the rate of chemical reaction in this regime is given in Equation E5.2.3:

$$R_{HP} = \left[HP^{*}\right]_{org} \sqrt{\frac{2}{m+1} D_{HP} k_{mn} \left[HP^{*}\right]_{org}{}^{m-1} \left[TOA\right]_{org}{}^{n}} \qquad \text{(E5.2.3)}$$

where m and n are the orders of the reaction with respect to aqueous and organic phase concentrations, respectively, D_{HP} is the diffusivity coefficient of pyruvic acid into 1-octanol, k_{mn} is the rate constant for a mth and nth order reaction.

5.2.6. The Order of the Reaction

The effect of the concentration of the reactants on the specific rate of extraction was investigated. Experiments were conducted by keeping the concentration of one of the components constant and varying the other at a constant speed of agitation and the volume ratio of phases. The initial concentration of trioctylamine was varied between 0.2 and 0.6 mol L^{-1}, while that of pyruvic acid, which is the equilibrium concentration of pyruvic acid in the diluent, was varied from 0.07 to 0.23 mol L^{-1}.

Graphs of the specific rate of extraction of pyruvic acid versus the initial extractant concentration in the organic phase (Figure 5.2.5) and initial concentration of pyruvic acid in the organic phase (Figure 5.2.6) are plotted. These graphs show the effect of the concentrations of the components in the organic phase on the specific rate of extraction. With the use of “The Method of Least Squares”, a linear approximation was applied on the data and the reaction is found to be first order with respect to the concentrations of the pyruvic acid and trioctylamine.

When m and n are replaced with one, Equation E5.2.3 becomes Equation E5.2.4:

$$R_{HP} = \left[HP^{*}\right]_{org} \sqrt{D_{HP} k_2 \left[TOA\right]_{org}{}^{n}} \qquad \text{(E5.2.4)}$$

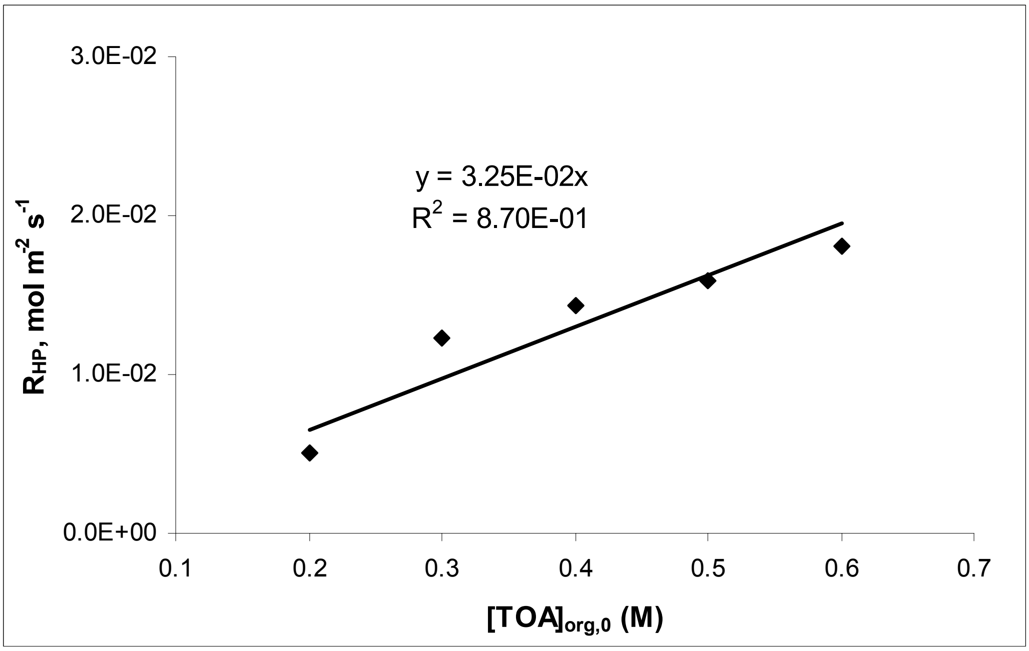

Figure 5.2.5 Effect of the initial amine concentration on the specific rate of extraction for reactive extraction of pyruvic acid with trioctylamine in 1-octanol (T=25^{0}C, *N*=1.00 rev s^{-1}, V_{org}/V_{aq}=1.00, $[HP]_{aq,0}$=0.5 M)

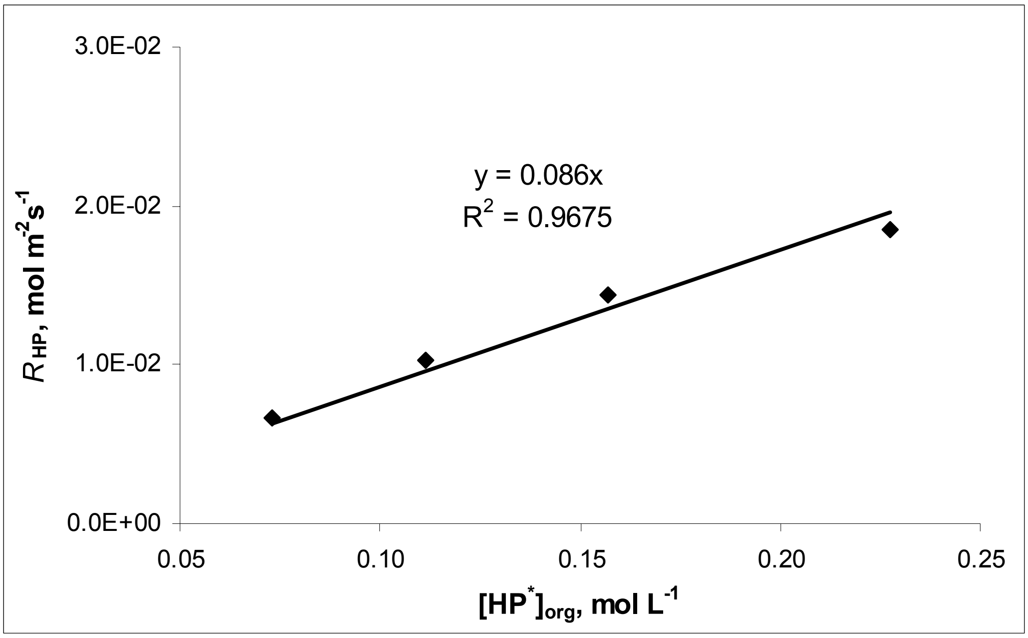

Figure 5.2.6 Effect of the initial pyruvic acid concentration on the specific rate of extraction for reactive extraction of pyruvic acid with trioctylamine in 1-octanol ($T=25^0C$, $N=1.00$ rev s^{-1}, $V_{org}/V_{aq}=1.00$, $[TOA]_{org,0}=0.4$ M)

5.2.7. Diffusivity Coefficient of Pyruvic Acid into 1-Octanol

In order to determine the rate constant of the reaction between pyruvic acid and trioctylamine, the diffusivity coefficient of pyruvic acid in 1-octanol should be estimated. For this purpose, two diffusivity equations, Wilke-Chang (E5.2.5) and Reddy-Doraiswamy (E5.2.6) equations are utilized and the average of the two is used.

$$D_{HP} = 7.4\times10^{-12}\frac{T\sqrt{\psi_{oct}M_{oct}}}{\eta_{oct}V^{0.6}{}_{pyr}} \qquad \text{(E5.2.5)}$$

$$D_{HP} = 10^{-11}\frac{T\sqrt{M_{oct}}}{\eta_{oct}\left(V_{oct}V_{pyr}\right)^{1/3}} \qquad \text{(E5.2.6)}$$

ψ_{oct} denotes the diluent association factor, which is predicted as 1.0 for 1-octanol, V signifies the molar volume of the component, and η represents the viscosity of the diluent.

The diffusivity of pyruvic acid in 1-octanol is estimated as 2.72 x 10^{-8} m^2 s^{-1} with the Wilke-Chang Equation (1955), and 1.79 x 10^{-8} m^2 s^{-1}, with the Reddy-Doraiswamy Equation (1967). Average of the two values, 2.26 x 10^{-8} m^2 s^{-1}, is used as the diffusivity coefficient in Equation 5.2.4.

5.2.8. Rate Constant

Second order rate constant is calculated with Equation E5.2.4. A plot of the Equation is shown as Figure 5.2.7. From the plot the second order rate constant is estimated as 0.94 L mol^{-1} s^{-1}. This is comparable to values reported in the literature for some organic acids and tertiary amines (Table 5.9).

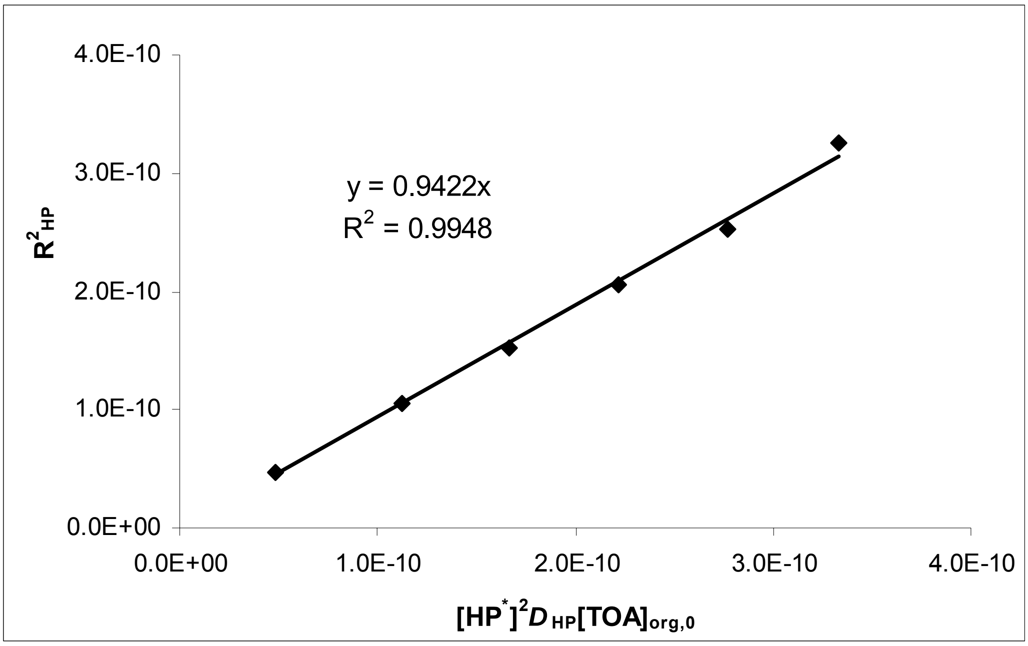

Figure 5.2.7 Calculation of the second order rate constant for the reaction between pyruvic acid and trioctylamine in 1-octanol

Table 5.9 Reaction rate constants reported in the literature for some carboxylic acids with tertiary amines

Authors	System	Regime	Order	Reaction Rate Constant
Wasewar *et al.* (2002a)	Lactic Acid-Alamine 336 MIBK	3	1	1.38 s^{-1}
Wasewar *et al.* (2002a)	Lactic Acid-Alamine 336 Decanol	3	1	0.21 s^{-1}
Nikhade *et al.* (2004)	Citric Acid-Aklamine 336 MIBK	1	2	0.013 L mol^{-1} s^{-1}

5.2.9. The Enhancement Factor

The effect of the reaction on the transfer of pyruvic acid can be quantified by the enhancement factor. Equation E5.2.7 is utilized to calculate the enhancement factor for the system. As is shown in Figure 5.2.8, enhancement factor is calculated as 25, which is remarkable for an enhanced recovery rate.

$$\Phi = \frac{R_{HP}}{k_L \left[HP^*\right]_{org}} \tag{E5.2.7}$$

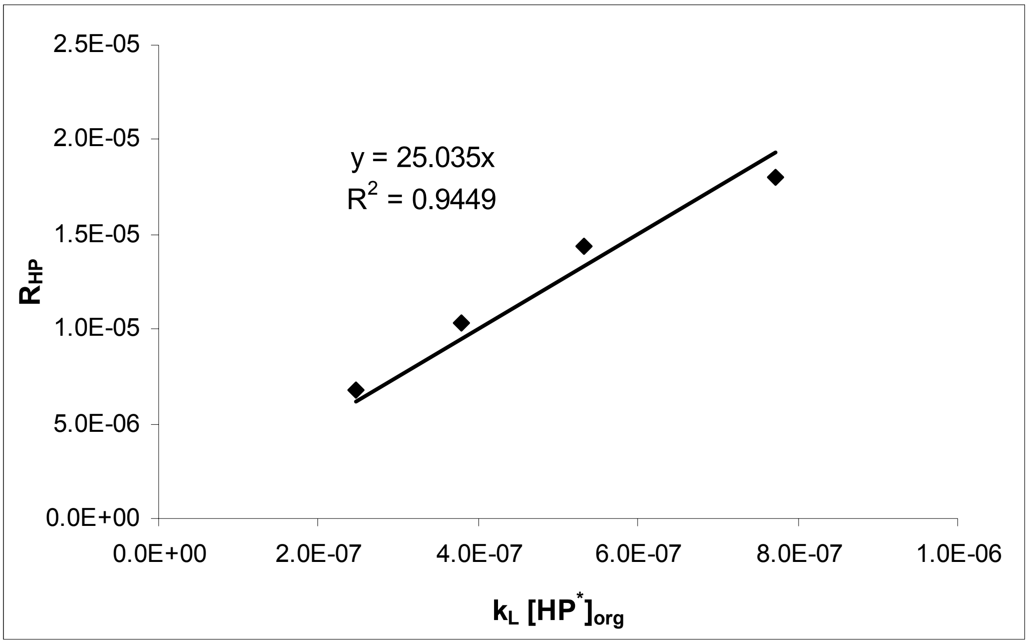

Figure 5.2.8 Calculation of the enhancement factor for the reaction between pyruvic acid and trioctylamine in 1-octanol

5.2.10. Verification of Mass Transfer Accompanied with Chemical Reaction Regime

The condition to be satisfied for Regime 3 with values of m and n taken as one is given by Doraiswamy and Sharma (1984):

$$D_{HP}k_2[TOA]_{org} >> k_L{}^2 \qquad \text{(E5.2.8)}$$

In Equation E5.2.8, the order of the magnitude of the left side is 10^{-9}, while that of the right side is 10^{-11}, which proves that the reaction between pyruvic acid and trioctylamine in 1-octanol falls in Regime 3, extraction accompanied by a fast chemical reaction occurring in the diffusion film.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

In this chapter, the conclusions of the studies on reactive extraction of pyruvic acid with tertiary amines in alcohols from single and mixed acid solutions are presented. In the first section, equilibrium studies with single and mixed acid solutions are considered, in the next, kinetic studies of the reactive extraction of pyruvic acid with trioctylamine in 1-octanol.

6.1. Conclusions on Equilibrium Studies

According to the results of forward extraction experiments, the following conclusions are :

- The distribution coefficient of pyruvic acid with 1-octanol was found to be much higher than with oleyl alcohol. 1-octanol could extract more carboxylic acid than oleyl alcohol because of its higher polarity. The trend did not differ when their solutions with tertiary amines were used in extraction. 1-octanol stabilized more acid-amine complex than oleyl alcohol did.
- The effect of the presence of the tertiary amine in the diluent on the extent of extraction of pyruvic acid was noted: the distribution coefficient of pyruvic acid increased with an increase in the concentration of the extractant. Equilibrium isotherms are plotted for pyruvic acid and the diluents for the two cases, with and without extractant.

- The formation of an emulsion phase at the interface was observed during the reactive extraction of pyruvic acid with oleyl alcohol, when tertiary amines were used in the diluents. This dense and opaque emulsion phase could not be eliminated completely by centrifuging. The reason for this formation was proposed to be due to the tendency of the complexes formed in the organic phase to cluster and move away from the bulk solvent because of the low solvating power of oleyl alcohol. The extent of formation of the emulsion phase increased with an increase in the concentration of the reactants in the two phases, indicating that at higher concentration levels, a significant proportion of the acid extracted could be located in this phase.
- The distribution coefficient of pyruvic acid extracted with Alamine 336 or trioctylamine were very close. Since Alamine 336 is a mixture of C_8-C_{10} straight-chain tertiary amines, a pure tertiary amine, trioctylamine was preferred to be used as the extractant in the rest of the study.
- Oleyl alcohol with 1-octanol may be proposed to be used in the extractive fermentation of pyruvic acid. 1-octanol is more efficient but a highly toxic extractant to the microorganisms producing carboxylic acids while oleyl alcohol does not have sufficient capability to stabilize the acid-amine complex. The use of 1-octanol, due to its higher solvating power, and oleyl alcohol, due to being benign together, might be expected to eliminate the disadvantages of the diluents.
- The distribution coefficient of the acids increased with an increase in the concentration of the extractant in the organic phase and a decrease in the equilibrium concentration of the aqueous phase, which is consistent with the literature. This behavior indicated that tertiary amines present in the organic phase was the limiting reagent for the reversible complexation reaction of the acid and the amine.

- It is clearly seen that the loading ratio was independent of the initial concentration of the extractant in the organic phase; however, it increased with an increase in the equilibrium concentration of the acid in the aqueous phase. At very low equilibrium concentration values of the acid in the aqueous phase, formation of (1-1) acid-amine complex was predicted for loading ratio values less than one ($z<1$). With an increase in the equilibrium concentration of the acid in the aqueous phase, overloading ($z>1$) was observed and the formation of some (2-1) acid-amine complex together with the (1-1) acid-amine complex was predicted.
- Mass Action Law was seen to be valid for the system studied at very low equilibrium acid concentration ranges in the aqueous phase and when the (1-1) acid-amine complex was presumed. There were some correlative trends at higher equilibrium acid concentration levels in the aqueous phase and higher loading ratios. However, lines representing the data did not pass through the origin indicating non conformity with the Mass Action Law.
- According to Henderson-Hasselbach Equation, the amount of undissociated acid initially present in the aqueous phase decreases with an increase in the pH of the solution. Since tertiary amines can react only with the undissociated portion of the acid, the extent of extraction at higher initial pH values, especially for those higher than the pK_A of the corresponding acid, was observed to decrease because of the decrease in the proportion of the undissociated acid in the aqueous phase. This is consistent with literature. It was seen that the extent of extraction of the carboxylic acids with the organic phase varied with respect to the pK_A of the corresponding acid and the initial pH of the aqueous phase. This behavior was proposed to be exploited to selectively extract carboxylic acids from mixed acid solutions.

- Extent of extraction of the three acids with trioctylamine in 1-octanol at similar initial pH and concentration levels in the aqueous phase were compared. The distribution coefficient decreased in the order of acetic > lactic > pyruvic acids. This order is identical with the order of the decreasing pK_A values of these acids.
- It was noted that the extraction of pyruvic acid was negatively affected by the presence of acetic acid from ternary acid solutions. When the concentration of the extractant in the organic phase was 0.1 M, acetic acid was preferably extracted to the organic phase while most of pyruvic and lactic acids remained in the aqueous phase. The distribution coefficient of pyruvic acid increased with an increase in the concentration of the extractant in the organic phase and more pyruvic acid was extracted to the organic phase. Antagonistic effects were observed for the extraction of pyruvic acid from a solution with an equivalent carboxylic acid composition of a biological production medium, containing pyruvic, lactic and acetic acids compared to results obtained from single solutions of pyruvic acid.
- Lactic acid was not significantly extracted from the mixed acid solution with trioctylamine in 1-octanol. It was proposed that the hydroxyl group in the structure of lactic acid enhanced its hydrophilicity and prevented its extraction when it is present in a mixed acid solution. The less hydrophilic acids were preferentially extracted.
- The effect of the presence of lactic acid on the distribution coefficient of pyruvic acid depended on the concentrations of the lactic acid and that of the extractant for aqueous binary acid mixtures of lactic and pyruvic. A low concentration of lactic acid had no effect on the distribution of pyruvic acid. However, at higher concentration values of the extractant (such as 0.4 and 0.6 M), a small synergistic effect was observed.

- The presence of pyruvic acid retarded the extraction of lactic acid. At higher concentration levels of lactic acid, the transfer was achieved in spite of the presence of pyruvic acid but with a very small extent compared to the amount achieved with single acid solutions.
- The recovery of pyruvic acid from the biological production medium must be conducted at low concentration ranges of lactic acid in order to obtain the main product in higher amounts and with high selectively. Following the separation step, unreacted lactic acid can be recycled to the production media.
- The possibility of extracting other components from a real production media should be investigated before using the proposed recovery system coupled with the production unit. It is reported in the literature that, under certain conditions, tertiary amines could be used in the recovery of some other chemicals such as amino acids and antibiotics. In order to be sure of a high extent of reaction with reasonable selectivity, these issues should be taken into consideration.

6.2. Conclusions on Kinetic Studies

In the present study, kinetic experiments were performed using a Lewis type stirred cell. The theory of extraction accompanied by a chemical reaction, proposed by Doraiswamy and Sharma (1984), was used to evaluate the data.

In accordance with the results obtained from experimental and mathematical studies, the following conclusions were drawn:

- In order to determine the regime of the reaction between the acid and amine, the effects of speed of agitation and volume ratio of the phases were examined. The kinetic experiments revealed that both of the parameters had no effect on the specific rate of extraction of pyruvic acid in the ranges studied, which means that the reaction between pyruvic acid and trioctylamine in 1-octanol in a Lewis type stirred cell fell in Regime 3, extraction accompanied by a fast chemical reaction that occurred in the diffusion film, according to the theory, proposed by Doraiswamy and Sharma (1984).

- The reaction was first order in the concentration of trioctylamine and pyruvic acid.
- The diffusivity of pyruvic acid in 1-octanol was calculated using Wilke-Chang and Reddy-Doraiswamy Equations. The average of these two results was utilized in the calculation of the second order rate constant, which was found to be 0.94 L mol^{-1} s^{-1}
- In order to quantify the effect of the rate of transfer of pyruvic acid to the organic phase, the enhancement factor was calculated and found to be 25.

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APPENDIX A

RESULTS OF REACTIVE EXTRACTION EXPERIMENTS

A.1 Solvent extraction of pyruvic, acetic and lactic acid with 1-octanol and oleyl alcohol

Table A1.1 Extraction of pyruvic acid with 1-octanol

$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D
0.099	0.080	0.238
0.260	0.200	0.300
0.510	0.400	0.275
0.780	0.600	0.300
1.010	0.770	0.312

Table A1.2 Extraction of pyruvic acid with oleyl alcohol

$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D
0.100	0.090	0.110
0.260	0.245	0.061
0.510	0.480	0.063
0.780	0.730	0.069
1.010	0.910	0.110

Table A1.3 Extraction of acetic acid with 1-octanol

$[HAc]_{aq,0}$ (M)	$[HAc]_{aq,ave}$ (M)	K_D
0.056	0.038	0.493
0.101	0.070	0.443
0.147	0.105	0.400
0.258	0.171	0.505

Table A1.4 Extraction of acetic acid with oleyl alcohol

$[HAc]_{aq,0}$ (M)	$[HAc]_{aq,ave}$ (M)	K_D
0.056	0.047	0.200
0.101	0.083	0.220
0.147	0.130	0.131
0.258	0.212	0.217

Table A1.5 Extraction of lactic acid with 1-octanol

$[HL]_{aq,0}$ (M)	$[HL]_{aq,ave}$ (M)	K_D
0.052	0.043	0.223
0.102	0.085	0.200
0.150	0.132	0.136
0.246	0.214	0.150

Table A1.6 Extraction of lactic acid with oleyl alcohol

$[HL]_{aq,0}$ (M)	$[HL]_{aq,ave}$ (M)	K_D
0.052	0.049	0.061
0.102	0.098	0.041
0.150	0.143	0.049
0.246	0.228	0.076

A.2 Reactive extraction of pyruvic, acetic and lactic acid with tertiary amines, trioctylamine and Alamine 336 in 1-octanol and oleyl alcohol

Table A2.1.1a Extraction of pyruvic acid with 0.1 M trioctylamine in 1-octanol

$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D	Z
0.052	0.001	60.183	0.391
0.102	0.005	19.198	0.732
0.119	0.013	8.225	0.782
0.167	0.036	3.791	0.927
0.260	0.116	1.235	0.827
0.510	0.310	0.645	0.804
0.780	0.505	0.556	0.958
1.010	0.650	0.554	1.232

Table A2.1.1b Extraction of pyruvic acid with 0.1 M trioctylamine in oleyl alcohol

$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D	Z
0.052	0.002	21.017	0.467
0.102	0.010	9.724	0.863
0.119	0.021	4.721	0.907
0.167	0.040	3.194	1.164
0.260	0.133	0.917	1.081
0.510	0.356	0.424	1.200
0.780	0.603	0.322	1.411
1.010	0.750	0.325	1.842

Table A2.1.1c Extraction of pyruvic acid with 0.1 M Alamine 336 in 1-octanol

$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D	Z
0.048	0.001	42.636	0.356
0.097	0.006	14.772	0.681
0.119	0.018	5.704	0.734
0.146	0.040	2.687	0.722
0.253	0.121	1.089	0.726
0.520	0.320	0.626	0.782
0.825	0.482	0.710	1.490
1.065	0.679	0.568	1.360

Table A2.1.1d Extraction of pyruvic acid with 0.1 M Alamine 336 in oleyl alcohol

$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D	z
0.048	0.003	30.686	0.419
0.097	0.012	6,977	0.788
0.119	0.025	3,810	0.867
0.146	0.048	2,015	0.884
0.253	0.142	0,785	0.954
0.520	0.358	0,452	1.294
0.825	0.555	0,485	1.973
1.065	0.801	0,330	1.972

Table A2.1.2 Extraction of pyruvic acid with 0.2 M trioctylamine in 1-octanol

$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D	z
0.049	0.000	101.979	0.182
0.099	0.001	127.571	0.374
0.125	0.001	126.551	0.472
0.161	0.001	123.806	0.608
0.245	0.032	6.778	0.777
0.495	0.235	1.110	0.716
0.748	0.424	0.764	0.734
0.980	0.575	0.704	0.864

Table A2.1.3 Extraction of pyruvic acid with 0.4 M trioctylamine in 1-octanol

$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D	z
0.045	0.000	109.000	0.084
0.094	0.000	187.600	0.177
0.114	0.001	206.273	0.215
0.144	0.001	170.429	0.271
0.210	0.001	237.636	0.396
0.290	0.002	130.818	0.544
0.501	0.057	7.743	0.806
0.747	0.236	2.164	0.826
1.022	0.370	1.765	1.013
1.034	0.438	1.363	0.866
1.513	0.696	1.173	1.127
2.09	1.050	0.990	1.335

Table A2.1.4 Extraction of pyruvic acid with 0.6 M trioctylamine in 1-octanol

$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D	z
0.510	0.003	158.875	0.635
0.738	0.101	6.321	0.758
1.098	0.250	3.392	0.961
1.450	0.588	1.466	0.839
1.940	0.958	1.025	0.838

Table A2.1.5 Extraction of pyruvic acid with 0.8 M trioctylamine in 1-octanol

$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D	z
0.510	0.002	259.204	0.474
0.738	0.010	75.083	0.677
1.098	0.097	10.320	0.905
1.450	0.405	2.580	0.848
1.940	0.745	1.604	0.881

Table A2.2.1 Extraction of acetic acid with 0.1 M trioctylamine in 1-octanol

$[HAc]_{aq,0}$ (M)	$[HAc]_{aq,ave}$ (M)	K_D	z
0.057	0.023	1.478	0.154
0.108	0.029	2.673	0.433
0.168	0.053	2.143	0.594
0.258	0.110	1.335	0.630
0.152	0.053	1.895	0.498
0.247	0.113	1.187	0.532
0.455	0.255	0.782	0.508
0.720	0.423	0.702	0.615
1.044	0.603	0.731	0.995

Table A2.2.2 Extraction of acetic acid with 0.2 M trioctylamine in 1-octanol

$[HAc]_{aq,0}$ (M)	$[HAc]_{aq,ave}$ (M)	K_D	z
0.052	0.006	7.667	0.144
0.102	0.011	8.273	0.286
0.152	0.025	5.146	0.384
0.258	0.065	2.963	0.536
0.247	0.073	2.399	0.461
0.455	0.198	1.297	0.529
0.720	0.329	1.187	0.759
1.044	0.518	1.017	0.900

Table A2.2.3 Extraction of acetic acid with 0.4 M trioctylamine in 1-octanol

$[HAc]_{aq,0}$ (M)	$[HAc]_{aq,ave}$ (M)	K_D	z
0.057	0.003	15.508	0.078
0.108	0.007	13.286	0.150
0.168	0.012	11.598	0.220
0.258	0.026	9.102	0.360
0.247	0.031	7.052	0.330
0.455	0.095	3.787	0.511
0.720	0.217	2.312	0.641
1.044	0.365	1.864	0.807
1.544	0.648	1.382	0.920
1.924	0.923	1.085	0.860

Table A2.2.4 Extraction of acetic acid with 0.6 M trioctylamine in 1-octanol

$[HAc]_{aq,0}$ (M)	$[HAc]_{aq,ave}$ (M)	K_D	z
0.482	0.079	5.072	0.388
0.731	0.147	3.978	0.545
0.950	0.219	3.342	0.661
1.544	0.524	1.944	0.792
1.924	0.770	1.499	0.794

Table A2.3.1 Extraction of lactic acid with 0.1 M trioctylamine in 1-octanol

$[HL]_{aq,0}$ (M)	$[HL]_{aq,ave}$ (M)	K_D	z
0.054	0.005	9.980	0.417
0.100	0.020	3.950	0.664
0.144	0.049	1.933	0.756
0.162	0.053	2.045	0.871
0.246	0.130	0.883	0.822
0.277	0.153	0.809	0.868
0.503	0.338	0.489	0.979
1.063	0.780	0.363	1.407

Table A2.3.2 Extraction of lactic acid with 0.2 M trioctylamine in 1-octanol

$[HL]_{aq,0}$ (M)	$[HL]_{aq,ave}$ (M)	K_D	z
0.050	0.002	20.000	0.206
0.110	0.006	17.400	0.448
0.144	0.011	12.714	0.571
0.154	0.013	10.815	0.600
0.246	0.060	3.119	0.764
0.277	0.063	3.418	0.885
0.503	0.264	0.907	0.858
0.692	0.451	0.535	0.740
1.063	0.697	0ç525	1.113

Table A2.3.3 Extraction of lactic acid with 0.4 M trioctylamine in 1-octanol

$[HL]_{aq,0}$ (M)	$[HL]_{aq,ave}$ (M)	K_D	z
0.054	0.002	34.933	0.113
0.106	0.004	28ç333	0.219
0.159	0.005	30.137	0.330
0.246	0.007	34.580	0.513
0.277	0.010	25.893	0.572
0.503	0.105	3.781	0.823
0.692	0.273	1.531	0.811
1.063	0.488	1.180	1.076

Table A2.3.4 Extraction of lactic acid with 0.6 M trioctylamine in 1-octanol

$[HL]_{aq,0}$ (M)	$[HL]_{aq,ave}$ (M)	K_D	z
0.503	0.025	19.533	0.682
0.692	0.122	4.677	0.745
1.063	0.304	2.498	0.847
1.389	0.568	1.445	0.985
1.853	0.931	0.990	1.054

A.3 Reactive extraction of pyruvic, acetic and lactic acid with trioctylamine in 1-octanol at various initial pH values of the aqueous phase

Table A3.1.1 Extraction of pyruvic acid with 0.1 M TOA in 1-octanol at various initial pH values of the aqueous phase

pH	$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D
1.35	0.518	0.317	0.634
2.00	0.511	0.361	0.415
2.40	0.490	0.380	0.287
3.00	0.492	0.386	0.274
3.53	0.482	0.410	0.174
4.00	0.482	0.440	0.096

Table A3.1.2 Extraction of pyruvic acid with 0.2 M TOA in 1-octanol at various initial pH values of the aqueous phase

pH	$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D
1.35	0.518	0.239	1.170
2.00	0.511	0.279	0.834
2.40	0.490	0.303	0.617
3.00	0.492	0.365	0.346
3.53	0.482	0.411	0.172
4.00	0.482	0.443	0.088

Table A3.1.3 Extraction of pyruvic acid with 0.4 M TOA in 1-octanol at various initial pH values of the aqueous phase

pH	$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D
1.35	0.518	0.083	5.233
2.00	0.511	0.184	1.774
2.40	0.490	0.261	0.877
3.00	0.492	0.350	0.404
3.53	0.482	0.402	0.199
4.00	0.482	0.432	0.116

Table A3.1.4 Extraction of pyruvic acid with 0.6 M TOA in 1-octanol at various initial pH values of the aqueous phase

pH	$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D
1.35	0.518	0.000	∞
2.00	0.511	0.000	∞
2.40	0.490	0.257	0.908
3.00	0.492	0.356	0.382
3.53	0.482	0.402	0.198
4.00	0.482	0.414	0.165

Table A3.2.1 Extraction of acetic acid with 0.1 M TOA in 1-octanol at various initial pH values of the aqueous phase

pH	$[HAc]_{aq,0}$ (M)	$[HAc]_{aq,ave}$ (M)	K_D
2.50	0.546	0.293	0.860
3.00	0.518	0.282	0.839
3.50	0.547	0.307	0.782
4.00	0.534	0.318	0.678
4.50	0.535	0.375	0.426
5.00	0.526	0.431	0.220
5.50	0.511	0.467	0.095
6.00	0.511	0.494	0.033

Table A3.2.2 Extraction of acetic acid with 0.2 M TOA in 1-octanol at various initial pH values of the aqueous phase

pH	$[HAc]_{aq,0}$ (M)	$[HAc]_{aq,ave}$ (M)	K_D
2.50	0.546	0.228	1.397
3.00	0.518	0.222	1.332
3.50	0.547	0.244	1.247
4.00	0.534	0.262	1.037
4.50	0.535	0.326	0.642
5.00	0.526	0.408	0.289
5.50	0.511	0.478	0.069
6.00	0.511	0.492	0.037

Table A3.2.3 Extraction of acetic acid with 0.4 M TOA in 1-octanol at various initial pH values of the aqueous phase

pH	$[HAc]_{aq,0}$ (M)	$[HAc]_{aq,ave}$ (M)	K_D
2.50	0.546	0.127	3.287
3.00	0.518	0.126	3.124
3.50	0.547	0.149	2.669
4.00	0.534	0.184	1.902
4.50	0.535	0.276	0.937
5.00	0.526	0.391	0.345
5.50	0.511	0.474	0.077
6.00	0.511	0.490	0.042

Table A3.2.4 Extraction of acetic acid with 0.6 M TOA in 1-octanol at various initial pH values of the aqueous phase

pH	$[HAc]_{aq,0}$ (M)	$[HAc]_{aq,ave}$ (M)	K_D
2.50	0.546	0.084	5.482
3.00	0.518	0.089	4.836
3.50	0.547	0.116	3.727
4.00	0.534	0.164	2.254
4.50	0.535	0.262	1.043
5.00	0.526	0.390	0.351
5.50	0.511	0.468	0.091
6.00	0.511	0.488	0.047

Table A3.3.1 Extraction of lactic acid with 0.1 M TOA in 1-octanol at various initial pH values of the aqueous phase

pH	$[HL]_{aq,0}$ (M)	$[HL]_{aq,ave}$ (M)	K_D
2.01	0.534	0.363	0.473
3.07	0.524	0.382	0.371
3.48	0.521	0.396	0.314
4.04	0.508	0.420	0.209

Table A3.3.2 Extraction of lactic acid with 0.2 M TOA in 1-octanol at various initial pH values of the aqueous phase

pH	$[HL]_{aq,0}$ (M)	$[HL]_{aq,ave}$ (M)	K_D
2.01	0.534	0.288	0.856
3.07	0.524	0.306	0.711
3.48	0.521	0.322	0.618
4.04	0.508	0.366	0.385

Table A3.3.3 Extraction of lactic acid with 0.4 M TOA in 1-octanol at various initial pH values of the aqueous phase

pH	$[HL]_{aq,0}$ (M)	$[HL]_{aq,ave}$ (M)	K_D
2.01	0.534	0.137	2.905
3.07	0.524	0.164	2.185
3.48	0.521	0.213	1.449
4.04	0.508	0.340	0.493

Table A3.3.4 Extraction of lactic acid with 0.6 M TOA in 1-octanol at various initial pH values of the aqueous phase

pH	$[HL]_{aq,0}$ (M)	$[HL]_{aq,ave}$ (M)	K_D
2.01	0.534	0.027	18.859
3.07	0.524	0.105	3.981
3.48	0.521	0.191	1.722
4.04	0.508	0.349	0.455

A.4 Reactive extraction of pyruvic acid with trioctylamine in 1-octanol at the Iowa State University – Reproducibility of Equilibrium Data

Table A4.1 Extraction of pyruvic acid with 1-octanol at the Iowa State University

$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D
0.159	0.127	0.244
0.256	0.198	0.289
0.533	0.414	0.286
0.797	0.594	0.342
0.952	0.740	0.287

Table A4.2 Extraction of pyruvic acid with 0.3 M TOA in 1-octanol at the Iowa State University

$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D
0.168	0.001	133.817
0.262	0.002	109.665
0.326	0.032	9.284
0.534	0.161	2.315
0.798	0.314	1.540
0.955	0.480	0.991

Table A4.3 Extraction of pyruvic acid with TOA dissolved in 1-octanol at various concentration levels at the Iowa State University $[HP]_{aq,0}$ = 0.5M

$[TOA]_{org,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D
0.1	0.300	0.671
0.2	0.221	1.274
0.3	0.128	2.929
0.4	0.054	8.311
0.5	0.013	37.597
0.6	0.003	189.876

A.5 Reactive extraction of pyruvic, acetic and lactic acid from their mixed acid solutions with trioctylamine in 1-octanol at various initial pH values of the aqueous phase

Table A5.1.1 Extraction of pyruvic acid with 0.1 M TOA in 1-octanol from mixed acid solutions at various initial pH values

pH	$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D
1.20	0.753	0.631	0.192
2.01	0.750	0.660	0.137
2.52	0.741	0.695	0.066
3.51	0.730	0.691	0.057
4.04	0.713	0.701	0.017
4.50	0.717	0.685	0.047
5.03	0.724	0.693	0.044

Table A5.1.2 Extraction of pyruvic acid with 0.2 M TOA in 1-octanol from mixed acid solutions at various initial pH values

pH	$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D
1.20	0.753	0.588	0.280
2.01	0.750	0.610	0.231
2.52	0.741	0.649	0.142
3.51	0.730	0.652	0.119
4.04	0.713	0.668	0.067
4.50	0.717	0.670	0.070
5.03	0.724	0.699	0.036
6.02	0.590	0.570	0.036

Table 5.1.3 Extraction of pyruvic acid with 0.4 M TOA in 1-octanol from mixed acid solutions at various initial pH values

pH	$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D
1.20	0.753	0.444	0.695
2.01	0.750	0.496	0.512
2.52	0.741	0.529	0.400
3.51	0.730	0.625	0.169
4.04	0.713	0.657	0.085
4.50	0.717	0.669	0.073
5.03	0.724	0.685	0.056
6.02	0.590	0.566	0.043

Table A5.1.4 Extraction of pyruvic acid with 0.6 M TOA in 1-octanol from mixed acid solutions at various initial pH values

pH	$[HP]_{aq,0}$ (M)	$[HP]_{aq,ave}$ (M)	K_D
1.20	0.753	0.225	2.531
2.01	0.750	0.398	0.885
2.52	0.741	0.511	0.450
3.51	0.730	0.627	0.164
4.04	0.713	0.657	0.086
4.50	0.717	0.658	0.091
5.03	0.724	0.672	0.077
6.02	0.590	0.560	0.054

Table A5.2.1 Extraction of acetic acid with 0.1 M TOA in 1-octanol from mixed acid solutions at various initial pH values

pH	$[HAc]_{aq,0}$ (M)	$[HAc]_{aq,ave}$ (M)	K_D
1.20	0.084	0.056	0.498
2.01	0.082	0.050	0.650
2.52	0.081	0.054	0.511
3.51	0.081	0.060	0.353
4.04	0.078	0.068	0.150
4.50	0.081	0.073	0.119
5.03	0.085	0.077	0.095
6.02	0.113	0.121	-

Table A5.2.2 Extraction of acetic acid with 0.2 M TOA in 1-octanol from mixed acid solutions at various initial pH values

pH	$[HAc]_{aq,0}$ (M)	$[HAc]_{aq,ave}$ (M)	K_D
1.20	0.084	0.058	0.464
2.01	0.082	0.051	0616
2.52	0.081	0.049	0.666
3.51	0.081	0.065	0.239
4.04	0.078	0.071	0.102
4.50	0.081	0.073	0.112
5.03	0.085	0.079	0.077
6.02	0.113	0.107	0.056

Table A5.2.3 Extraction of acetic acid with 0.4 M TOA in 1-octanol from mixed acid solutions at various initial pH values

pH	$[HAc]_{aq,0}$ (M)	$[HAc]_{aq,ave}$ (M)	K_D
1.20	0.084	0.055	0.546
2.01	0.082	0.048	0.715
2.52	0.081	0.052	0.552
3.51	0.081	0.066	0.233
4.04	0.078	0.071	0.098
4.50	0.081	0.073	0.111
5.03	0.085	0.077	0.095
6.02	0.113	0.107	0.047

Table A5.2.4 Extraction of acetic acid with 0.6 M TOA in 1-octanol from mixed acid solutions at various initial pH values

pH	$[HAc]_{aq,0}$ (M)	$[HAc]_{aq,ave}$ (M)	K_D
1.20	0.084	0.048	0.744
2.01	0.082	0.040	1.044
2.52	0.081	0.051	0.585
3.51	0.081	0.067	0.214
4.04	0.078	0.071	0.109
4.50	0.081	0.071	0.140
5.03	0.085	0.075	0.127
6.02	0.113	0.107	0.051

Table A5.3.1 Extraction of lactic acid with 0.1 M TOA in 1-octanol from mixed acid solutions at various initial pH values

pH	$[HL]_{aq,0}$ (M)	$[HL]_{aq,ave}$ (M)	K_D
1.20	0.050	0.044	0.134
2.01	0.050	0.044	0.135
2.52	0.051	0.046	0.109
3.51	0.051	0.049	0.050
4.50	0.051	0.050	0.029
5.03	0.054	0.051	0.051
6.02	0.049	0.059	-

Table A5.3.2 Extraction of lactic acid with 0.2 M TOA in 1-octanol from mixed acid solutions at various initial pH values

pH	$[HL]_{aq,0}$ (M)	$[HL]_{aq,ave}$ (M)	K_D
1.20	0.050	0.046	0.098
2.01	0.050	0.044	0.144
2.52	0.051	0.045	0.114
3.51	0.051	0.048	0.061
4.04	0.049	0.049	0.004
4.50	0.051	0.049	0.045
5.03	0.054	0.052	0.033
6.02	0.049	0.054	-

Table A5.3.3 Extraction of lactic acid with 0.4 M TOA in 1-octanol from mixed acid solutions at various initial pH values

pH	$[HL]_{aq,0}$ (M)	$[HL]_{aq,ave}$ (M)	K_D
1.20	0.050	0.046	0.104
2.01	0.050	0.041	0.225
2.52	0.051	0.041	0.233
3.51	0.051	0.047	0.093
4.04	0.049	0.048	0.025
4.50	0.051	0.050	0.029
5.03	0.054	0.051	0.054
6.02	0.049	0.054	-

Table A5.3.4 Extraction of lactic acid with 0.6 M TOA in 1-octanol from mixed acid solutions at various initial pH values

pH	$[HL]_{aq,0}$ (M)	$[HL]_{aq,ave}$ (M)	K_D
1.20	0.050	0.041	0.223
2.01	0.050	0.034	0.500
2.52	0.051	0.040	0.269
3.51	0.051	0.047	0.078
4.04	0.049	0.049	0.002
4.50	0.051	0.049	0.052
5.03	0.054	0.049	0.091

APPENDIX B

RELATIVE UNCERTAINTY CALCULATION

The uncertainty of the measured data was determined. In the following table, four measured values are presented for concentration of the acid in the aqueous phase at equilibrium for reactive extraction of 0.51 M pyruvic acid with 0.1 M trioctylamine dissolved in 1-octanol.

Table B.1 Measured values of concentration of the acid in the aqueous phase at equilibrium for reactive extraction of 0.51 M pyruvic acid with 0.1 M trioctylamine in 1-octanol

# of Measurement	Area	Concentration (ppm)	Conc. (ppm) x Dilution Rate (400)	Conc. (M)
1	624402	68.18	27270.93	0.3099
2	623229	68.05	27219.70	0.3093
3	623516	68.08	27232.23	0.3095
4	624065	68.14	27256.21	0.3097

Average value of concentration of pyruvic acid in the aqueous phase is

= sum of the values / (number of the values)

$$= \frac{0.3099 + 0.3093 + 0.3095 + 0.3097}{4}$$

$$= \frac{1.2384}{4}$$

$$= 0.3096M$$

The reproducibility of the measurement is ± 0.0003 M. Thus relative uncertainty is

$$= \frac{0.0003}{0.3096}$$

$$= 1.0x10^{-3}$$

$$= 1.0x10^{-1} \text{ \%}$$

APPENDIX C

SAMPLE CHROMATOGRAMS AND CALIBRATION CURVES

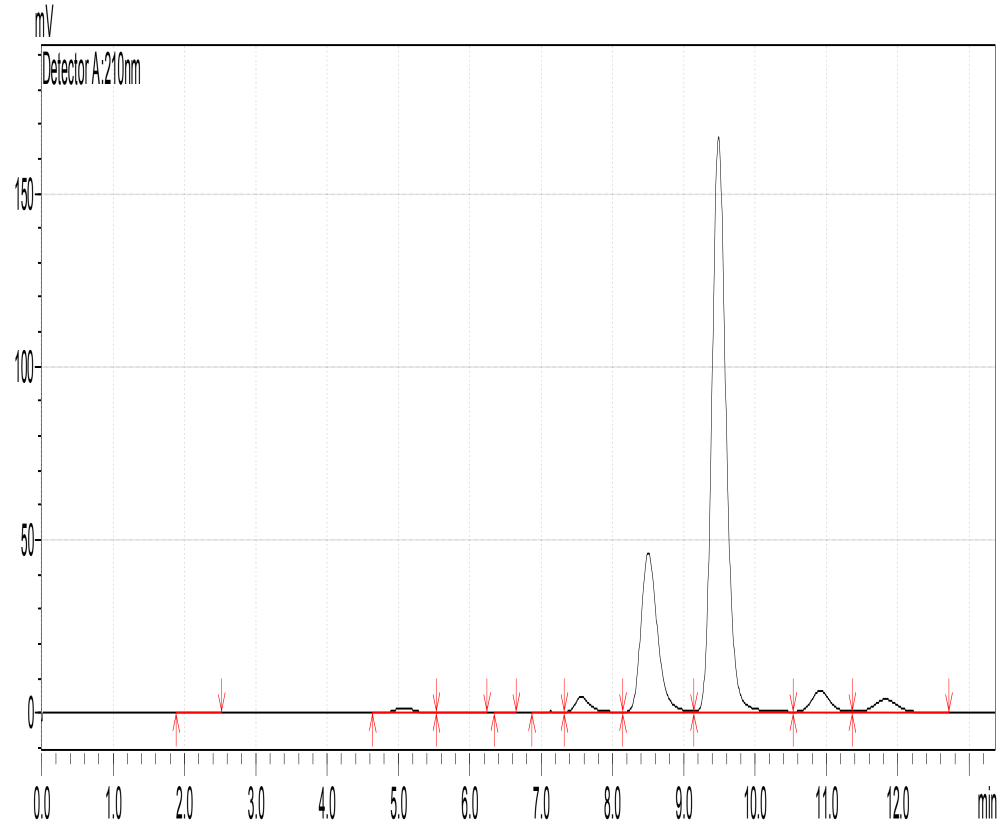

Figure C.1 A sample chromatogram for 100 ppm pyruvic acid in the aqueous phase (Column: MetaCarb 87H (7,8 x 300 mm), Mobile Phase: 0.008 N H_2SO_4, Flow Rate: 0.6 ml min^{-1}, Wavelength: 210 nm, Retention time: 9.4 min)

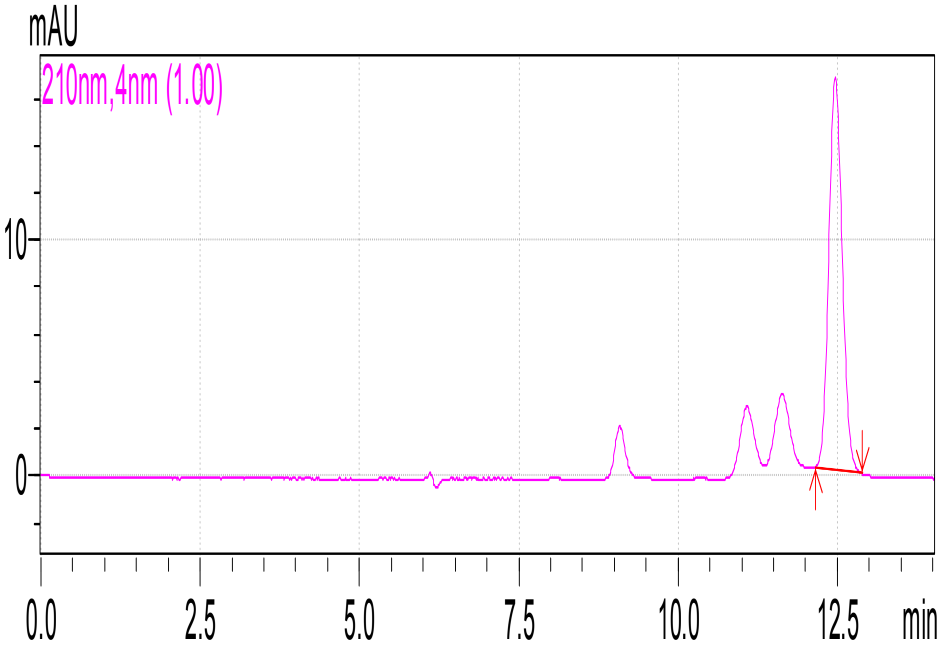

Figure C.2 A sample chromatogram for 200 ppm lactic acid in the aqueous phase (Column: MetaCarb 87H (7,8 x 300 mm), Mobile Phase: 0.008 N H_2SO_4, Flow Rate: 0.6 ml min^{-1}, Wavelength: 210 nm, Retention time: 12.5 min)

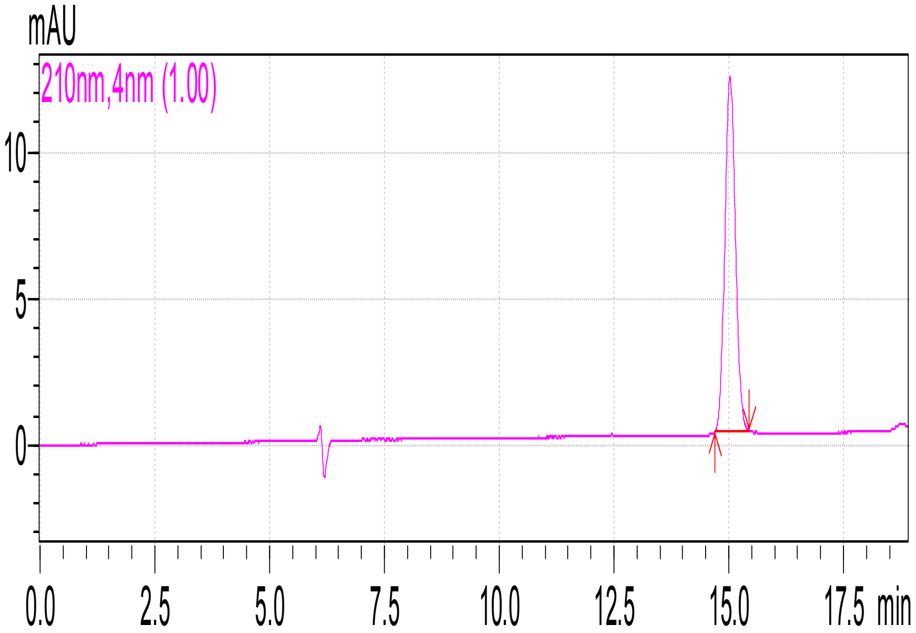

Figure C.3 A sample chromatogram for 200 ppm acetic acid in the aqueous phase (Column: MetaCarb 87H (7,8 x 300 mm), Mobile Phase: 0.008 N H_2SO_4, Flow Rate: 0.6 ml min^{-1}, Wavelength: 210 nm, Retention time: 15.0 min)

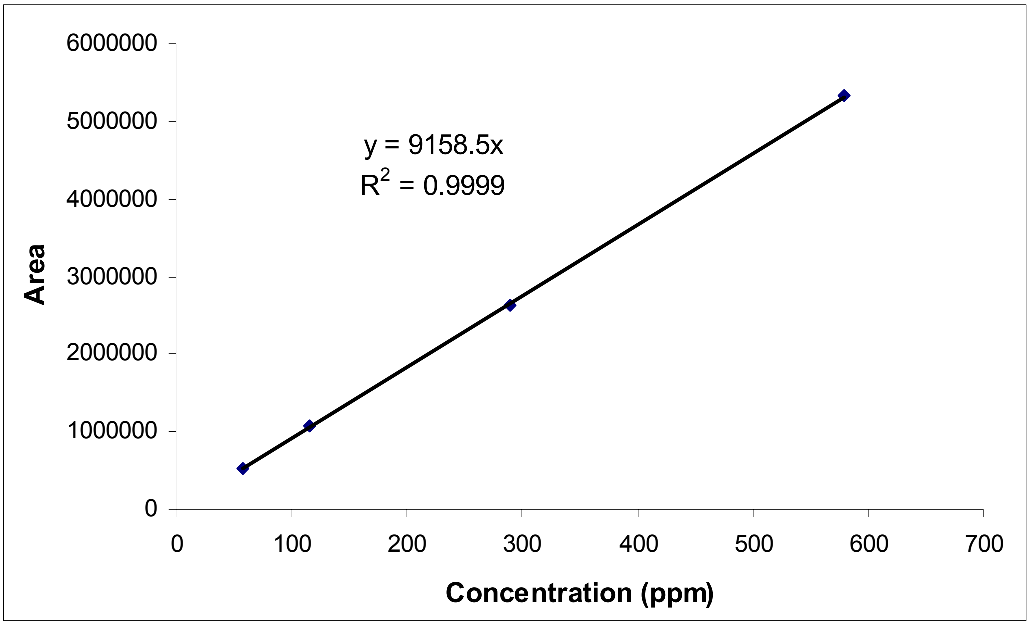

Figure C.4 Pyruvic acid calibration curve for HPLC

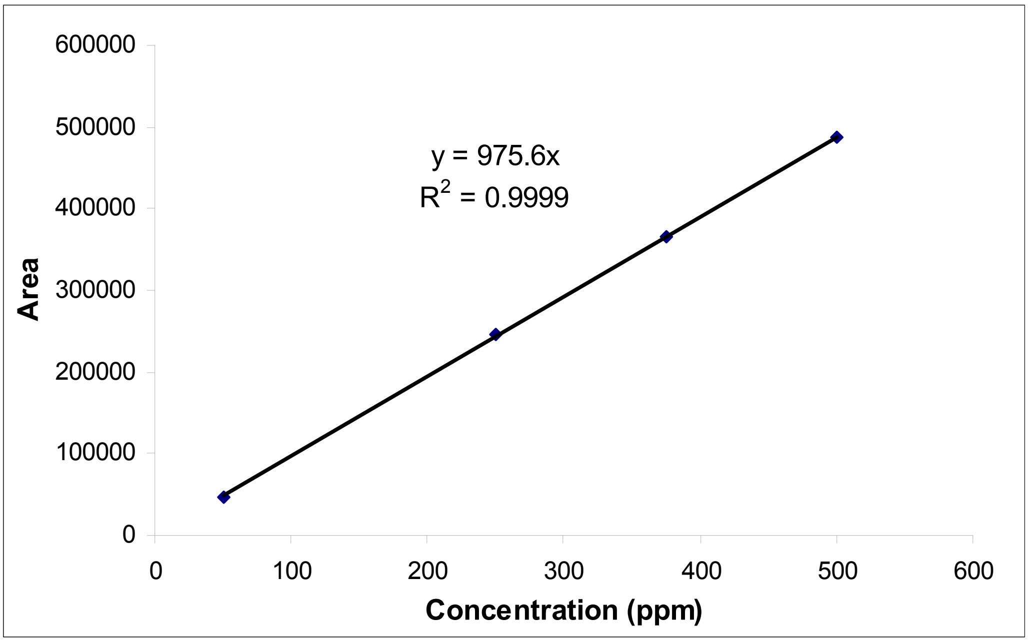

Figure C.5 Lactic acid calibration curve for HPLC

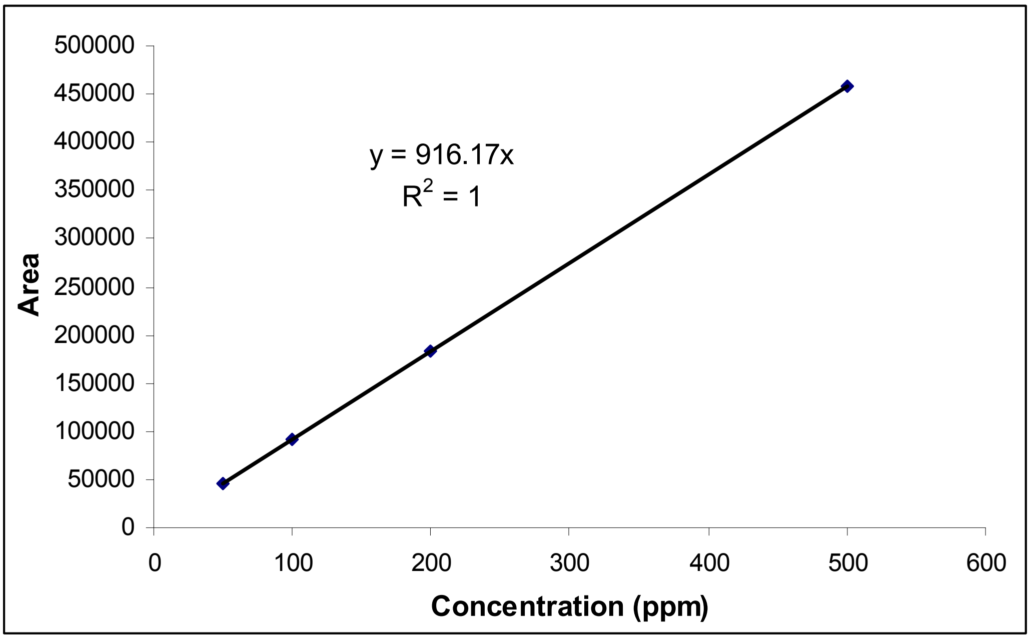

Figure C.6 Acetic acid calibration curve for HPLC

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1. Marti, M. E., Gurkan, T., Doraiswamy, L. K., Reactive Extraction of Pyruvic Acid with TOA in 1-Octanol: Equilibrium and Kinetic Studies

2. Marti, M. E., Gurkan, T., Reactive Extraction of Pyruvic Acid with TOA in 1-Octanol from Mixed Acid Solutions

CONGRESSES

1. Marti, M. E., Gurkan, T., Doraiswamy, L. K., Reactive Extraction of Pyruvic Acid using TOA dissolved in 1-Octanol Equilibrium and Kinetics, AIChE 2009, Nashville, TN, USA (November, 8-13, 2009)

2. Marti, M. E., Gurkan, T., Recovery of Pyruvic Acid from Mixed Acid Solutions by Reactive Extraction, UKMK 8, Malatya, Turkey (August, 26-29, 2008) - National

3. Marti, M. E., Gurkan, T., Reactive extraction of Pyruvic Acid using tertiary amines dissolved in 1-octanol and oleyl alcohol, UKMK 7, Eskisehir, Turkey (September, 5-8, 2006) – National

4. Gurkan, T., Acan, B., Karabulut, F., Martı, M. E., Recovery of Lactic Acid from Biotechnological Medium by Reactive Extraction, CHISA 2006, Prague, Czech Republic (August, 28-31, 2006)

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