EQUILIBRIUM STUDIES ON THE REACTIVE EXTRACTION OF LACTIC ACID FROM FERMENTATION BROTH

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ABSTRACT

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Lactic acid recovery from dilute fermentation broths is a growing requirement due to the increasing demand for pure lactic acid. Reactive extraction is proposed as an alternative to conventional methods of recovery, since the selectivity of separation is remarkably enhanced in reactive extraction.

The aim of this study is to perform the equilibrium studies for the recovery of lactic acid from its synthetic aqueous solutions (not from real fermentation broths) by reactive extraction and investigate the effects of various parameters such as initial lactic acid concentration in the aqueous phase (0.25 - 1.3 M), initial pH of the aqueous phase (2 – 6), organic phase extractant concentration (0.1 – 0.5 M), type of the extractant (chloride, hydrogensulphate and hydroxide

salts of tri-n-octylmethylammonium) and the type of diluent (oleyl alcohol or octanol).

The results of the experiments showed that the degrees of extraction decreased with increasing use of diluent with the extractant and increasing initial lactic acid concentration of the aqueous phase. Highest degrees of extraction were achieved for undiluted extractants. The performance of the diluents were investigated by performing extraction experiments with solutions of TOMAC in oleyl alcohol or octanol at different pH values and it was observed that octanol had a higher solvating power than oleyl alcohol especially at lower aqueous phase pH values. Higher extraction efficiencies were obtained for TOMAC dissolved in octanol rather than oleyl alcohol. Initial aqueous pH of 6 was identified as the optimum pH for the extraction, also due to its being equal the average fermentation pH for the extractions with Lactobacillus species.

Among the different salts of tri-n-octylmethylammonium, hydroxide salt exhibited the highest degrees of extraction (83% with undiluted TOMA(OH) and 78% with 0.5 M TOMA(OH) in octanol for the extraction of 0.316 M lactic acid solutions).

The present work is the first step in the design of an industrial reactive extraction process that is going to attempt forward and backward extraction of lactic acid simultaneously in a hollow fiber membrane module that is going to be attached to the lactic acid fermentor to achieve continuous product recovery. The equilibrium data obtained from this study can be combined with the kinetic studies as the next step of designing a separation module.

Keywords: Reactive extraction, lactic acid, equilibrium studies, TOMAC, tri-n-octylmethylammonium salts

ÖZ

LAKTIK ASIDIN FERMENTASYON ORTAMINDAN REAKTIF ÖZÜTLENMESI ILE ILGILI DENGE ÇALISMALARI

Açan, Basak

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Laktik asidin seyreltik fermentasyon çözeltilerinden ayirilmasi, saf laktik aside olan talep yüzünden giderek artan bir gereksinimdir. Reaktif özütleme, gelismis ayirim seçiciligi dolayisiyla geleneksel yöntemlere alternatif olarak önerilmistir.

Bu çalismanin amaci, laktik asidin sentetik sulu çözeltilerinden (gerçek fermentasyon ortamindan degil) reaktif özütleme yöntemiyle ayirilmasina dair denge çalismalarini yapmak ve sulu fazdaki baslangiç laktik asit derisimi (0.25 – 1.3 M), baslangiç sulu faz pH'i (2 - 6), organik faz ekstraktant (özütleyen) derisimi (0.1 – 0.5 M), ekstraktant çesidi (tri-n-oktilmetilamonyum'un klorür,

hidrojensülfat ve hidroksit tuzlari) ve seyreltici çesidi (oleil alkol, oktanol) parametrelerinin özütleme performansina etkilerini arastirmaktir.

Deney sonuçlari özütleme derecesinin seyreltici maddenin ekstraktantla beraber artan kullanimiyla ve artan baslangiç laktik asit derisimi ile azaldigini göstermistir. En yüksek özütleme dereceleri seyreltilmemis ekstraktantlarda gözlemlenmistir. Seyrelticilerin performanslari TOMAC'in oleil alkol ve oktanol çözeltileriyle farkli sulu faz pH'larinda özütleme deneyleri yaparak saptanmistir ve oktanol'un özellikle düsük pH'larda oleil alkol'den daha yüksek çözme gücüne sahip oldugu bulunmustur. TOMAC'in oktanol içindeki çözeltileriyle yüksek özütleme verimleri elde edilmistir. En uygun özütleme pH'i Laktobasillus çesitleriyle gerçeklestirilen ortalama fermentasyonlarin pH'ina da esit olmasi dolayisiyla 6 olarak belirlenmistir.

Tri-n-oktilmetilamonyum tuzlari arasinda en yüksek özütleme derecelerini hidroksit tuzu göstermistir (0.316 M laktik asidin seyreltilmemis TOMA(OH) ile özütlenmesi için %83 ve 0.5 M TOMA(OH)'li oktanol çözeltisi ile özütlenmesi için %78).

Bu çalisma, laktik asidin ileri ve geri özütlenmesini ayni anda gerçeklestirecek ve fermentöre bagli olarak sürekli ürün saflastimasini saglayacak tüplü fiber membran modülünün gelecekteki tasariminin ilk basamagidir. Bu çalismada elde edilen denge verileri kinetik çalismalarla birlestirilerek özütleme modülünün tasarimindaki bir sonraki adim atilabilir.

Anahtar kelimeler: Reaktif özütleme, laktik asit, denge çalismalari, TOMAC, tri-n-oktilmetilamonyum tuzlari

To Asude, Sadik and Beliz Açan,

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LIST OF SYMBOLS AND ABBREVIATIONS

D_m	Extraction factor
E	Fraction extracted
K_D	Distribution coefficient
K_E	Equilibrium constant
K_1	Intrinsic Distribution Coefficient at extremely low pH, as defined in Equation (2.8)
K_2	Intrinsic Distribution Coefficient at extremely high pH, as defined in Equation (2.8)
r	Phase ratio
Z	Loading of the extractant
$[A]^*_{aq}$	Equilibrium concentration of any acid in aqueous phase
$[A]^*_{org}$	Equilibrium concentration of any acid in organic phase
$[Cl]^*_{aq}$	Equilibrium concentration of chloride in aqueous phase
$[LA]^0_{aq}$	Initial concentration of lactic acid in aqueous phase
$[LA]^*_{aq}$	Equilibrium concentration of lactic acid in aqueous phase
$[LA]^*_{org}$	Equilibrium concentration of lactic acid in organic phase
$[SO_4]^*_{aq}$	Equilibrium concentration of sulphate in aqueous phase
$\alpha_{A,B}$	Selectivity or Separation factor

DBP	Dibutyl Butylphosphonate
MIBK	Methlyisobutyl ketone
TBP	Tri-n-butylphosphate
TBPO	Tributylphosphinoxide
TIOA	Triisooctylamine
TOA	Tri-n-octylamine
TOPO	Tri-n-octylphosphineoxide
TOMAC	Tri-n-octylmethylammonium chloride
TOMA(HSO_4)	Hydrogensulphate salt of tri-n-octylmethylammonium
TOMA(OH)	Hydroxide salt of tri-n-octylmethylammonium
TPPO	Triphenylphosphine Oxide

CHAPTER 1

INTRODUCTION

Lactic acid is a commodity chemical utilized in many fields like food, chemical, pharmaceutical, agricultural, textile industries. It can be converted to ethanol, propylene glycol and acrylic polymers, its derivatives like lactic salts, esters, lactamides and lactonitriles have widespread applications (Elvers, 1990; Wasewar, 2002). An increasingly interesting application is the use of lactic acid as a monomer for the synthesis of biodegradable homopolymers and copolymers, which are substitutes for conventional petrochemical plastics and ideal candidates for novel specific uses (McKetta, 1988).

Due to its expanding area of applications, the demand for lactic acid is increasing and the recovery of lactic acid from aqueous solutions is a growing requirement in fermentation based industries. Recovery of lactic acid from fermentation broth presents a challenging separation problem due to the dilute and complex natures of fermentation broths (Dai, 1996). Separation methods of lactic acid receive increasing attention because the cost of product recovery is a determining factor in the overall economics of production by fermentation. Traditional methods of recovery of carboxylic acids from dilute aqueous solutions

such as fermentation broths have high separation costs due to complex and energy intensive recovery techniques (Jarvinen, 2000).

The traditional recovery process of lactic acid from fermentation broth is quite complicated which involves the precipitation of calcium lactate with calcium hydroxide, recovery by filtration followed by conversion to lactic acid by addition of sulphuric acid. The dilute lactic acid product is then sequentially purified using activated carbon, evaporation and crystallization. These separation and final purification stages account for nearly half of the production costs. Consequently they are undesirable and also environmentally unfriendly due to the consumption of lime and sulphuric acid and the production of large quantities of calcium sulphate sludge as solid waste (Wasewar[1], 2002; Wasewar[2], 2002).

Various processes without the costly precipitation steps have been developed for lactic acid recovery from fermentation broth, some of which are:

- solvent extraction
- membrane separation (Juang[3], 1997; Moueddeb, 1996)
- liquid surfactant membrane extraction
- adsorption (Kaufman, 1994)
- direct distillation
- electrodialysis (Kim, 2001; Siebold, 1995)
- chromatographic separations
- ultrafiltration (Hauer, 1994)
- reverse osmosis (Hauer, 1994)
- aqueous two phase extraction (Planas[1], 1997; Planas[2], 1999)
- drying
- reactive extraction

Only a few of these methods have proved to be successful for the recovery of lactic acid and the others have found to be inferior due to their low selectivity, low yields or due to being impractical.

Reactive extraction is an alternative to conventional methods. It is advantageous since the extraction process, if properly applied, does not affect the stability of the bioproducts and the energy demand is substantially low. Like liquid-liquid extraction, two liquid phases; one being the aqueous phase containing lactic acid and the other being the organic phase into which lactic acid is extracted, are contacted in reactive extraction. But in the case of reactive extraction, the organic phase contains an extractant (or reactant) which yields higher distribution coefficients for lactic acid than the traditional organic solvents. Compared to physical liquid-liquid extraction, the selectivity of separation is remarkably enhanced in reactive extraction because the reactant present in the organic phase promotes the transfer of lactic acid to the organic phase (Jarvinen, 2000).

Like many bioconversions, lactic acid fermentation is a product inhibited process. The fermentation medium should have a low lactic acid concentration (<10%) to prevent product inhibition and also not to be harmful to the lactic acid producing bacteria. This can be overcome by in-situ product recovery (Pai, 2002). Fermentation processes involving in-situ extraction or extractive fermentation has been receiving growing attention because they are capable of relieving end-product inhibition and bringing about high productivity. The extraction process can either be carried out inside the fermentor or in an external unit, attached to the reactor (Figure 1.1).

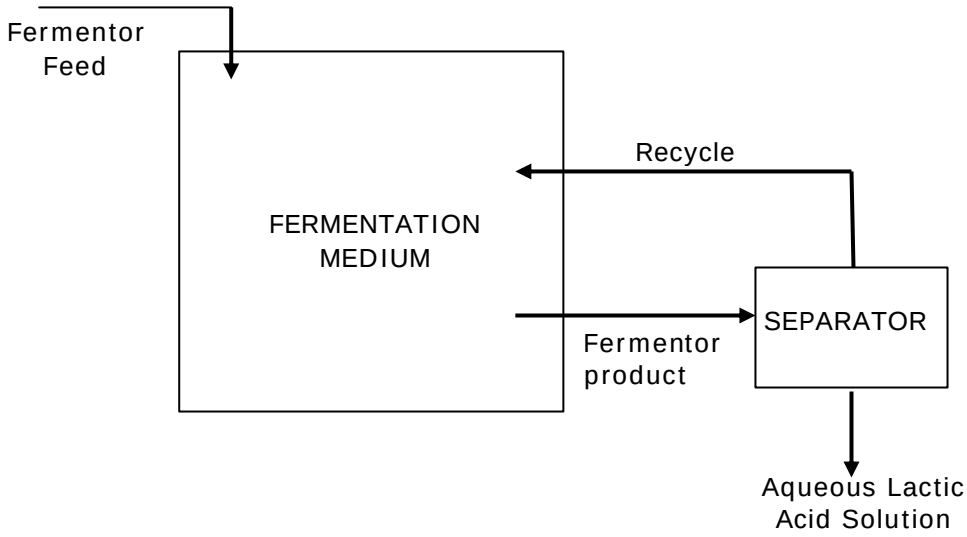


Figure 1.1 Schematic representation of the combined production and purification system

The extraction system should have some important properties such as: it should have no adverse effects on fermenting microorganisms, the optimum pH of extraction must be consistent with that of the fermentor, it should be water-immiscible and has minimal tendency to form a stable emulsion (Tong, 1998). Most of these conditions can be fulfilled by performing non-dispersive solvent extraction, which combines solvent extraction and back extraction in the same unit with microporous membranes as interphase stabilizers and phase barriers for aqueous and organic phases.

The contact of aqueous phase containing the lactic acid to be recovered with the organic phase containing the extractant, and the contact of the loaded organic phase with the aqueous stripping or back-extraction phase can be accomplished via a hollow fiber supported liquid membrane as shown in Figure 1.2. According to this design, the organic phase will be entrained in the pores of the individual hollow fibers and there will be a continuous flow of aqueous feed

phase and the aqueous stripping phase in the lumen and shell sides, respectively. A hydrophobic membrane will be employed as support medium for the organic phase and the mass transfer between the organic and the aqueous phases will take place on the surfaces of the hollow fibers.

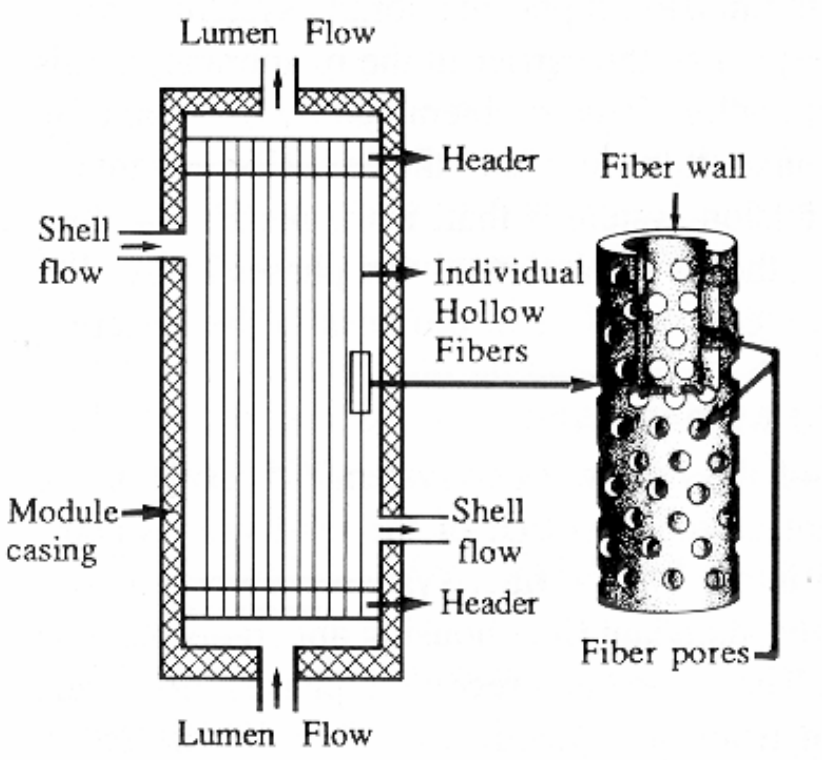


Figure 1.2 Hollow Fiber Supported Liquid Membrane Module

Lactic acid is transferred from the aqueous feed phase coming from the fermentor in the form of lactate ion to the organic phase containing the extractant, which forms a reversible complex with the lactate ion. The lactate-extractant complex moves across the membrane and the extractant releases the lactate ion on the Aqueous Phase II side of the membrane that is the stripping phase. As the lactate ion is transferred from the aqueous feed phase through the organic phase to the aqueous stripping phase, the counter ion coming from the stripping agent in the stripping phase is transferred in the opposite direction to the aqueous phase as shown in Figure 1.3. By varying the type of stripping

agent, the counter ion to be given back to the Aqueous I phase can be controlled which gives the freedom to control the composition and properties of the fermentation medium, if the Aqueous Phase I is recycled back to the fermentor.

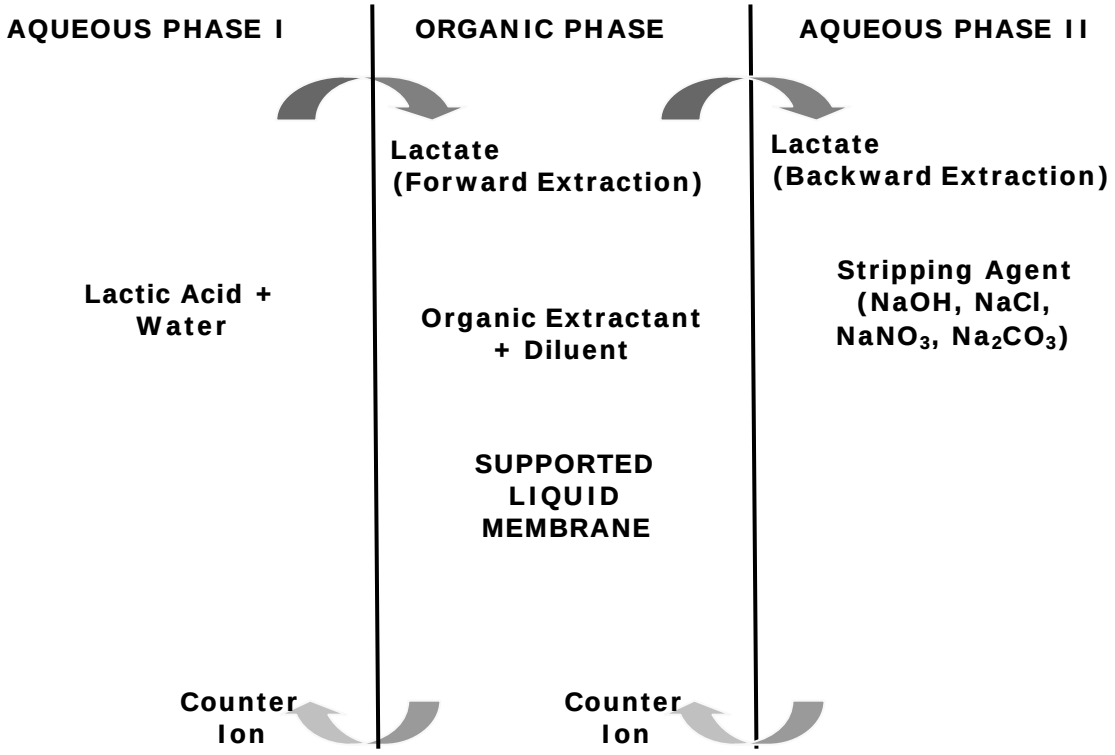


Figure 1.3 Transfer of the lactate and the counter ion through the supported liquid membrane

Membrane extraction has numerous advantages such as no danger of back mixing, no direct exposure of microbes to extraction reagents therefore assuring biocompatibility, no need for agitation and modest pressure drop along the axial length which reduces the power consumption, independent flow rate variation of the phases and no problem of flooding, flexibility in processing apparatus configuration (vertical or horizontal), potentially high efficiency due to high surface area per unit equipment volume, relatively easy scale up due to modular design. It is therefore considered a very promising alternative to conventional

dispersive solvent extraction for the separation and purification of fermentation-derived organic acids (Tong, 1998).

The design of an industrial reactive extraction process relies on the knowledge of the following four major steps:

- Reactive phase equilibria
- Microkinetic parameters
- Macrokinetic parameters
- Apparatus selection and design

The reactive equilibria are either determined in a stirred vessel or in a shaking funnel. The microkinetic parameters can be obtained experimentally in a Lewis-type stirred cell to determine the true kinetic parameters in the reaction controlled regime without any diffusional contribution. The effective mass transfer in a dispersed phase droplet is then determined by superpositioning kinetic, diffusional and turbulent contributions and verifying with experiments either in a rising droplet apparatus or in a Venturi-tube. The final stage is the column design. (Bart[2], 2001)

The present study aims to accomplish the first one of the above mentioned steps that is to obtain equilibrium data for a specific reactive extraction system for lactic acid recovery. These data will later be used in the design of a reactive extraction unit that is to be coupled with the lactic acid fermentor to achieve in-situ recovery and purification of the product. The separation unit that is going to be designed in the future will attempt to perform extraction and back extraction of lactic acid simultaneously in a single separation unit in a continuous or semi-continuous mode.

In the present study, forward extraction of lactic acid from its aqueous solutions is performed to obtain the equilibrium data and to determine the optimum conditions for the recovery of lactic acid from fermentation broth. The effects of various parameters on the extraction are investigated with the intention of implementing the data obtained to a future industrial separation unit. The equilibrium investigations are carried out in shaking flasks in which aqueous and organic phases are contacted until the equilibrium is attained. Quaternary ammonium salts (chloride, sulphate and hydroxide salts of tri-n-octylmethylammounium or Aliquat 336) dissolved in oleyl alcohol or octanol are used as the organic phases which are contacted with aqueous phases of varying lactic acid concentrations at different initial pH values. The effects of initial aqueous phase lactic acid concentration and organic phase extractant concentration on the extraction efficiency are observed. The initial pHs of the aqueous lactic acid solutions are also varied with an effort to harmonize the pH of the extraction process with that of the fermentation. The type of quaternary ammonium salt is another important parameter since using the chloride, hydrogensulphate or hydroxide salt of the extractant, Aliquat 336, can greatly influence the extraction performance and also determine the counter ion that is going to be given back to the fermentation medium when the extraction unit and the fermentor are coupled. For this reason, the chloride ion of tri-n-octylmethylammonium chloride is replaced with hydrogensulphate and hydroxide ions and the extraction of lactic acid is performed with these extractants to ascertain the effects of different counter-ions on lactic acid extraction.

CHAPTER 2

LITERATURE SURVEY

2.1 Reactive Extraction

The history of extraction dates back in “life science” applications to about 3500 BC when it was used to produce solid feed materials from vegetables or animals, followed by the production of first perfumes, pharmaceutical oils and waxes in 2100 BC, then its use in hydrometallurgy in the medieval age. Major developments were accomplished in the late nineteenth century, with the developments in thermodynamics and apparatus design. Nowadays, liquid ion exchangers are being used in the extraction of virtually all metals in the mining and environmental business, but also in the fields of extraction of organic and inorganic acids, organic chemistry intermediates, pharmaceuticals, etc (Bart, 2001).

It may be useful to define some terms critical in the understanding of liquid-liquid distribution (solvent extraction) at this stage, which are also applicable to reactive extraction (Rice, 2000):

Diluent: The liquid or homogeneous mixture of liquids in which extractant(s) and possible modifier(s) may be dissolved to form the solvent phase.

Extractant: The active component(s) primarily responsible for transfer of a solute from one phase to the other. The extractant is sometimes called reactant or carrier.

Extract: The separated phase (often but not necessarily organic) that contains the material extracted from the other phase.

Solvent: The term applied to the whole initial liquid phase containing the extractant. The solvent may contain only extractant or it may be a composite homogeneous mixture of extractant(s) with diluent(s) and also sometimes modifiers and accelerators.

Modifier: A substance added to the solvent to improve its properties e.g. by increasing the solubility of an extractant, changing interfacial parameters, or reducing adsorption losses.

Accelerator or Catalyst: A substance included in the solvent to increase the rate of transfer without affecting the position of equilibrium.

Distribution Coefficient or Distribution Ratio: The ratio of the total analytical concentration of a solute in the extract (regardless of its chemical form) to its total analytical concentration in the other phase.

Partition Ratio or Distribution Constant: The ratio of the concentration of a substance in a single definite form, A, in the extract to its concentration in the same form in the other phase at equilibrium, e.g. for an aqueous/organic system

Loading Capacity: The maximum concentration of solute(s) that a solvent can contain under specified conditions. Loading capacity can also be called maximum loading, saturation capacity or saturation loading.

The loading of the extractant, Z, is defined as the total concentration of acid (all forms) in the organic phase, $[A]_{org}$, divided by the total concentration of extractant (all forms) in the organic phase, $[B]_{total}$ (Tamada, 1990).

$$Z = = [A]_{org} / [B]_{total} \quad (2.1)$$

Phase ratio (r): The ratio of the quantity of solvent to that of the other phase. Unless otherwise specified the phase ratio refers to the phase volume ratio.

Separation Factor ($\alpha_{A,B}$) (Selectivity): The ratio of respective distribution ratios of two extractable solutes measured under the same conditions.

$$\alpha_{A,B} = D_A / D_B \quad (2.2)$$

By convention the solutes designated as A and B above are chosen so as to make $\alpha > 1$.

Extraction Factor (D_m): The ratio of the total mass of a solute in the extract to that in the other phase. It is the product of the (concentration) distribution ratio and the appropriate phase ratio.

Fraction Extracted (E): The fraction of the total quantity of a substance extracted (usually by the solvent) under specified conditions, i.e. $E_A = Q_A / Q_A'$ where Q_A is the mass of A extracted and Q_A' is the total mass of A present at the start. E may be expressed as a percentage, %E.

Chemical extraction systems make use of liquid ion exchangers, more commonly called as extractants or reactants, to perform selective separations (Bart[2], 2001). For ease of handling and other practical purposes, the extractant is diluted preferably in a non-aromatic, high-boiling, water-immiscible diluent. Since most extractants are highly viscous, the physical properties of the organic phase can be tailored by diluting the extractant to give desired properties like high interfacial tension, low viscosity and low density (Bart[1], 2001). The reactive substance forms a reversible complex with the target substance and promotes its transfer from the aqueous phase to the organic phase, remarkably enhancing the selectivity of the separation compared to the physical liquid-liquid extraction.

At very high solute loadings the organic phase may itself split into two fractions, one solvent-rich and one solvent-less fraction, forming an undesirable three-phase system. In these cases a modifier, which is usually a long chain alcohol, is added to increase the solute-extractant complex. Aromatic diluents have more solvating power and will avoid three-phase formation, but they are usually avoided in industrial applications. The solubility of the modifier is different from the other organic components and its addition is not always favored since the composition of the organic phase will gradually change and its designed properties will no longer be the same.

There are many different extractants commercially available. The four main types of liquid ion exchangers may be classified as the following:

- anion exchange
- cation exchange
- solvatizing
- chelate-forming

Anion exchange extractants (e.g. aliphatic primary, secondary, tertiary amines) form ion pairs (salts) in acidic medium. Cation exchange extractants (e.g. phosphinic- and phosphoric acid) are organic acids, which exchange the acid protein against the cation. Solvatizing extractants (e.g. phosphoric and phosphinic acid esters and phosphinoxides) are Lewis bases which form non-stoichiometric compounds with neutral solutes. Chelate-forming extractants (e.g. aliphatic aromatic hydroxymes) exchange the cation and form coordinative binding (Siebold, 1995).

2.2 Reactive Extraction of Carboxylic Acids

There are many studies in the literature on the reactive extraction or extractive fermentation of carboxylic acids. These studies focus on the various aspects of the issue like chemical interactions involved in the complexation of carboxylic acid with extractants, possible reaction mechanisms, solvent selection, effects of temperature, pH, aqueous and organic phase compositions on extraction, effects of modifiers, in-situ product recovery, kinetics of extraction, acid recovery from the organic phase by back extraction, the use of supported liquid membranes and membrane modules for non-dispersive solvent extraction, etc.

Among the various fermentation product carboxylic acids, the most commonly investigated ones are acetic, butyric, citric, formic, fumaric, lactic,

maleic, propionic, pyruvic, succinic and tartaric acids (Wardell, 1978; Kertes, 1985; Prochazka, 1994; Poposka, 2000; Tamada, 1990). Some of the studies on the extraction of these acids are discussed and summarized below.

The pioneering studies on the reactive extraction of carboxylic acids were carried out by King, C. J., and his group. In their first study in 1978, Wardell and King (Wardell, 1978) worked on the extraction of acetic and formic acids with the aim of identifying and characterizing solvents and solvent mixtures that provide high equilibrium distribution coefficients. The solvents under investigation were phosphoryl solvents (Tributyl Phophate (TBP), Dibutyl Butylphosphonate (DBP), Tributylphosphinoxide (TBPO) and Triphenylphosphine Oxide (TPPO)) and tertiary amine solvents (Trioctylamine (TOA) and Triisooctylamine (TIOA)). They reported that, among the phosphoryl compounds the distribution coefficients (K_D) increased in the order of phosphate<phosphonate<phosphine oxide. They also found out that Tributyl- and Trioctylphosphine oxides give higher K_D values than the corresponding triphenyl compound. The K_D values for Trioctylamines were higher than those for tributylamine due to higher solubility of the reaction product in the solvent phase of the former.

In a following comprehensive study by the same group in 1985, Kertes and King (Kertes, 1985) reviewed the extraction chemistry of fermentation product carboxylic acids aiming to improve the existing extractive recovery technology. They investigated the extraction characteristics of various carboxylic acids (propionic, lactic, pyruvic, succinic, fumaric, maleic, malic, itaconic, tartaric, citric, and isocitric acids), which are obtained by aerobic fermentation of glucose via the glycolytic pathway and glyoxylate bypass. They classified the extraction of carboxylic acids according to three extraction categories:

- Acid extraction by solvation carbon-bonded oxygen-bearing extractants
- Acid extraction by solvation with phosphorus-bonded oxygen-bearing extractants
- Acid extraction by proton transfer or ion pair formation, the extractant being high molecular weight aliphatic amines

Kertes et.al reviewed the conventional extraction systems, which use alcohols, ketones or ethers and they pointed out that these systems were inefficient for acid recovery from dilute solutions. In their work, they identified novel, more powerful extractants such as organophosphorus and aliphatic amine extractants, which can recover organic acids more efficiently from a wide variety of aqueous solutions. In their review, they found that the undissociated monomeric acid was extracted into carbon-bonded and phosphorus-bonded oxygen donor extractants with no exception. The acids were dimerized in the organic phase. The partition coefficients of the acids, which obey the Nernst law range from 0.003 for aliphatic hydrocarbons to about 2 to 3 for aliphatic alcohols and ketones and to about 10 or more for organophosphates. They found out that long chain tertiary amines form bulky salts in the organic phase and have equally high distribution ratios. Most of the following studies on the reactive extraction of organic acids were based on the statements of Kertes et.al.

C. J. King and his co-workers then made another important contribution to issue with a trilogy that focuses on the extraction of carboxylic acids with amine extractants, more specifically a commercially available tertiary amine, Alamine 336 which has 8-10 carbon length aliphatic chains (Tamada[1, 2, 3], 1990). In the first part of this work, Tamada et.al studied the extraction of several carboxylic acids including lactic, acetic, succinic, malonic, fumaric, and maleic acids by a tertiary amine extractant (Alamine 336) in a variety of diluents and compared

the equilibrium behaviors of different systems. The diluents used in this study were chloroform, methylene chloride, methyl isobutyl ketone (MIBK), nitrobenzene, 1-octanol (active diluents), and n-heptane (inert diluent), which were chosen from different chemical classes to examine the effects of diluent-complex interactions. These interactions were found to affect the stoichiometry of reaction and magnitude of the corresponding equilibrium constants. Their findings related to the stoichiometry of complexation were as follows:

- Common behavior is the formation of complexes with more than one acid per amine for monocarboxylic acids.
- The ratio of (1,1) to (2,1) complex formation is diluent dependent (the notation (p,q) denotes p, the number of acid molecules, and q, the number of amine molecules, in the complex).
- Halogenated hydrocarbons and alcohols inhibit overloading, ketones enhance overloading.
- They also observed that at a given aqueous acid activity loading decreases with increasing amine concentration at higher amine concentrations and reported this as a nonideal behavior. For monocarboxylic acids in inert diluents amine concentration had no effect on loading.

As far as the degree of extraction is concerned, more acid is extracted with the increasing pK_a of the acids. They observed that for most of the acids studied the solubility of the complex by the diluent decreases in the order of alcohol ≥ nitrobenzene ≥ proton donating halogenated hydrocarbon > ketone > Halogenated aromatic > benzene > alkyl aromatic > aliphatic hydrocarbon.

In the consequent study by Tamada et.al (Tamada[2], 1990), the results of mass action law analysis of the previous study were combined with the results

from spectroscopic studies to analyze chemical interactions involved in the complexation of carboxylic acids with amine extractants with various diluents. These results indicate that the formation of the (1,1) complex involves ion-pair or hydrogen-bond formation between the acid and the amine, while (2,1) complex formation involves hydrogen bonding between the carboxyl of the second acid and the carboxylate of the first. They also found that the magnitude of the (1,1) equilibrium constant is closely related to the aqueous phase pK_a of the acid increasing with decreasing pK_a.

In the last part of their work Tamada et.al (Tamada[3], 1990) studied the coextraction of water during the extraction of succinic acid by Alamine 336 in different diluents and found out that the amounts of coextracted water increases as the solubilities of water in the pure diluents increases. The effects of temperature on the extraction of lactic and succinic acids were measured and the enthalpies and entropies of complex formation were derived. In the extraction with MIBK alone the amount of lactic acid extracted increased with increasing temperature but the opposite case was observed when a tertiary amine was mixed with the diluent.

Few studies were done to observe the synergistic extraction of carboxylic acids. The presence of more than one acid in the aqueous phase affects the extraction characteristics. This was demonstrated by Juang et.al (Juang[3], 1997), who used solvent extraction and supported liquid membranes for the separation of lactic and citric acids with tri-n-octylamine (TOA) used as the extractant. They reported that different synergistic effect exists on solvent extraction distribution compared to single acid systems, at certain initial citric acid to lactic acid concentration ratios; this ratio is referred to as α. Large synergistic effects were observed at α=1 and 1/4. At α=1/2 and 1/3 this effect was observed only at

high total acid concentration. An antagonistic effect was observed at $\alpha=2$. They concluded that the presence of the second acid enhances the transport of citric acid but retards that of lactic acid. Also, increasing the TOA concentration and the temperature increases the transport rate.

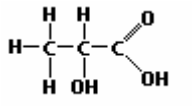
Another synergistic extraction system was developed by Matsumoto et.al (Matsumo, 2001) this time to observe the use of mixed extractants on the synergism of acid extraction. The extraction equilibria of acetic, glycolic, propionic, lactic, succinic, fumaric, L-malic and itaconic acids with tri-n-octylamine (TOA) and/or tri-n-butylphosphate (TBP) used as extractants was analyzed. Synergism was observed in the extraction of all of the acids investigated, when a mixed extractant of TOA and TBP was used. Especially the extractions of glycolic, lactic, succinic and fumaric acids showed a remarkable synergism with the mixed extractant. Several stoichiometries were also proposed for the coupling of the acids with the extractants, those being, one to one complexes of the acid with either TOA or TBP and the 1:2:1 complex of acid, TBP and TOA respectively.

The rest of the literature that is mentioned here, related to the reactive extraction of carboxylic acids will be focused on the reactive extraction of lactic acid after this general overview of carboxylic acid extraction.

2.3 Reactive Extraction of Lactic Acid

2.3.1 Properties of Lactic Acid

Lactic acid, also called 2-hydroxypropionic acid, was discovered in 1780 by the Swedish chemist Scheele and has the following chemical formula:



Lactic acid is the simplest hydroxy acid having an asymmetric carbon atom. It is a white crystalline solid with low melting point when it is in the pure and anhydrous form. But this form is rare because of its physical properties and the difficulties in its preparation. Lactic acid generally appears in the form of more or less concentrated aqueous solutions, as syrupy liquids, solutions of good quality being practically colorless and odorless (Holten, 1971).

Lactic acid goes intermolecular esterification spontaneously, resulting in the formation of lactoyllactic acid and chain polyesters containing more lactic acid units in the molecule, which affects the physical properties.

2.3.2 Distribution Coefficients

The distribution coefficients of lactic acid between water and organic solvents have to be determined (Appendix A), in order to find suitable solvents for the extraction of the acid. Among the groups of organic solvents, alcohols, ketones and esters give the highest distribution coefficients, followed by ethers, amines and nitromethane, the smallest distribution coefficients are observed in hydrocarbons, halogen compounds and nitro compounds.

Molecular weight has two opposite effects on distribution coefficients. For the solvents with similar properties, distribution coefficients generally decrease with increasing molecular weight, making low molecular weight solvents more preferable over high molecular weight ones. However an opposite effect is observed in distribution coefficients when the miscibility of water and solvent is considered. The miscibilities of solvents and water increases with decreasing

molecular weight, so larger amounts of low molecular weight solvents need to be used for extraction procedures. An optimization is therefore made between these and intermediate molecular weight solvents are generally preferred (Holten, 1971).

2.3.3 Influence of Concentration

In liquid-liquid extraction systems, two liquid phases exist in equilibrium for low lactic acid concentration in the aqueous phase. As the concentration of lactic acid increases the miscibility of solvent and water increases, forming one homogeneous system from three components, provided that sufficient lactic acid is present in the system. The limiting lactic acid concentration is generally 10-30%. Solvents with low distribution coefficients show the highest limiting concentrations and the lactic acid in the system must be below this concentration.

Another problem is the water co-extraction with lactic acid, to the organic phase. Water remains with the lactic acid after the solvent is removed and it must be evaporated. Water co-extraction is minimum when solvents with low distribution coefficients are used in extraction (Holten, 1971).

The distribution of lactic acid in other solvents is also influenced by temperature, pH, additives and the presence of lactoyllactic acid, etc.

2.3.4 Effects of Various Parameters on the Reactive Extraction of Lactic Acid

Lactic acid is one of the most commonly studied acids for reactive extraction. Various aspects of reactive lactic acid extraction were investigated in

a number of studies in the literature. These studies are classified here according to the explored parameters that are known to affect the lactic acid extraction.

2.3.4.1 Effects of the Type of Extractant and the Aqueous and Organic Phase Concentrations

The preliminary studies on finding the most suitable extractant-diluent combinations for lactic acid recovery as well as the recovery of other carboxylic acids were conducted and summarized in the previous section. A number of extractants and diluents were tested by many investigators for their success specifically in lactic acid recovery and some of these are mentioned here.

The most commonly utilized extractants for lactic acid extraction were phosphoryl extractants like TBP, Cyanex 923 and TOPO and amines; especially tertiary and quaternary amines like TOA, Alamine 336, Hostarex A327 and TOMAC.

In the studies that attempt lactic acid extraction with phosphoryl extractants (Siebold, 1995; Frieling, 1999; Malmary, 2000) either TBP or Cyanex 923, which is a widely used phosphine oxide carrier was used. Malmary et.al performed lactic acid extraction with TBP dissolved in dodecane at various ratios. They concluded that TBP appears to be an effective extractant for lactic acid recovery and that the viscosity of the pure TBP has to be reduced by diluting with dodecane due to easier phase settling and mass transfer. Cyanex 923 was used in two other studies. In their work, Frieling et.al varied the amount of Cyanex 923 diluted by kerosene and they observed high loadings in the organic phase at low lactic acid concentrations. The distribution factor increased with increasing concentration of lactic acid and then approached to a constant value. The loading of the organic phase was limited by the extractant concentration.

The degree of extraction of lactic acid with 40% (w/w) Cyanex 923 dissolved in kerosene from the fermentation media with an initial lactic acid concentration of 0,24 mol/L was as high as 47%. In another study by Siebold et.al, it was reported that the degree of extraction was reduced from 50 to 25% as the lactic acid concentration was increased from 0.1 to 1 mol/L with 10% Cyanex in kerosene. The degree of extraction was reduced from 80 to 60% as the lactic acid concentration was increased from 0.1 to 1 mol/L with 40% Cyanex 923 in kerosene. That is, better extraction was achieved at high extractant and lower initial lactic acid concentrations.

On the other hand, different trends were observed when amine extractants were used for lactic acid recovery. Juang et.al (Juang[1], 1997) used the tertiary amine TOA diluted in xylene for lactic acid extraction and observed that the distribution ratio increased with TOA concentration but it did not change with respect to the initial lactic acid concentration at fixed TOA concentration. Simultaneous formation of (1,1), (1,2), and (3,1) lactic acid-TOA complexes in the organic phase were proposed. The dominant complex was (1,1) at low initial lactic acid concentrations and (3,1) at higher initial lactic acid concentrations. Hostarex A327 is a commercial product, which is a mixture of tri-n-octyl and tri-n-decylamines. Siebold et.al (Siebold, 1995) found out that, for the tertiary amine Hostarex A327, the stoichiometric factor S_c, which gives the ratio of total lactic acid equilibrium concentration in the organic phase to the initial extractant concentration, did not depend on the amine but on the modifier concentration. Jarvinen et.al (Jarvinen, 2000) also used 40% (w/w) Hostarex A327 in 1-decanol and achieved degrees of extraction as high as 60% in a single stage depending on the mode of acidification of the fermentation broth.

Alamine 336, which is a straight chain tertiary amine containing C_8-C_{10} alkyl groups is one of the most popular diluents used in the reactive extraction of lactic acid. In the two studies conducted by Wasewar et.al (Wasewar[1], 2002; Wasewar[2], 2002) Alamine 336 was diluted in MIBK and decanol respectively to obtain organic phase compositions of 20, 30 and 40% (v/v). It was previously reported that the degree of extraction increased up to a concentration of 40% (v/v) of the Alamine 336 and then remained constant, so the Alamine concentrations in these studies were limited to this value. The findings of these two studies were reported in terms of distribution coefficients (Table 2.1), which are defined as the ratio of equilibrium lactic acid concentration in the organic phase to that in the aqueous phase.

Table 2.1 Distribution coefficients for the lactic acid extraction with various concentrations of Alamine 336 in decanol and MIBK

% Alamine 336 in decanol or MIBK	Distribution Coefficient, K_D for Alamine 336 in decanol	Distribution Coefficient, K_D for Alamine 336 in MIBK
0	0.13	0.31
20	12.57	0.72
30	16.44	2.68
40	23.37	4.24

The effect of diluent on the extraction performance and the distribution coefficients can be seen clearly form these studies. For the extraction of lactic acid with Alamine 336 very high distribution coefficients could be achieved when decanol was used as the diluent whereas much lower results were obtained in the case of MIBK, possibly due to the different solvation capacities and polarities of the two diluents. For both cases, Wasewar et.al indicated that more lactic acid

is transferred to the organic phase than would be expected from a 1:1 stoichiometry of the reaction and interpreted the results by the consecutive formation of two acid-amine species with stoichiometries of 1:1 and 2:1.

Kahya et.al worked on the optimization of process parameters for reactive extraction of lactic acid and used Alamine 336 dissolved in oleyl alcohol as the organic phase. The experiments were carried out at Alamine 336 concentrations ranging between 15% and 50%. They reported that K_D values increased with increasing Alamine concentration in oleyl alcohol and the high extraction power of Alamine 336 was attributed to its strong Lewis-base nature. The function of Alamine 336 with oleyl alcohol is to form an acid amine complex in the extraction and the rate of this complexation increases at higher Alamine concentrations consequently increasing the mass transfer between the phases and the K_D.

The final type of extractants that are widely used for lactic acid extraction are long chain aliphatic amines, which are effective extractants for separation of carboxylic acids from dilute aqueous solutions. The acid extracted into an amine-containing organic phase is no longer regarded as an acid but as an ammonium salt. Extractability is measured by the extent of ion pair association between the alkylammonium cation and the acid radical. The extraction process is based on an acid-base type reaction between the alkylamine, R, and the acid, HA:

$$HA_{(aq)} + R_{(o)} \leftrightarrow RHA_{(o)} \quad (2.3)$$

$$K_E = [RHA]_{(o)} / [HA]_{(aq)} [R]_{(o)} \quad (2.4)$$

where, K_E is the equilibrium constant (Kertes, 1985).

A striking behavior of acid amine extraction systems is the capability of the organic phase to take up acid in excess of that necessary for the stoichiometric

neutralization of the amine base. The distribution data can be interpreted with the following equations.

$$RHA_{(org)} + nHA_{(aq)} \leftrightarrow RHA(HA)_{n(org)} \quad (2.5)$$

$$K_{En} = [RHA(HA)_{n(org)}]/[RHA]_{(org)} [HA]^{n}_{(aq)} \quad (2.6)$$

Loading of the extractant, Z depends on the strength of the acid-base interaction and the aqueous phase concentration of the acid. It is independent of the amine content in an inert diluent. For low concentration organic phases, $Z<0.5$, the equilibrium constant can be expressed as follows:

$$Z/(1-Z)=K_E [HA]_{(aq)} \quad (2.7)$$

This ideal behavior is observed due to the ability of diluent to solvate the complex effectively. However, if the diluent is a poor solvating medium for the species formed, the polar complexes tend to form clusters, due to molecular association of the alkylammonium salts, RHA, formed in the organic phase at higher phase loadings in inert diluents. The salts of most acids form micelles of a variety of sizes, shapes, and properties. There is a considerable similarity between what is required of a good extractant and the surface-active properties, thus, aggregation of the salt it forms. In extreme cases a separate phase or a precipitate may form.

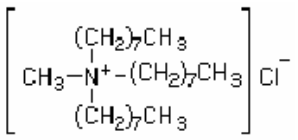
In the preliminary studies conducted to investigate the extractive capacity of amines, tertiary amines were found to possess a higher capacity than primary and secondary ones. Aliphatic tertiary amines with less than 6 carbon atoms per chain and tertiary aromatic amines were found to be poor extractants.

In the extraction studies of lactic acid by a variety of amines in a number of diluents, it was found that primary alkylammonium lactates were either excessively water soluble at room temperature or exhibit surface-active

properties or both. Secondary alkylammonium lactates were more stable and organic solvents soluble although gel formation was a drawback.

The extraction power of an amine is dictated by its basicity. The proton association constant is highest for tertiary amines and increases with the number of carbon atoms. The diluent affects the basicity of the amine and thus the stability and the solvation of the ion pair. Polar diluents are more favorable than nonpolar, low dielectric constant aliphatic and aromatic hydrocarbons.

The last and most commonly used class of amine extractants are quaternary amine salts. Aliquat 336 is the commercial name of a quaternary ammonium salt known as tri-n-octylmethylammonium chloride (TOMAC). It is composed of a large organic cation associated with a chloride ion and has the following structural formula:



TOMAC (Aliquat 336) was first used as an extractant for organic acid recovery by Yang et.al in 1991 (Yang, 1991). They performed the reactive extraction of lactic acid with both TOMAC and Alamine 336 and compared the performances of these two. In general, pure TOMAC had much higher K_D values than Alamine 336. They defined two more K values namely, K_1 which can be referred to as the intrinsic distribution coefficient at extremely low pH and K_2 which can be referred to as the intrinsic distribution coefficient at extremely high pH. They derived the following equation to predict the values of K_D at any pH value.

$$K_D = \frac{K_1 + K_2 K_a / [H^+]}{1 + K_a / [H^+]} \qquad (2.8)$$

where, K_a is the dissociation constant of the weak acid. Contrary to the high K_D value of pure TOMAC , both K_1 and K_2 values for TOMAC decreased dramatically when the amine concentration is lowered with a diluent, no matter which diluent was used. This may limit the use of TOMAC. However, K_1 value for Alamine 336 increased several-fold when 2-octanol was used as the diluent making it an effective extractant for lactic acid under acidic conditions.

The extraction ability of TOMAC was mainly determined by its concentration and not the diluent. (Highest in the case of pure amine and decreasing as the concentration of amine is decreased with the diluent) However, the use of diluent with TOMAC improves the physical properties of extractant and makes the mixture easier to handle than the pure amine, decreasing the viscosity and the surface tension therefore allowing faster phase separation.

Another extensive reactive extraction study of lactic acid was conducted by Tong et.al (Tong[1], 1998). The extractants used were tri-n-octylmethylammonium chloride (TOMAC), dioctylamine (DOA), tri-n-octylamine, tri-n-octylphosphineoxide (TOPO) and tri-n-butylphosphate (TBP) and the diluents they used were oleyl alcohol, hexane and butyl acetate. They found out that TOMAC dissolved in oleyl alcohol, butyl acetate, and a mixture of hexane and oleyl alcohol to be the most appropriate combination in terms of high extraction capacity and simplicity of operation. For a 0.3 M TOMAC in oleyl alcohol solution, the highest degree of extraction that could be achieved was around 20% for an initial lactic acid concentration of 0.3 M at around pH 6.0. When TOMAC was dissolved in a mixture of hexane and oleyl alcohol comparably high extraction

efficiencies were observed. Increasing the TOMAC concentration in the organic phase increased the degree of extraction. As a result, it was concluded that satisfactory extractive fermentation was achieved with TOMAC dissolved in oleyl alcohol as the optimum extraction system at the optimum pH of fermentation and the equilibrium constant of this system was determined to be 0.073 at 25^{o}C.

Choudhury et.al (Choudhury, 1998) studied lactic acid extraction with higher molecular weight aliphatic amine extractants. TOMAC and TOA were used as extractants in three diluents namely MIBK, octanol, and paraffine liquid. They examined the organic phase extractant concentration on the extraction process and found out that TOA was a better extractant than TOMAC. The maximum extraction was only 41% (K_D =0.71) with 70% (v/v) TOMAC in MIBK whereas the maximum extraction achieved with 50% (v/v) TOA in MIBK was much higher (79%, K_D =3.75). It was also observed that extraction with TOMAC does not depend on the nature of diluents; increasing the volume percent of TOMAC increased K_D for all the diluents.

Tong et.al (Tong[2], 1998) investigated the partition ratio of lactic acid between the aqueous phase and the organic phase composed of TOMAC dissolved in oleyl alcohol. It was found out that the partition ratio of lactic acid during the extraction at pH 6.0 decreases with aqueous lactic acid concentration whereas it increases with that of TOMAC. A satisfactory recovery of lactic acid from both aqueous solution and actual fermentation broth was accomplished.

2.3.4.2 Effect of pH

It was identified that the absence of an adequate extraction system continues to be the major bottleneck in the development of an efficient extractive fermentation process for organic acids. A more efficient extractive

fermentation process should be able to meet the optimum pH requirements of both extraction and fermentation processes. Tong et.al (Tong[1], 1998) investigated the extraction of lactic acid from aqueous solutions by different extractants dissolved in a number of diluents over a wide range of pH values with the aim of harmonizing extraction pH with fermentation pH. They performed lactic acid extraction with TOMAC dissolved in oleyl alcohol, butyl acetate, and a mixture of hexane and oleyl alcohol. They observed that TOMAC exhibited its highest extraction power around pH 6.0. This high extraction capacity of TOMAC at high pH value is due to being an anion exchange extractant. The degree of extraction with TOMAC was insensitive to pH until around pH 2.0. This implies that the extraction mechanism at low pH differs from that in a relatively high pH range. As a result, it was concluded that satisfactory extractive fermentation was achieved with TOMAC dissolved in oleyl alcohol as the optimum extraction system at the optimum pH of fermentation.

Yang et.al (Yang, 1991) directed their studies towards understanding the effects of pH on the extraction as well as on the fermentation before designing an optimum extractive fermentation process. In their work, they used Aliquat 336 (TOMAC) and Alamine 336 as extractants and 2-octanol and kerosene as diluents to investigate their abilities to extract lactic, acetic, propionic and butyric acids at various pH values. In a previous study by Kertes and King (Kertes, 1985) it was proved that most aliphatic amines extract acids from aqueous phase by forming an acid-base complex with the undissociated acid. Since the concentration of undissociated acid is a function of the pH, the extraction of organic acids will greatly depend on the pH of the aqueous phase. In the case of tertiary amines such as Alamine 336, the total acid concentration in the organic phase would be dependent only on the undissociated acid concentration in the aqueous phase. However, since TOMAC is composed of an

organic cation associated with a chloride ion, it can function as an anion exchange reagent under both acidic and basic conditions and therefore can extract both undissociated and dissociated forms of the acid. As a result of their experiments, they found out that the K_D value increased with a decrease in the pH value except at extremely high or low pHs, where K_D did not change significantly with pH. They concluded that TOMAC or other extractants that can work at high pH values must be used for fermentation processes which require a pH value higher than 6, whereas Alamine 336 will be good for use in fermentations such as lactic acid fermentation which can tolerate a pH value as low as 4.0. They also observed that neither the polar (2-octanol) nor the nonpolar (kerosene) diluent was active when use with TOMAC.

The findings of Yang et.al related to the better extraction performance Alamine 336 at low pH values were confirmed by other investigators using Alamine 336 as the extractant.

Tung et.al (Tung, 1994) also pointed out that an important aspect of the recovery concerns the pH of the fermentation broth. Many fermentations produce carboxylic acid at pH values greater than pK_{a1} of the acid being produced. Lactic acid, which has a pKa1 value 3.86, is typically produced at pH 5-6. If the recovery method is to be used out without pH change, it must function well at pH > pK_{a1}. For this purpose, they investigated the use of solid sorbents or liquid extractants that are enough basic to provide substantial capacity even at high pH values. The acids under investigation were lactic and succinic acid and these extracted by using Alamine 336 or Amberlite LA-2 diluted in MIBK, 1-octanol or chloroform. Data for the sorption of the acids by several commercially available basic polymeric sorbents were also presented and it was

reported that the performance at pH values higher than pK_{a1} was a function of sorbent basicity.

In the case of liquid extractants, it was observed that the extractants sustain capacity to higher pH in diluents that stabilize the acid amine complex. When the effect of pH upon capacity was investigated, it was reported that at low pH 100% loading of the tertiary amine, Alamine 336, is achieved for all diluents. Chloroform and 1-octanol sustained significant capacity to two or more pH units above the pK_{a1} of the acid (3.86) and the loading values were 0.61 and 0.50 for chloroform and 1-octanol respectively at the optimum fermentation pH of 5.5. The corresponding loading with MIBK was only 0.15. This difference was explained by the fact that MIBK is a polar diluent that provides general solvation of the acid-amine ion pair, whereas chloroform and 1-octanol form hydrogen bonds with the acid amine complex and provide additional stabilization, which results in higher loading. When Amberlite LA-2 was used as extractant, the loading values were 0.72, 0.61, and 0.32 in diluents of 1-octanol, chloroform and MIBK respectively. It was concluded that secondary amines provide higher capacities than tertiary amines in diluents that solvate the additional proton. Competitive uptakes of sulphate, phosphate, and carboxylate by the sorbents and extractants were also measured.

As a result of the efforts to find the optimum pH values for the fermentation and extraction of lactic acid, Choudhury et.al (Choudhury, 1998) studied the extraction of lactic acid with TOMAC and TOA at different pHs. It was concluded that a lower pH favors the extraction of lactic acid for both extractants. They also observed that in the case of TOMAC due to its quaternary amine nature, the extraction of lactic acid was less influenced by the pH of the aqueous phase in comparison with the tertiary amine, TOA.

2.3.4.3 Effect of Temperature

There is only a very slight effect if any, of the temperature in the range of 20-90 ^{o}C on the distribution ratio of lactic acid into alcohols, ketones, diethyl carbinol and ethers (Kertes, 1985). In the amine based solvent extraction systems, it was known that the extractability of the single acid decreases with increasing temperature (Tamada[3], 1990). This was also observed by Kahya et.al (Kahya, 2000) for the extraction of lactic acid with Alamine 336. They reported that the K_D values decreased with increasing temperature and attributed this observation to the fact reversible complexation reactions between the organic acid and the amine involve a proton transfer reaction or hydrogen bond formation which are mildly exothermic. Frieling et.al (Frieling, 1999) also investigated the effect of temperature on the equilibrium distribution of lactic acid between the aqueous phase and the organic phase containing Hostarex A327 and isodecanol in kerosene. They observed that the loading of the organic phase decreased with increasing temperature. The temperature dependence of loading of the Cyanex 923 in kerosene system was less significant. On the other hand there is no general trend for the effect of temperature in supported liquid membrane transport. It was found that the transport rate of lactic acid by liquid surfactant membranes containing Alamine 336 was not significantly affected by temperature. Juang et.al (Juang[3], 1997) investigated the supported liquid membrane transport of lactic and citric acids and observed that the transport rates of both increased with increasing temperature and explained this by the decreasing viscosity of the membrane phase and the increase in viscosity of the species. When the equilibrium solvent extraction distribution of this system, in which TOA in xylene was used as the solvent phase, was investigated, it was observed that K_D values decrease with increasing temperature (Juang (3), 1997).

2.3.4.4 Toxicity of Extractants

The toxicity of extraction reagents to microbes is an important parameter for in-situ reactive extraction operations, since the extraction reagents will be in direct contact with the fermentation media and the fermenting microbes or those will be recycled back to the fermentor after the product recovery. It is very important to identify and minimize the toxic effects of the extractants, diluents and possible modifiers in the organic phase, also taking into account the solubilities of these reagents with water, therefore with the aqueous fermentation media.

Some studies in the literature are directed towards understanding the toxicity of the extractants. Tong et.al (Tong[1], 1998) investigated the toxicities of the various extractants they used for lactic acid recovery. According to that, TOMAC and DOA appear to be the most toxic. Butyl acetate and hexane exhibit a certain degree of toxicity but oleyl alcohol does not, which may be accounted for by its low solubility in the aqueous fermentation broth and by its lower toxicity as a compound.

Jacquet and his coworkers (Jacquet, 1999) stated that the intimate contact of an organic phase with the fermentation broth implies that the organic components of this phase may be present in the aqueous phase at saturation levels. They used TOMAC (Aliquat 336) dissolved in octanol for lactic acid extraction and observed that it showed no inhibition on the growth of *Pseudomonas putida* in the molecular level. They modified the counter ion of Aliquat 336 with HSO_4^- ion and observed the toxic effects of both Cl^- and HSO_4^- ions on the microbial growth. An organic phase containing Aliquat 336 with

HSO_4^- counter ion rather than Cl^- was found to be more convenient for use in the in-situ liquid extraction.

Tik et.al (Tik, 2001) worked on lowering the toxic effects of Alamine 336 in oleyl alcohol on lactic producing bacteria *Lactobacillus delbrueckii*. They observed that Alamine 336 has toxic effect on free cells and that the toxicity increases with increasing Alamine 336 concentration. Immobilization of the cells in the presence of sunflower oil reduced the toxic effect of the water soluble portion of the organic phase.

2.4 Membrane Extraction

The problems associated with the formation of stable emulsions in conventional dispersive extraction systems and the problems arising from the toxic effects of the extractants which are in direct contact with the ferme ntation medium has lead to the use of microporous membranes as interface stabilizers and phase barriers in solvent extraction. Contrary to classical dispersive extraction, there is a little tendency to form emulsion when membranes are used. Lactic acid could be recovered satisfactorily from aqueous solutions and actual fermentation broth by using microporous hollow fiber membranes, integrating the membrane extraction with fermentation process. Such a study was conducted by Tong and his coworkers (Tong[2], 1998) in which continuous lactic acid recovery was undertaken in a microporous hollow fiber membrane extraction device with TOMAC dissolved in oleyl alcohol used as the organic phase. TOMAC and oleyl alcohol were found to be the most suitable extractant diluent combination for lactic acid extractive fermentation due to their high extraction power at optimum fermentation pH and low toxicity toward the lactic acid bacteria but both TOMAC and oleyl alcohol form a stable emulsion in

conventional mixer-settlers. Tong et.al therefore carried the extraction operation in a continuous mode with aqueous and organic phases fed co-currently to the tube and shell sides of a microporous hollow fiber membrane module.

It was observed that, at given organic and aqueous phase flowrates the average extraction flux increased with increasing TOMAC and lactic acid concentrations. High extractant and lactic acid concentrations and high aqueous phase flowrates were advantageous for achieving fast extraction. The aqueous phase flowrate had a considerable effect on the extraction, whereas the organic phase flowrate had a very little effect. The degree of extraction increased rapidly with aqueous residence time initially than the rate slowed down.

Membrane extraction was also implemented on actual fermentation broth and very little deviation was observed from model lactic acid solutions. Microporous hollow fiber membrane devices offer an additional advantage over the conventional extractors in the sense that they are not subject to adverse effects from yeast extracts in fermented broths.

Juang et.al (Juang[3], 1997) also used supported liquid membranes for the separation of citric and lactic acid with TOA in xylene and observed the effects of temperature and strip phase composition on supported liquid membrane extraction. The strip phase was either water or Na_2CO_3 solution. A hydrophobic membrane support, the pores of which were filled with TOA under vacuum, was immersed in the organic phase. Both TOA concentration in the membrane phase and initial total acid concentration feed phase were varied. A competitive extraction exists between lactic and citric acids. The transport rate of the acid whose concentration is varied, increases with its concentration but that of the

acid whose concentration is fixed reduces slightly. Increasing the TOA concentration increases the transport rate.

The proposed supported liquid membrane process contains three steps:

1. Diffusion of the acids across aqueous stagnant films of the feed and strip phases

2. Diffusion of TOA and TOA-acid complexes in the membrane phase

3. Chemical interactions between the acids and TOA at the membrane-aqueous phase interfaces

It was reported that the chemical reactions at interfaces and membrane diffusion are of critical importance in the transport mechanism of these two competing acids for the extraction by a supported liquid membrane containing the extractant.

The transport rate increases initially with Na_2CO_3 concentration and reaches a maximum after which a further increase results in lowering the transport rate.

The separation factor achieved by this supported liquid membrane operation was not far from unity but this process is still promising if some modifications are made like supported liquid membranes operated with hollow fiber modules or the use of liquid surfactant membranes, which can provide sufficiently large surface areas for mass transfer. Improving the membrane stability by using modified types of supported liquid membranes could also

overcome the major problems hindering the application of supported liquid membranes.

But the present literature lacks a membrane extraction study which combines forward and backward extraction of lactic acid in a single unit. A hollow fiber supported liquid membrane module can be designed for this purpose, which utilizes microporous hydrophobic membranes as hollow fiber supports, the pores of which are filled with the organic phase. The forward and backward extraction can be accomplished simultaneously with aqueous feed and the aqueous stripping phases flowing on the both sides of the hollow fiber membrane tubes. Lactic acid molecules in the aqueous feed phase will couple with the extractant molecules on the surface of the membrane and diffuse through the membrane containing the organic phase. The extractant will release the lactic acid molecules on the other side of the membrane contacting with the aqueous stripping phase.

Quaternary ammonium salt TOMAC (tri-n-octylmethylammonium chloride) was identified as an effective extractant. But it is a phase transfer catalytic agent and it has a tendency to form a stable emulsion when used in conventional dispersive extraction. It has to be used in conjunction with a low viscosity and low toxicity diluent to reduce its high viscosity and also to hinder its toxic effects toward the lactic acid bacteria and the fermentation medium. Oleyl alcohol was found to exhibit zero toxicity and also to provide good extraction when used together with TOMAC. But the problem of emulsion formation is still a major hurdle to overcome when TOMAC in oleyl alcohol is used as the organic phase. So using this extractant-diluent combination in membrane extraction, by filling the organic phase into the pores of the individual hollow fiber membranes would be a wise alternative. This was attempted for the forward extraction of lactic

acid, and a module was designed by Tong et.al (Tong[2], 1998) but there is no similar study to perform the forward and backward extraction simultaneously in the same module.

The use of TOMAC has another importance since the counter ion of TOMAC (chloride in this case) is exchanged with lactic acid; as lactic acid is being transferred from the aqueous feed phase through the organic membrane phase to the aqueous stripping phase, the chloride ion is transferred in the opposite direction and given back to the aqueous feed phase. If in-situ extraction is to be performed, then the aqueous feed phase will be the fermentation medium itself. So by controlling the counter ion to be transferred to the fermentor, the composition of the fermentation medium can be adjusted. The anion of TOMAC can be exchanged with another ion to tailor the composition of the fermentation medium to obtain better yields. The quaternary ammonium salt obtained by exchanging the chloride ion with hydrogensulphate, hydroxide or any other anion will have considerably different properties and will show a different extraction performance. Such a study is not present in the literature. Sulphate counter ion was reported to show a lesser toxic effect on lactic acid producing bacteria (Jacquet, 1999) and the toxic effects of the quaternary ammonium salt with the hydroxide counter ion can be investigated similarly. The most important aspect of the present study is to investigate the reactive equilibria for the forward extraction of lactic acid from its aqueous solutions when different salts (chloride, hydrogensulphate and hydroxide) of tri-n-octylmethylammounium are used as extractants.

CHAPTER 3

EXPERIMENTAL

The experimental methodology that was followed to obtain the equilibrium data for the forward extraction of lactic acid from its aqueous solutions is described in this chapter. Since the aim of this study is to observe the effects of various factors on the extraction characteristics and equilibrium properties, the parameters to be studied were first identified. For the extraction system of our choice, these parameters were aqueous phase lactic acid concentration and pH, organic phase extractant concentration and type of quaternary ammonium salt. The ranges of these parameters between which the extraction experiments were carried out are listed in Table 3.1.

The ranges of parameters were determined as such, to simulate the conditions of an actual fermentation broth and a potential reactive extraction system that could be coupled with the fermentor.

Table 3.1 Ranges of parameters applied to extraction experiments

Parameter	Range
Aqueous phase lactic acid concentration	0.25 – 1.3 M lactic acid in water
Organic phase extractant concentration	0.1 – 0.5 M extractant in diluent
Type of the quaternary ammonium salt	Cl^-, OH^-, HSO_4^- salts of tri-n-octylmethyammonium
Initial pH of the aqueous phase	2 - 6
Type of diluent	Oleyl alcohol or octanol

The upper limit of the aqueous phase lactic acid concentration was selected as 1.3 M which corresponds to approximately 11% (w/w) lactic acid in water. This concentration is a little higher than the maximum lactic acid concentration in the actual fermentation broths which should be below 10% due to product inhibition and toxic effect towards the lactic acid producing microbes. The lower limit was chosen to be 0.25 M (approximately 2% (w/w)) lactic acid in water to represent the lowest concentration to apply the recovery process.

The organic phase extractant concentration was varied between 0.1 M and 0.5 M extractant (tri-n-octylmethylammnium salt) dissolved in the diluent (oleyl alcohol or octanol) mainly due to the physical properties of the extractant and its toxic effect. TOMAC has a very high viscosity (1450 cP at 30 ^oC) so it is very difficult to handle and use it directly in liquid extraction in its pure form. Therefore it has to be diluted with a suitable organic solvent prior to its use in extraction. It was found that higher concentrations of TOMAC extracted more lactic acid and better relieved product inhibition (Tong[1], 1998) but in the extraction experiments its maximum concentration was limited to 0,5 M in the

diluent in order to suppress its toxic effect and to obtain an organic phase that can be more easily handled and that can mix better with the aqueous phase to provide better mass transfer.

The aqueous phase lactic acid concentration and organic phase extractant concentration were generally tested at 5 levels. The effect of pH was investigated in a different set of experiments, which includes the combination of aqueous phase lactic acid concentration and organic phase extractant concentration at 3 levels. Additional experiments were conducted to observe the effects of different diluents and the extraction behavior when pure extractant or pure diluent was used as the organic phase. Some sets of experiments were duplicated to check the reproducibility of the experiments and the average of the two results was taken as the final value.

3.1 Equipment

The extraction experiments were carried out in 100 mL Erlenmeyer flasks which are placed in a constant temperature shaking water bath (GFL 1083) the temperature of which was set to 30 ^{o}C.

The ingredients of these flasks were separated by using a high speed centrifuge (Sigma High Speed Laboratory Centrifuge) after the extraction was complete.

The analysis of the aqueous phases before and after extraction, as well as the analysis of the aqueous phases resulting from the ion exchange attempts on pure TOMAC were performed by using Ion Chromatograph (Dionex DX-100), equipped with a Dionex IonPac AS15 4 mm Column, a conductivity detector and

a suppressor, the mobile phase being 30 mM NaOH solution with a pH of 11.95, flowing at 1.5 mL/min.

3.2 Materials

The materials used in this study were lactic acid (Merck Co.) which has an analytical purity of 88-92%, Aliquat 336 (TOMAC) (Aldrich) which has a quaternary ammonium content of 88.3% and which is used as the extractant, oleyl alcohol (Merck Co.) and octanol (Riedel) which are used as diluents, H_2SO_4 (Merck Co.), NaOH (Merck Co.) and UHP water produced by Millipore Milli-Q Water System.

3.3 Experimental Procedure

3.3.1 Preparation of Lactic Acid Stock Solution

It was known that lactic acid usually self-esterifies or dimerizes in aqueous solutions of concentrations over 20% (w/w) (Holten, 1971). To avoid this, 88-92% concentrated lactic acid was diluted to approximately 15% (w/w) with UHP water and boiled under constant reflux for 24 hours to hydrolyze any lactic acid polymers present. The refluxed lactic acid solution was then analyzed for its lactic acid content by titration with 0.1 N NaOH solution and used as the stock solution to prepare the sublevels of concentration for the aqueous phase that is going to be used in the extraction experiments.

3.3.2 Preparation of the Aqueous Phase for Extraction

The 15% lactic acid stock solution was diluted to the desired concentrations by UHP water. The pH of these aqueous solutions were adjusted

to the desired value by adding 5 M NaOH solution and measuring the pH simultaneously by a pH meter (Beckman Expandomatic SS-2).

3.3.3 Preparation of the Organic Phase for Extraction

Organic phase was prepared by well-mixing the extractant (tri-n-octylmethylammonium salt) with the diluent (either oleyl alcohol or octanol). A 0.5 M extractant in diluent solution was first prepared which was then diluted to the desired sublevels with the diluent. In some of the experiments pure extractant or pure diluent were used alone as the organic phase.

3.3.3.1 Procedure to Exchange the Chloride Ion of TOMAC to Hydrogensulphate Ion

One of the aims of this study is to observe the effects the type of quaternary ammonium salt on the extraction. To obtain HSO_4^- salt of tri-n-octylmethylammonium the following procedure was applied.

100 mL of pure TOMAC and 100 mL of 1.5 M H_2SO_4 aqueous solution were placed in a separatory funnel and shaken vigorously for 5 minutes. Phases were then left to settle and separate for 12 hours after which the aqueous phase was decanted and stored for analysis. The remaining organic phase was contacted with 1.5 M H_2SO_4 again and the same procedure was repeated for a total of five contacts. As the last step, the organic phase was contacted with an equal amount of UHP water to get rid of any remaining water soluble impurities, and the same procedure was applied. After the removal of the aqueous phase, the remaining organic phase is mainly the TOMA(HSO_4^-) mixed with the remaining or unconverted TOMA(Cl^-). The TOMA(HSO_4^-) concentration of the organic phase is determined by analyzing the aqueous phase with Ion Chromatograph after each

decantation and using a mass balance to calculate the organic phase salt concentration. The raw data obtained from these analyses is given in Appendix B.1

3.3.3.2 Procedure to Exchange the Chloride Ion of TOMAC to Hydroxide Ion

A similar procedure was followed to obtain TOMA(OH^-). This time 100 mL of pure TOMAC and 100 mL of 2 M and 5 M NaOH aqueous solutions were contacted and the above procedure was repeated. The TOMA(OH^-) concentration was then determined by analyzing the aqueous phase with Ion Chromatograph after each decantation and using a mass balance to calculate the organic phase salt concentration. The raw data obtained from these analyses are given in Appendix B.2

These quaternary ammonium salts were then diluted to the desired concentrations by mixing with octanol.

3.3.4 Extraction Experiments

Equilibrium investigations were carried out by adding equal volumes (10 mL) of aqueous and organic solutions of various concentrations in Erlenmeyer flasks and equilibrating in the constant temperature shaking water bath for 24 hours (which, by performing preliminary kinetic experiments, was found to be the time sufficient for equilibrium to be reached) at 30 ^oC. As equilibrium was attained, the mixtures were transferred to centrifuge tubes and phases were separated by centrifuging at 11000 rpm for 5 to 60 minutes depending on the degree of emulsion formation between aqueous and organic phases. Some samples need not be centrifuged due to self separation of phases. When a clear separation of the two phases was achieved, the lower aqueous phase was

carefully pipetted out, diluted and analyzed for residual lactic acid concentration. The concentration of lactic acid transferred to the organic phase was calculated by using mass balance.

3.3.5 Analytical Method

The analyses of the aqueous phases were done with an ion chromatograph (IC) (Dionex DX-100).

A calibration curve was drawn with the standard solutions of known concentration for each substance. The chromatograph can detect very low concentrations and the calibration curve can be drawn in the linear region for very dilute solutions. Above a certain concentration, the calibration curve becomes non-linear due to concentration effects and the decrease in the signal of the ion chromatograph. Since working in the linear region gives more reliable results, all samples were diluted in various ratios to fit their concentrations in this low concentration linear region of the calibration curve. Some samples were diluted 250 fold while some others were diluted 1000 fold before injecting them to the ion chromatograph.

The concentrations of the samples were then found by calculating the area under the curve of their IC chromatograms and converting these areas to concentration by using the calibration curve for that substance. Sample calibration curves for lactic acid, sulphate and chloride are given in Appendix C.

CHAPTER 4

RESULTS AND DISCUSSION

The results of the experiments performed to describe the equilibria for lactic acid extraction from aqueous solutions are presented and discussed in this section.

Known concentrations of aqueous and organic phases were equilibrated in a constant temperature shaking water bath and the two phases were separated after the equilibrium was reached. The lower aqueous phase was diluted to the desired concentration range and analyzed for its residual lactic acid content as well as for the concentrations of the ions formed in the aqueous phase after extraction. The success of extraction was quantified in terms of degree of extraction which is defined as:

$$\text{Degree of extraction (\%)} = \frac{[LA]_{aq}^{0} - [LA]_{aq}}{[LA]_{aq}^{0}} \times 100 = \frac{[LA]_{org}}{[LA]_{aq}^{0}} \times 100 \qquad (4.1)$$

Degree of extraction is defined in terms of concentrations since the volumes of the aqueous and organic phases are equal ($V_{org}/V_{aq}=1$) and assuming that they don't change after extraction, so that concentrations can be used instead of number of moles of lactic acid. Fraction extracted, which is defined as

the ratio of the mass of substance extracted to the total mass of substance initially present (Rice, 2000) could also be used instead of the degree of extraction defined above, but it is not preferred here.

A higher degree of extraction means that more lactic acid is transferred from the aqueous phase to the organic phase, which implies a successful forward extraction.

The distribution coefficient, K_D, which is defined as the ratio of the concentrations of lactic acid in the two phases, is also a measure of extraction. The distribution coefficients are also calculated and tabulated for the experiments performed.

$$K_D = \frac{[LA]_{org}}{[LA]_{aq}} \tag{4.2}$$

The loading of the extractant, Z, was defined as the total concentration of acid (all forms) in the organic phase, $[A]_{org}$, divided by the total concentration of extractant (all forms) in the organic phase, $[B]_{total}$ (Tamada, 1990).

$$Z = [A]_{org} / [B]_{total} \tag{4.3}$$

In the case of lactic acid extraction form its aqueous solutions by quaternary ammounium salts, the loading of the extractant can be calculated as the ratio of the concentration of lactic acid that was calculated to be present in the organic phase to the total concentration of extractant (TOMAC, TOMA(HSO_4), TOMA(OH) or their mixtures) in the organic phase. Loading values for all the extractions with quaternary ammonium salts are calculated and listed in the tables (Appendix D) together with degree of extraction and K_D values. The loading phenomenon is discussed in detail in Section 4.2.5.

4.1 Extractions with TOMAC dissolved in oleyl alcohol

4.1.1 Initial Aqueous Phase pH Between 2-2.5

The equilibrium complexation reaction between TOMAC and lactic acid can be written as follows:

$$H^+A^-_{(aq)} + TOMA^+(Cl^-)_{(org)} \leftrightarrow TOMA^+(A^-)_{(org)} + H^+Cl^-_{(aq)} \quad (4.4)$$

The reaction of TOMAC and lactic acid involve the formation of a TOMA-lactate complex in the organic phase, and the chloride counter ion of TOMAC, which is replaced by the lactate is liberated and transferred to the aqueous phase to couple with hydrogen ions and form hydrochloric acid. The counter ion of TOMAC exerts two main effects linked to its ion exchange properties. The first is to lower the equilibrium pH of the aqueous phase and the second is to liberate the counter ion to this phase, which may affect the extraction of the acid. The discussion on lactic acid extraction with TOMAC will be built up on this equilibrium reaction.

The initial extraction experiments were carried by contacting organic phases composed of TOMAC (Aliquat 336) dissolved in oleyl alcohol, with aqueous phases containing lactic acid, the initial pH of which are not controlled and that vary between 2 and 2.5. The results of these experiments are given in the tables D.1.1-2-3-4 and 5, which show the variation of the concentrations of aqueous phases of five different initial lactic acid concentrations before and after extraction. The difference between these two is calculated to be the organic phase lactic acid concentration, from mass balance. The degree of extraction, distribution coefficient and loading values are calculated from equations 4.1, 4.2 and 4.3.

These results are plotted in Figure 4.1.1 for different organic phase compositions, to observe the variation of the degree of extraction with initial lactic acid concentration in the aqueous phase. From this plot, it can be seen that the degree of extraction decreases with increasing initial lactic acid concentration. This is consistent with the previous studies in the literature which report a similar trend for carboxylic acid extraction with amine extractants (Tong[1], 1998; Kahya, 2001). The decrease in the degree of extraction with increasing lactic acid concentration can be attributed to the fact that the concentration of the extractant in the diluent is the limiting reagent for the reversible complexation of the lactic acid molecules with the extractant molecules in forward extraction. At low lactic acid concentrations there is a larger chance for the extractant to couple with for most of the acid molecules initially present in the aqueous phase, so higher extraction efficiencies can be achieved. At higher initial lactic acid concentrations, however, lower degrees of extraction are observed due to a lower ratio of extractant molecules in the organic phase to couple with the lactic acid molecules in the aqueous phase.

It can also be seen from Figure 4.1.1 that the highest degree of extraction with TOMAC dissolved in oleyl alcohol could be achieved for the extraction of approximately 0.3 M lactic acid solution with 0.5 M TOMAC in oleyl alcohol solution, which represent the lowest and highest concentration limits of aqueous and organic phases, respectively. The degree of extraction achieved in this case is nearly 16%. The extraction efficiency decreases to about 2% for the extraction of approximately 1.3 M lactic acid solution with 0.1 M TOMAC in oleyl alcohol solution, which represent the highest and lowest concentration limits of aqueous and organic phases, respectively. The extraction efficiencies for the TOMAC in oleyl alcohol set without pH control vary in this narrow range and all are

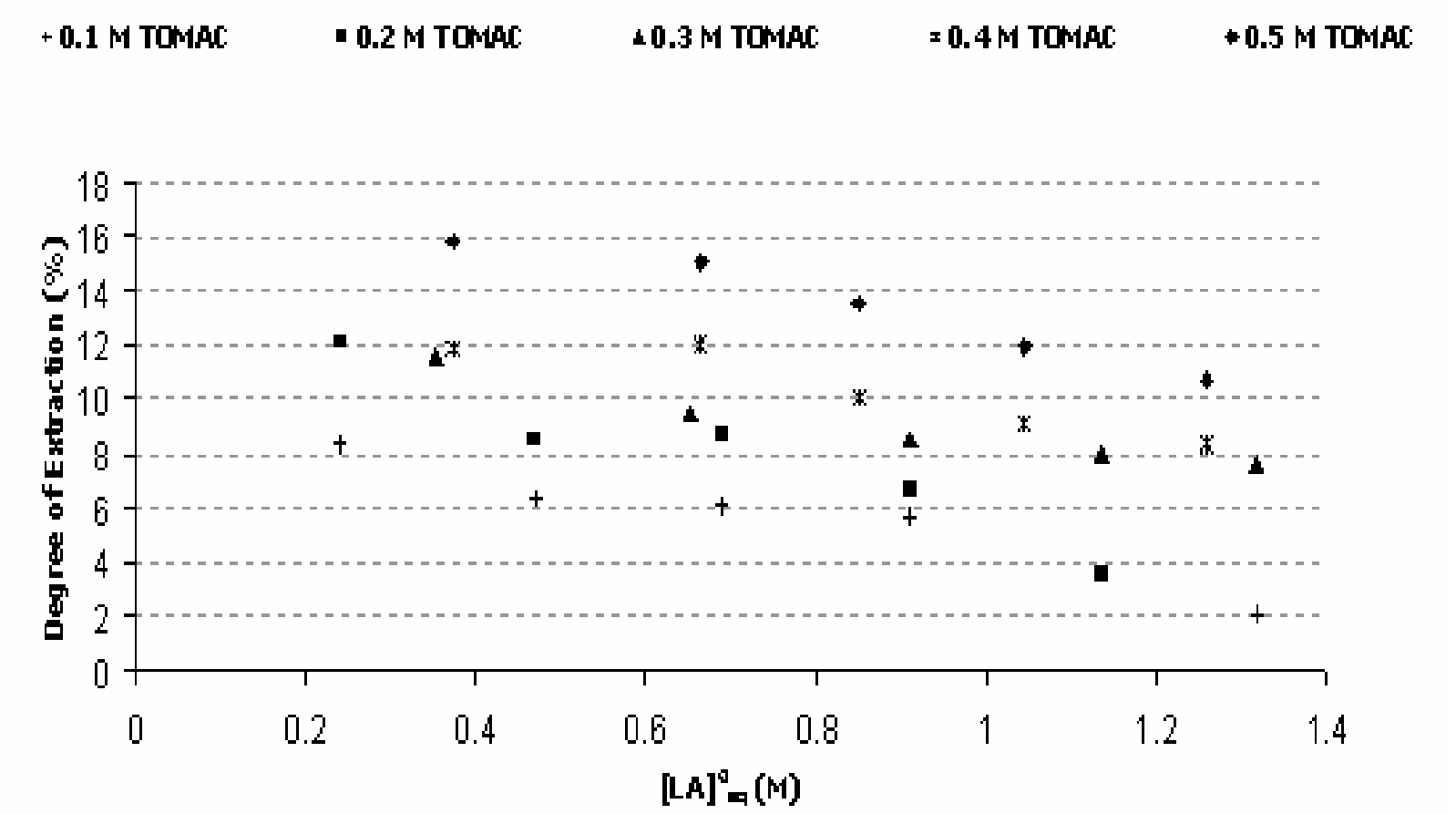


Figure 4.1.1 Variation of degree of extraction with initial lactic acid concentration for TOMAC in oleyl alcohol, without pH adjustment (pH=2-2.5)

considered to be inefficient for the recovery of lactic acid from aqueous phase to the organic phase.

For all extractions performed with TOMAC in oleyl alcohol, a third, emulsion phase was observed at the interface between the aqueous and organic phases when the initial pH of the aqueous phase is between 2 and 2.5 (without pH control). This third phase could hardly be destroyed by centrifuging the samples at 11000 rpm for one hour. The degree of emulsion formation increased with increasing aqueous and organic phase concentrations. It is known that, if the solvent does not have good solvating capacity, the acid-amine complexes tend to cluster together and move away from the bulk solvent (Yang, 1991). As a result of this, most of the extracted lactic acid was probably kept in this dense, opaque third emulsion layer, and the amount of lactic acid that passed to the organic phase was much lower than expected. Phases could be separated much easier and the degrees of extractions would be much higher if there had not been this emulsion formation. Later efforts were directed towards overcoming this problem by using a proper diluent, which can provide the necessary solvating power for TOMAC and by adjusting the initial pH of the aqueous phase to higher values.

But, before investigating the effects of a different diluent and aqueous phase pH, extraction experiments were also performed by using pure TOMAC and pure oleyl alcohol as the organic phase to compare the extraction efficiencies with those of TOMAC dissolved in oleyl alcohol set. The results of these experiments are presented in Tables D.1.6 and D.1.7 and plotted in Figure 4.1.2.

For the extraction of lactic acid form its aqueous solutions, the pH of which vary between 2 and 2.5 depending on their concentrations, to organic phases

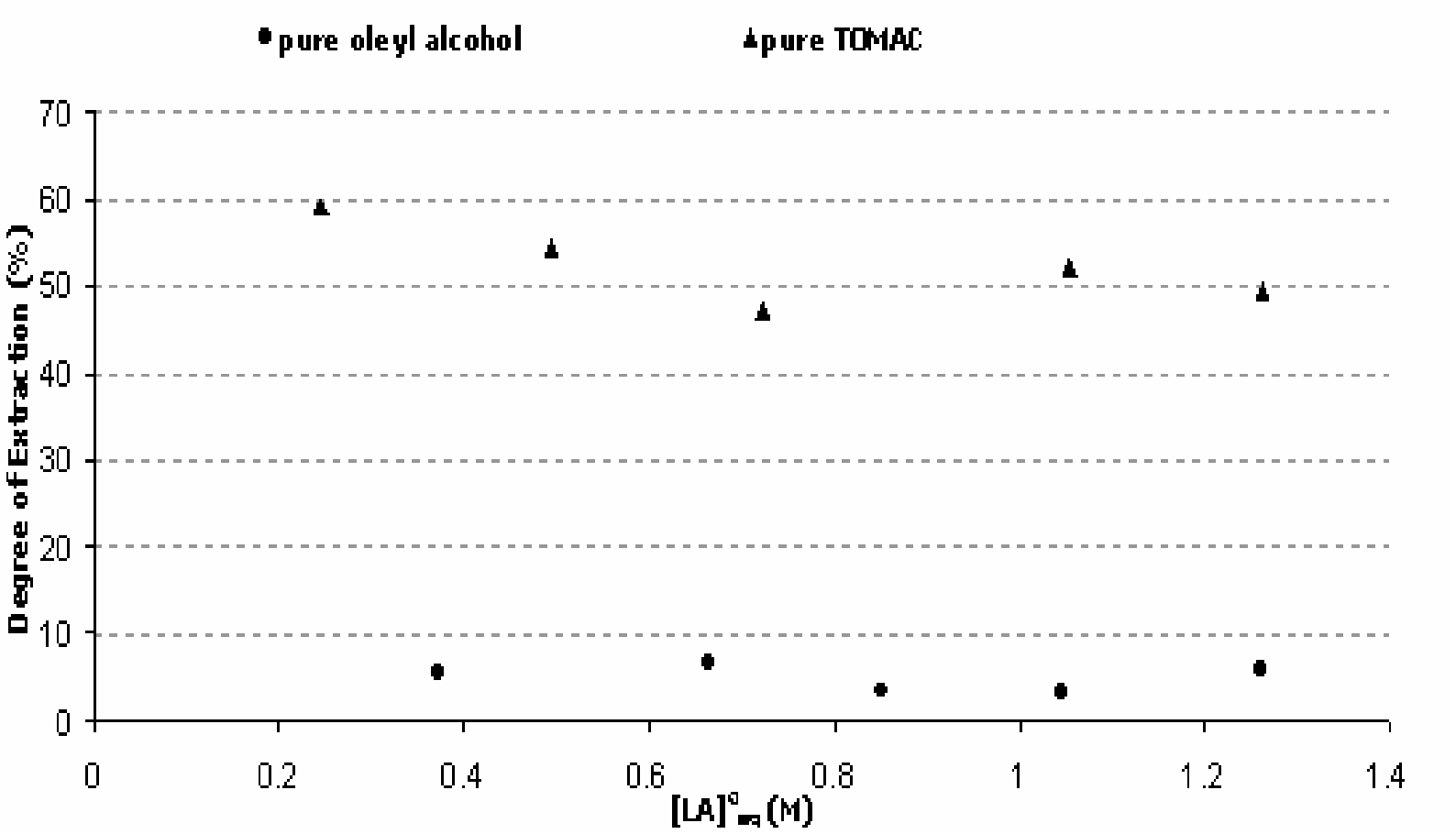


Figure 4.1.2 Variation of degree of extraction with initial lactic acid concentration for pure TOMAC and pure oleyl alcohol, without pH adjustment (pH=2-2.5)

composed of pure TOMAC or pure oleyl alcohol, no apparent effect of initial lactic acid concentration on the degree of extraction or the distribution coefficients was observed. Quite high degrees of extraction (59%) could be achieved when using TOMAC without diluting it with oleyl alcohol. But the viscosity of undiluted TOMAC is very high making it much difficult to handle. The surface tension that arises between the two phases decreases when TOMAC is used with a diluent and the phases separate faster. The surface tension is higher in the case of pure TOMAC and less emulsion formation was observed when compared to experiments performed with TOMAC dissolved in oleyl alcohol. The degrees of extraction that could be achieved with pure oleyl alcohol without any extractant were as high as 7%, but no obvious trend could be identified with increasing initial aqueous phase concentration, in the extraction of lactic acid to oleyl alcohol.

A potential cause of errors and inconsistencies in the experimental results may be due to the slight changes in the phase volumes for extractions performed with TOMAC. The volumes of the organic phases, which were equal to the volumes of the aqueous phases before extraction, were observed to increase after extraction with pure TOMAC or TOMAC dissolved in oleyl alcohol. This indicates that the aqueous phase is soluble in the organic phase. The increase in the volume of the organic phase was different for each experiment and it was not measured. The calculations were based on the assumption that the change in phase volumes was negligible.

4.1.2 Initial Aqueous Phase pH Adjusted to 4

Due to the low degrees of extraction obtained for the extraction of lactic acid solutions the pH of which were not controlled, the initial pH of the aqueous solutions were adjusted to 4 by adding 5 M NaOH on the lactic acid solutions whose initial pH values range between 2 and 2.5.

It was reported by Yang et.al (Yang, 1991) that TOMAC (or Aliquat 336) is composed of an organic cation associated with a chloride ion and it can function as an anion-exchanger under both acidic and basic conditions, so that it can extract both dissociated and undissociated forms of the acid. The dissociation constant, pK_a, of lactic acid is 3.86 at 25^oC. At extremely low pH values the acid is mainly in the undissociated form, whereas as the pH becomes higher the acid starts to dissociate. The amount of dissociated acid is equal to the amount of undissociated acid at $pH=pK_a$ of the acid. When the initial pH of the lactic acid solutions were adjusted to 4 by adding NaOH, approximately 58% of the acid was calculated to become dissociated (Appendix D).

The results of the extraction experiments performed with these aqueous solutions are given in Tables D.1.8-9-10-11 and 12 and plotted in Figure 4.1.3. The organic phases used in these experiments were 0.1, 0.3 and 0.5 M solutions of TOMAC dissolved in oleyl alcohol, pure TOMAC and pure oleyl alcohol.

When compared to the extraction experiments performed at pH 2-2.5, the set of experiments performed at pH 4 yield much better degrees of extraction, as can be seen from these results and from Figure 4.1.3. The degrees of extraction again decreased with increasing initial lactic acid concentration in the aqueous phase and the highest percent recoveries were obtained for lowest initial lactic acid concentrations. The degrees of extraction also decreased as the use of

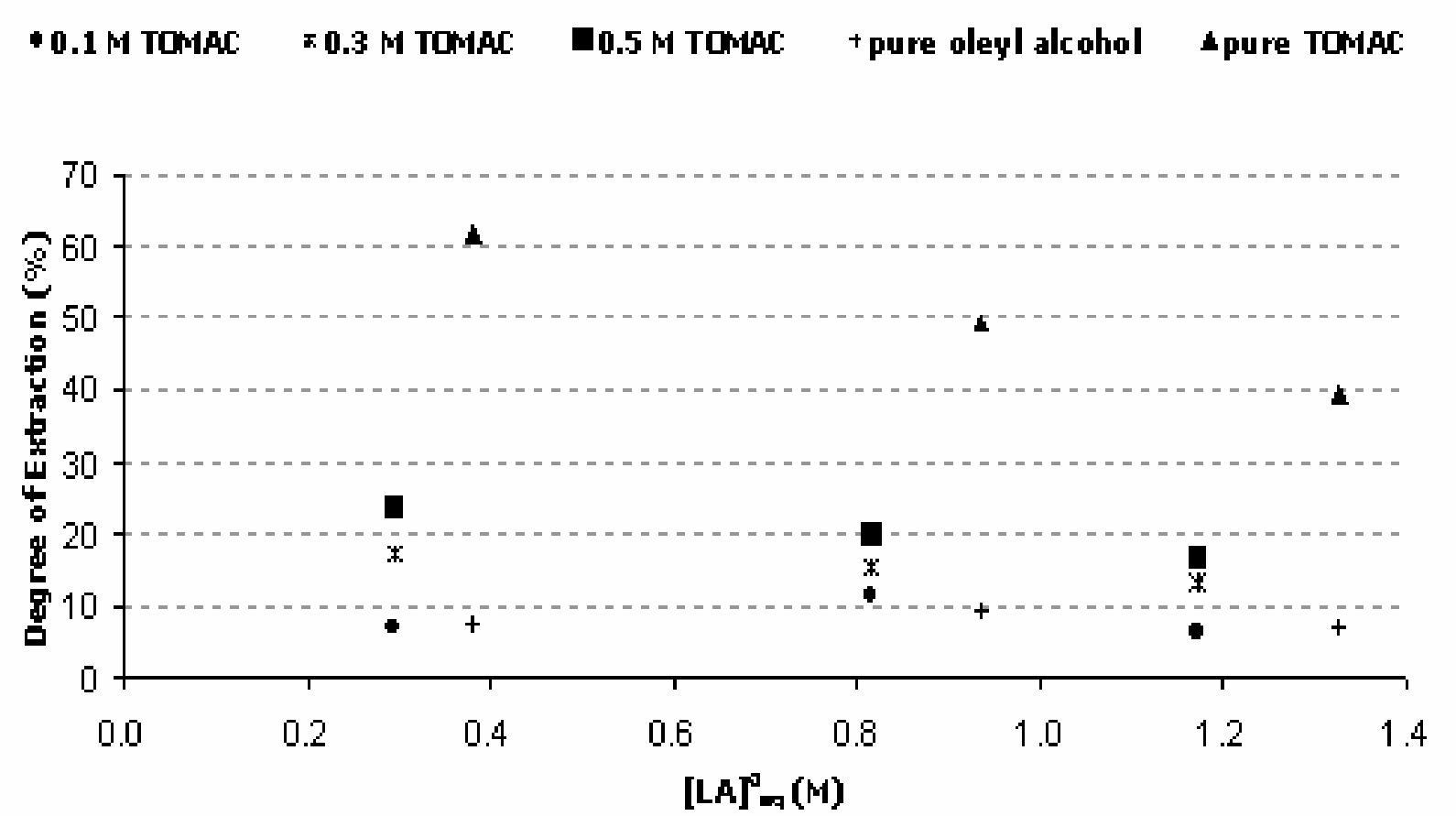


Figure 4.1.3 Variation of degree of extraction with initial lactic acid concentration for TOMAC in oleyl alcohol, initial aqueous phase pH adjusted to 4

diluent with the extractant was increased, in this case oleyl alcohol with TOMAC. The highest degree of extraction was observed for pure TOMAC, which followed a decreasing trend with increasing aqueous phase acid concentration similar to TOMAC dissolved in oleyl alcohol extractions. This decreasing trend was not observed for the results of extraction experiments performed with pure TOMAC on the aqueous solutions at pH 2-2.5, which varied in a narrower range (59-47%). This sharper decrease of degrees of extraction (from 62% to 39%) may be due to the change of pH of the aqueous phase during extraction. As the extraction proceeds and the equilibrium is reached, lactic acid is transferred to the organic phase, and hydrochloric acid is formed in the aqueous phase due to the transfer of the chloride ion of TOMAC to the aqueous phase. These ion exchanges affect the equilibrium pH, and a different equilibrium pH value may result in lower degrees of extraction. The degrees of extraction achieved with pure oleyl alcohol for pH 4 set were higher than those obtained at pH 2-2.5, but still did not exceed 10%. Oleyl alcohol is a polar diluent, so it may exhibit a higher extraction capacity at higher pH values as more of the acid will be in the dissociated form in that case. This may explain the increase in the extraction capacity of pure oleyl alcohol with increasing aqueous phase pH.

One important improvement that was achieved with increasing the initial aqueous phase pH to 4 was the disappearance of the third emulsion phase that was observed in TOMAC in oleyl alcohol extractions at pH 2-2.5. When the pH of the aqueous solutions was adjusted to 4 by the addition of NaOH, clear separation of the two phases was achieved after 24 hours of extraction, with no emulsion at the interface. This may be explained by the higher solubility of the lactate-ammonium complex in the organic phase than the lactic acid-ammonium complex. It can also be suggested that the dissociated portion of the lactic acid

was preferentially extracted to the organic phase at pH 4, with TOMAC dissolved in oleyl alcohol.

4.1.3 Initial Aqueous Phase pH Adjusted to 6

The increase in the extraction efficiency for the experiments performed by contacting organic phases composed of TOMAC dissolved in oleyl alcohol with aqueous lactic acid containing phases, which have an initial pH value of 4 suggested that the organic phase may function better at even higher aqueous phase pH values. In previous studies, different conclusions were reached with respect to the effect of pH on the extraction efficiency. It is known that TOMAC could extract the acid well in both the low and intermediate pH range but the findings related to the pH at which it exhibits its highest extraction power are contradictory (Choudhury, 1998; Tong[1], 1998; Yang, 1991). Adjusting the initial pH of the aqueous phase to 6 seems to be a proper choice since approximately 99% of the lactic acid is in the dissociated form at this pH (Appendix D), so the effect of pH and dissociation on the degrees of extraction can clearly be observed.

Performing the extraction experiments at pH 6 has another importance since 5-6 is the optimum pH range for lactic acid production by fermentation when Lactobacillus species are used as the microorganisms (Tong[1], 1998; Tong[2], 1998: Tung, 1994). An efficient extractive fermentation process should be able to exploit both optimum pH values for the extraction and fermentation processes. So to enable smooth implementation of extractive fermentation, which is especially important for the design of the future extraction unit that is going to be coupled with the lactic acid fermentor to achieve in-situ extraction, it is necessary to identify an extraction system whose optimum pH corresponds to approximately 6.

As in the case of the experiments whose pH was adjusted to 4, there was no emulsion formation in the extractions performed with aqueous solutions whose pH was adjusted to 6. Phases separated easily and no centrifuging was necessary.

The results of these experiments in terms of calculated degrees of extraction and distribution coefficients are given in Tables D.1.13-14-15-16 and 17 and plotted in Figure 4.1.4.

From these results, it can be seen that the extraction capacity of undiluted TOMAC when contacted with aqueous solutions at pH 6 is lower than that of its extraction capacity at pH 2 or 4. So it may be concluded that the degree of extraction that can be achieved by pure TOMAC is very sensitive to initial aqueous phase pH and it decreases as the aqueous pH increases. At pH 6, the degrees of extraction achieved with pure TOMAC range between 51% and 23%, again showing a sharp decrease with increasing initial aqueous phase lactic acid concentration. These results suggest that pure TOMAC can perform better extractions at lower initial aqueous pH values (highest degrees of extraction that were achieved with pure TOMAC were performed at pH 2-2.5 and consistently high percent recoveries were obtained over the whole aqueous concentration range). This was also suggested by Yang, et.al (Yang, 1991) that the K_D value increased with a decrease in the pH value except at extremely high or low pHs, where K_D did not change significantly with pH. But for the extractions performed with TOMAC dissolved in oleyl alcohol, the opposite trend was observed. The degree of extraction was increased by increasing the equilibrium pH of the aqueous phase. The reason behind this improvement may be the increasing solvating capacity of the diluent, oleyl alcohol, for the lactate-extractant complex at higher pH values. The highest degree of extraction (approximately 36%) was

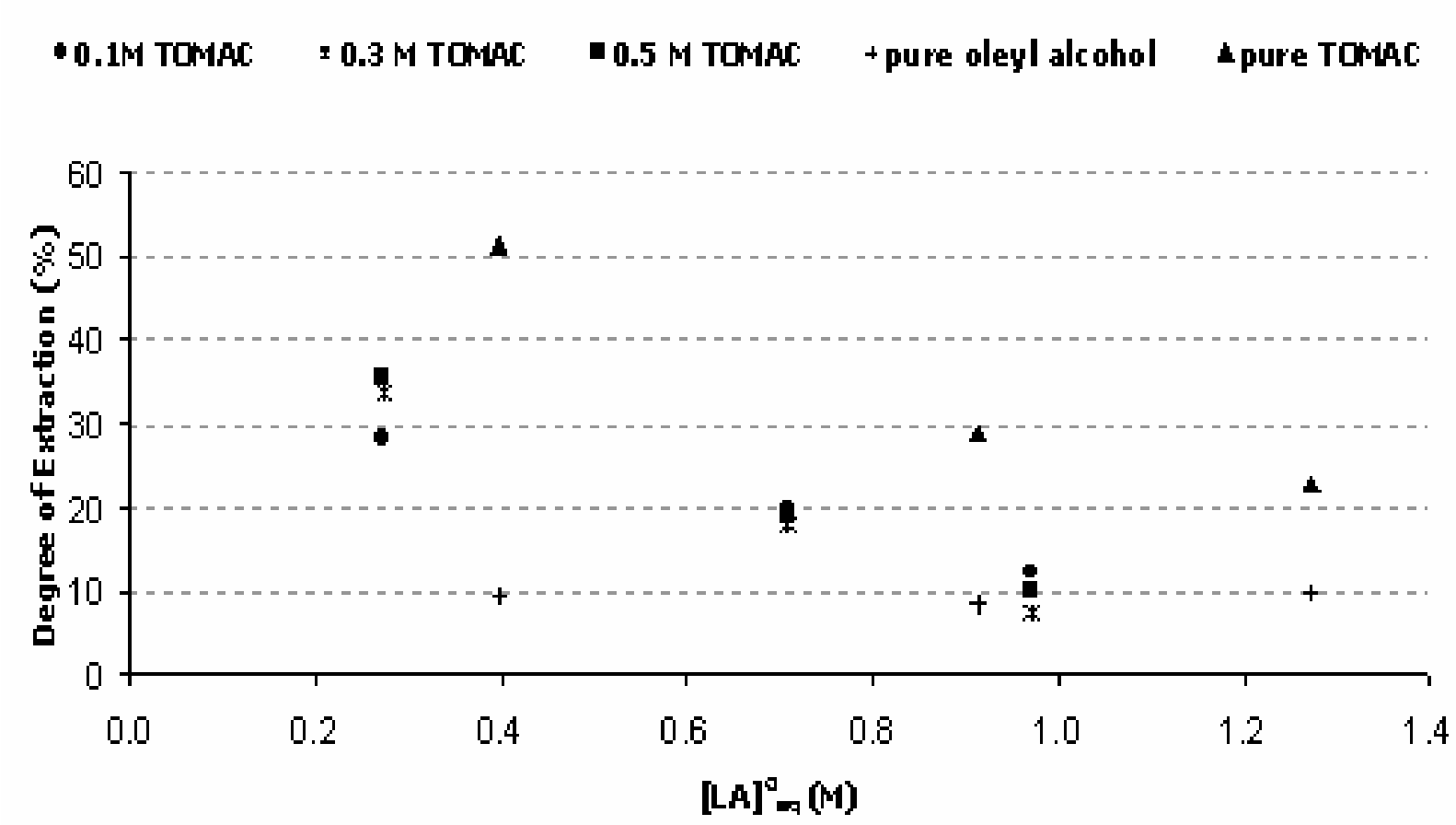


Figure 4.1.4 Variation of degree of extraction with initial lactic acid concentration for TOMAC in oleyl alcohol, initial aqueous phase pH adjusted to 6

achieved for the extraction of 0.273 M lactic acid solution with 0.5 M TOMAC dissolved in oleyl alcohol. This value is higher than the results of extraction experiments performed at pH 2-2.5 or 4 with aqueous and organic phases of similar composition. In fact, the percent recoveries were higher for most of the extractions performed at combinations of various aqueous and organic phase compositions at pH 6, than those of pH 2-2.5 or 4. This suggests that the TOMAC dissolved in oleyl alcohol has a better extraction performance at pH 6, and at higher extractant concentrations of the organic phase.

4.2 Extractions with TOMAC Dissolved in Octanol

The extraction efficiencies achieved up to this point, with TOMAC in oleyl alcohol were still not satisfactory for an effective recovery system even though the increase of pH improved the degrees of extraction. So, alternatives were sought to improve the extraction efficiency and the use a different diluent to dissolve TOMAC was attempted to observe the effect of the diluent on the lactic acid recovery. Octanol, being a lower molecular weight alcohol than oleyl alcohol was chosen to be used in the following set of extraction experiments.

Octanol is a straight chain alcohol with 8 carbon atoms ($C_8H_{18}O$), whereas oleyl alcohol is a more bulky molecule ($C_{18}H_{36}O$). Octanol is reported to act well as a diluent for lactic acid recovery. But it exhibits more toxicity towards the lactic acid producing microorganisms than oleyl alcohol, which was reported to be non-toxic (Tong[1], 1998). But the organic phase will be in minimal contact with the aqueous fermentation broth across a membrane, in a hollow fiber membrane module to be designed in the future, the toxic effect of octanol would be hindered. So, it is worth to explore the extraction ability of this diluent, when combined with TOMAC.

The same methodology was followed to investigate the extraction properties of TOMAC in octanol solutions, as that of TOMAC in oleyl alcohol set. This time, three levels of aqueous and organic phase compositions, at two different initial aqueous phase pH values were used in extraction. The results of these are given and discussed in the following sections.

4.2.1 Initial Aqueous Phase pH between 2-2.5

The results of the extraction experiments performed with pure octanol and TOMAC in octanol solutions, on the aqueous phases whose pH were not controlled and vary between 2 and 2.5, are reported in Tables D.2.1-2-3 and 4 in terms of calculated phase concentrations, degree of extractions and distribution coefficients and plotted in Figure 4.2.1.

For the extractions with octanol as the diluent, degrees of extraction follow the same decreasing trend with increasing aqueous phase initial lactic acid concentration. But octanol is obviously superior to oleyl alcohol both in its pure form and when it is used with TOMAC, in terms of its ability to extract lactic acid. This can be seen by comparing Figures 4.1.1 and 4.1.2 with Figure 4.2.1. The highest degree of extraction that can be achieved with pure octanol is 12.9% and it remains almost constant throughout the aqueous phase concentration range. Whereas the degrees of extractions that can be achieved with pure oleyl alcohol range between 3-6%. Also, for all three levels of TOMAC concentrations in the diluents, degrees of extractions for the octanol set are consistently higher than those for the oleyl alcohol set.

For the extractions performed with TOMAC dissolved in octanol as the organic phase contacted with aqueous lactic acid phases without any pH control (pH between 2-2.5), no emulsion or third phase formation occurred. This was a

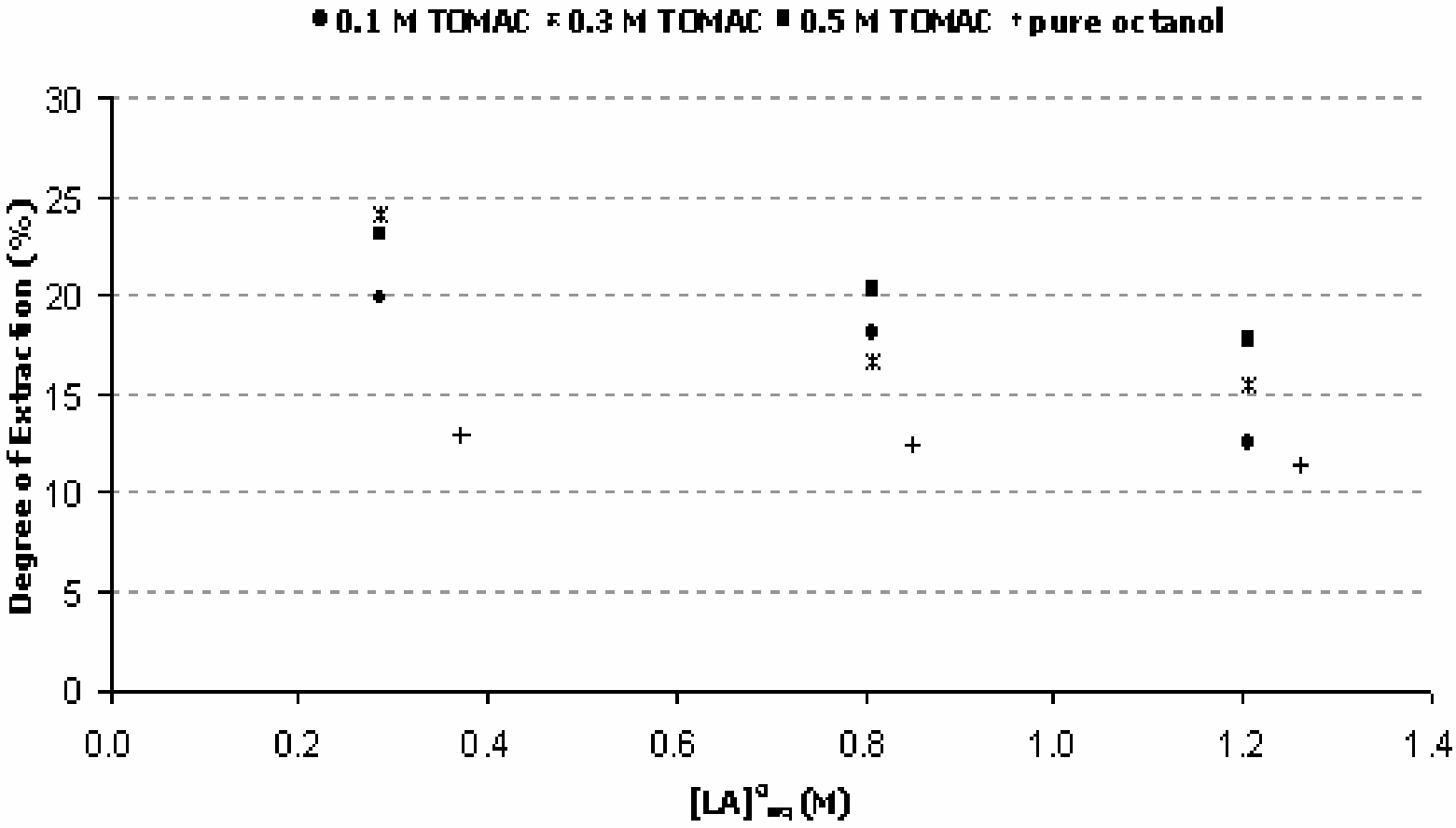


Figure 4.2.1 Variation of degree of extraction with initial lactic acid concentration for pure octanol and TOMAC in octanol, without pH adjustment (pH=2-2.5)

major problem when TOMAC in oleyl alcohol was used at that pH. The disappearance of the third phase is a good sign of the higher solvating power of octanol for the acid-amine complex than oleyl alcohol. Since there was no emulsion at the interface of the aqueous and organic phases, material loss was avoided and better extractions to the organic phase could be achieved.

4.2.2 Initial Aqueous Phase pH Adjusted to 6

Octanol was proved to be a better diluent than oleyl alcohol for lactic acid extraction at initial aqueous phase pH of 2-2.5. The same experiments were then conducted at pH 6 (optimum fermentation pH) for pure octanol and TOMAC in octanol solutions to observe the effect of increasing aqueous phase pH on the extraction efficiency of the organic phase, again with the aim of harmonizing the extraction pH with that of fermentation, for the simultaneous operation of the fermentor and the separator in the future. The results of these experiments are given in the Tables D.2.5-6-7-8 and plotted in Figure 4.2.2.

These results show that the degrees of extraction achieved by TOMAC dissolved in octanol were quite high for experiments performed with high TOMAC concentrations contacted with low aqueous phase concentrations, i.e., 0.5 M TOMAC in octanol contacted with 0.396 M lactic acid solutions. But these exhibited a sharper decrease in the degrees of extraction with increasing aqueous phase concentration. These sets were somewhat more sensitive to aqueous phase concentration at pH 6 than they were at pH 2-2.5. This may be related to the ability of pure octanol and TOMAC dissolved in octanol to extract dissociated lactic acid. The initial lactic acid concentration of the aqueous phase is in this case almost equal to the initial lactate concentration and as the amount of lactate in the aqueous phase increases, the extractability of the lactate to the

organic phase decreases, more than the decrease in the extractability of lactic acid (at pH 2-2.5).

It was reported by Tung et.al (Tung, 1994) that the extractants sustain capacity to higher pH in diluents that stabilize the acid amine complex. Both octanol and oleyl alcohol form hydrogen bonds with the acid amine complex and provide additional stabilization, which results in higher loading. Extractions in which octanol was used as the diluent at the aqueous phase pH of 6 exhibited a sharper decrease in degrees of extraction than those in which oleyl alcohol was used as the diluent. This may be explained by the decrease in the ability of the diluents to form hydrogen bonding with the complex at higher lactate concentrations, and this decrease is more apparent when octanol is used as the diluent. Octanol can achieve good recoveries for the lactate-amine complex at pH 6 for low concentrations of the lactate and its ability to form hydrogen bonding with the complex may decreases at higher lactate concentrations.

But TOMAC dissolved in octanol still has a good extraction performance. The degrees of extraction for low initial lactic acid concentrations were improved with the introduction of octanol instead of oleyl alcohol as the diluent, so from this point forward, the extraction experiments were carried out with octanol partly due to this fact and partly due to its superior physical properties.

From the results of experiments performed up to this point with TOMAC as the extractant, it can be concluded that TOMAC dissolved in octanol has a good extraction capacity and octanol has a high solvatizing power for the acid-amine complex. In addition, the extraction pH of 6 yields higher degrees of lactic acid extraction for the most part of the concentration range in the aqueous and organic phase. Highest percent recoveries were obtained with 0.5 M TOMAC

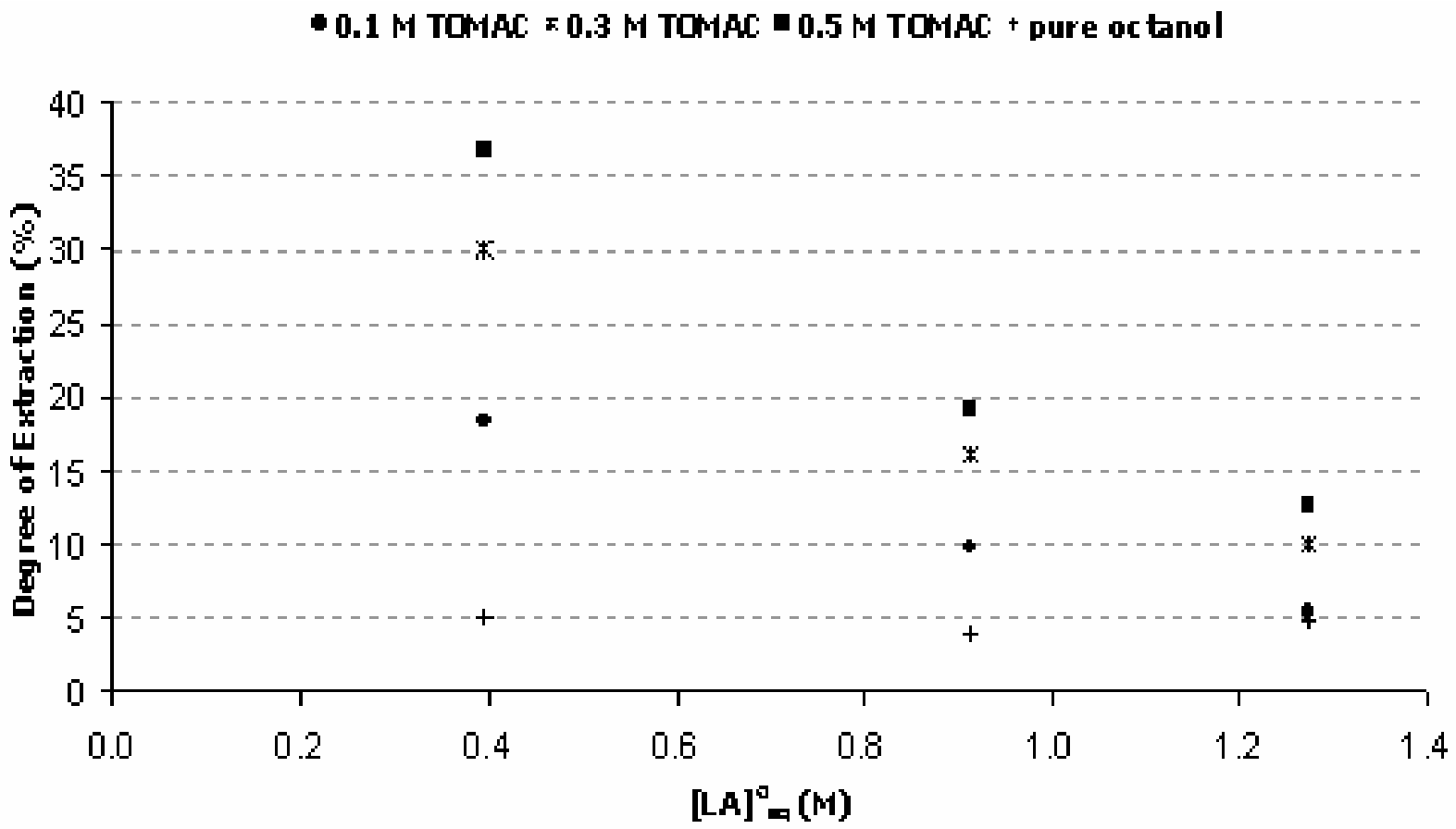


Figure 4.2.2 Variation of degree of extraction with initial lactic acid concentration for pure octanol and TOMAC in octanol, initial aqueous phase pH adjusted to 6

dissolved in octanol, so this organic phase composition was identified to be best in terms of extraction power.

The effects of different salts of tri-n-octylmethylammonium were then investigated by first conducting an ion exchange procedure with pure TOMAC to exchange its chloride ion with either sulphate or hydroxide and then performing the forward extraction experiments with these new extractants dissolved in octanol at pH 6.

4.3 Ion Exchange of TOMAC to TOMA(HSO_4)

4.3.1 Ion Exchange Experiments to Exchange the Chloride Ion with the Hydrogensulphate Ion

The procedure described in Section 3.3.3.1 was followed to exchange the chloride ion of TOMAC (Aliquat 336) with hydrogensulphate ion. At the end of this procedure 1.69 M TOMA(HSO_4) was obtained together with approximately 0.11 M unconverted TOMAC. The results of the analyses performed with the ion chromatograph to determine the concentrations of the sulphate and chloride ions in the aqueous phase, and the calculation of the concentrations of the quaternary ammonium salts are explained in detail in Appendix B.1.

The analyses and the calculations revealed that there was a one-to-one exchange of ions between TOMAC in the organic phase and H_2SO_4 in the aqueous phase. So the tri-n-octylammonium salt formed in the organic phase at the end of the exchanges is the hydrogensulphate salt, TOMA(HSO_4), not the sulphate salt, $TOMA_2(SO_4)$.

The summation of the molarities of the hydrogensulphate (1.69 M) and chloride salts (0.11 M) of tri-n-octylmethylammonium should be equal to the initial concentration of pure TOMAC (1.93 M), but there is a difference of 0.13 M between these values. This can be partly due to the slight changes of phase volumes during the contacts of aqueous and organic phases, or due to dilution and analysis errors. In this case, the total concentration of the extractant present in the organic phase was taken to be equal to that of pure TOMAC, 1.93 M, but the calculations for the dilution of the extractant mixture with the diluent were based on the individual concentration of TOMA(HSO_4).

4.3.2 Extraction Experiments with TOMA(HSO_4)

The extraction experiments with this new extractant were performed with octanol used as the diluent, for three different organic phase compositions, the first one being the undiluted extractant and the other two being 0.1 M and 0.5 M solutions of TOMA(HSO_4) in octanol, to represent the upper and lower organic phase concentration limits.

The initial aqueous phase pH was adjusted to 6 with 5 M NaOH solution and the extraction experiments were carried out on the aqueous phases at 5 different levels of lactic acid (most of which is in the dissociated form) concentrations. The results of these experiments are given in Tables D.3.1-2 and 3, and plotted in Figure 4.3.1.

Clear phase separations were achieved for the extractions with TOMA(HSO_4) diluted in octanol and no emulsion formation was observed. The extractions with undiluted TOMA(HSO_4) required 30 minutes of centrifuging, to clear out the cloudy aqueous phase.

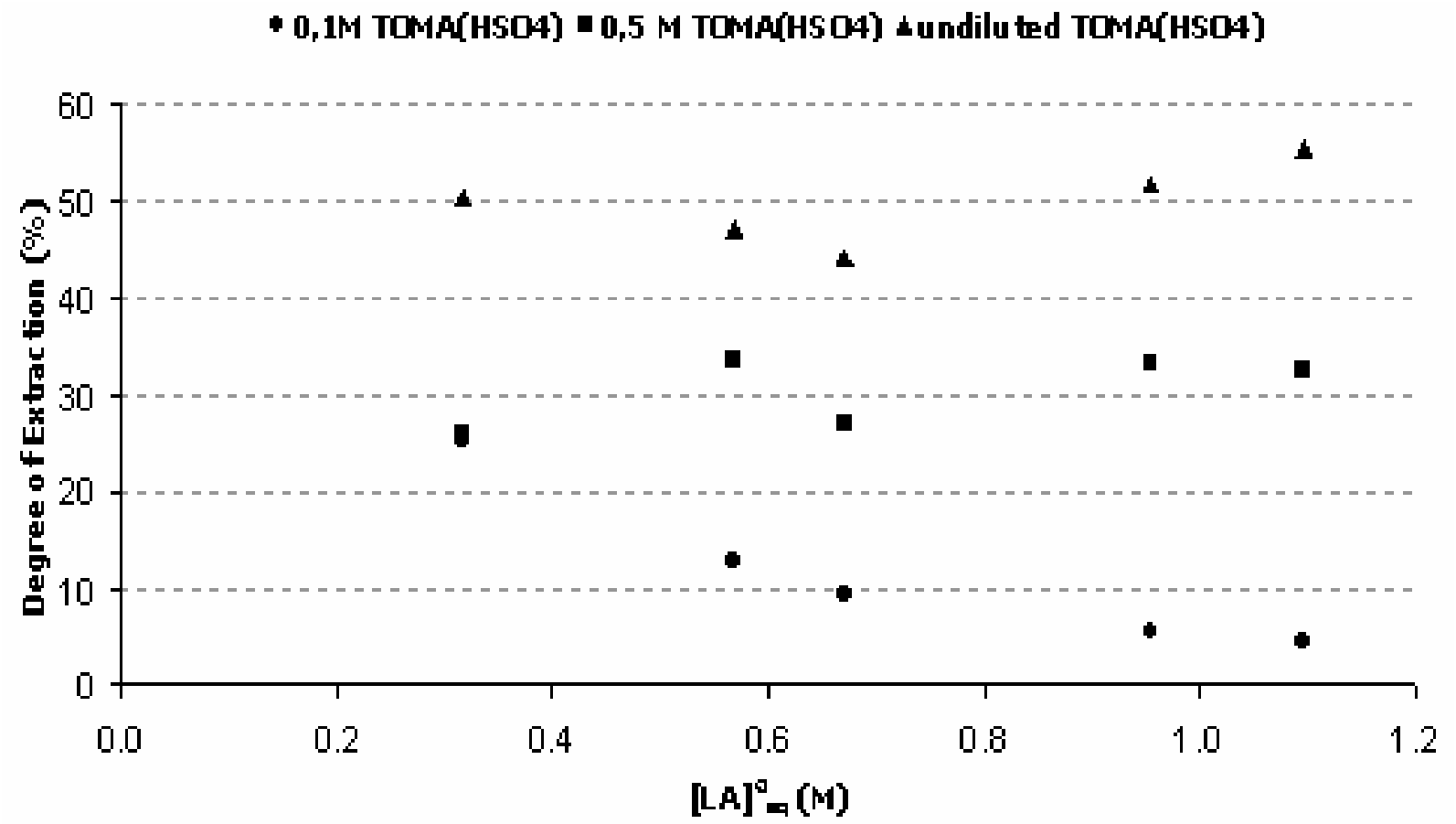


Figure 4.3.1 Variation of degree of extraction with initial lactic acid concentration for undiluted TOMA(HSO_4) and TOMA(HSO_4) in octanol, initial aqueous phase pH adjusted to 6

The reaction between lactic acid and TOMA(HSO_4) is:

$$HA_{(aq)} + TOMA(HSO_4)_{(org)} \leftrightarrow TOMA^+(A^-)_{(org)} + H^+(HSO_4^-)_{(aq)} \quad (4.5)$$

According to this reaction as the lactate ion passes to the organic phase, the hydrogensulphate counter ion of TOMA(HSO_4) passes to the aqueous phase and forms sulphuric acid.

The degrees of extraction with 0.1 M TOMA(HSO_4) dissolved in octanol showed the expected decreasing trend with increasing initial aqueous phase lactic acid concentration and they were higher than the degrees of extraction achieved with 0.1 M TOMAC dissolved in octanol at pH 6. This may be due to the additional extraction capacity of the residual TOMAC that is present together with TOMA(HSO_4).

The degrees of extraction with 0.5 M TOMA(HSO_4) and undiluted TOMA(HSO_4) dissolved in octanol, showed no regular variation with initial aqueous phase concentration. The percent recovery values for 0.5 M TOMA(HSO_4) varied between approximately 26-34%, and those for undiluted TOMA(HSO_4) varied between 44-55%. These values are greater than those obtained with 0.5 M TOMAC in octanol and pure TOMAC. Extractions with 0.5 M TOMA(HSO_4) again included the contribution of the unconverted TOMAC in the organic phase, therefore the unpredictable trend of the degrees of extraction can be explained by the extraction of lactic acid molecules simultaneously by these two salts of Aliquat 336. On the other hand undiluted TOMA(HSO_4) can clearly be identified as a better extractant than undiluted TOMAC since higher degrees of extraction could be achieved and sustained with TOMA(HSO_4) used without octanol.

The amounts of counter ions transferred from the organic phase to the aqueous phase were also measured and summarized in Tables D.3.1-2 and 3. The amount of hydrogensulphate ion that passed to the organic phase was proportional to the amount of lactate that was transferred in the opposite direction. But there was not a one to one correspondence for the concentrations of these ions for the extractions with undiluted TOMA(HSO_4) and 0.5 M TOMA(HSO_4) in octanol (Figures 4.3.2 and 3). According to these figures for four lactate ions passing to the organic phase, nearly three hydrogensulphate ions are passing to the aqueous phase. The amounts of chloride counter ion were much lower than the amounts of hydrogensulphate counter ion, and they remained nearly constant with increasing amounts of lactate that pass to the organic phase.

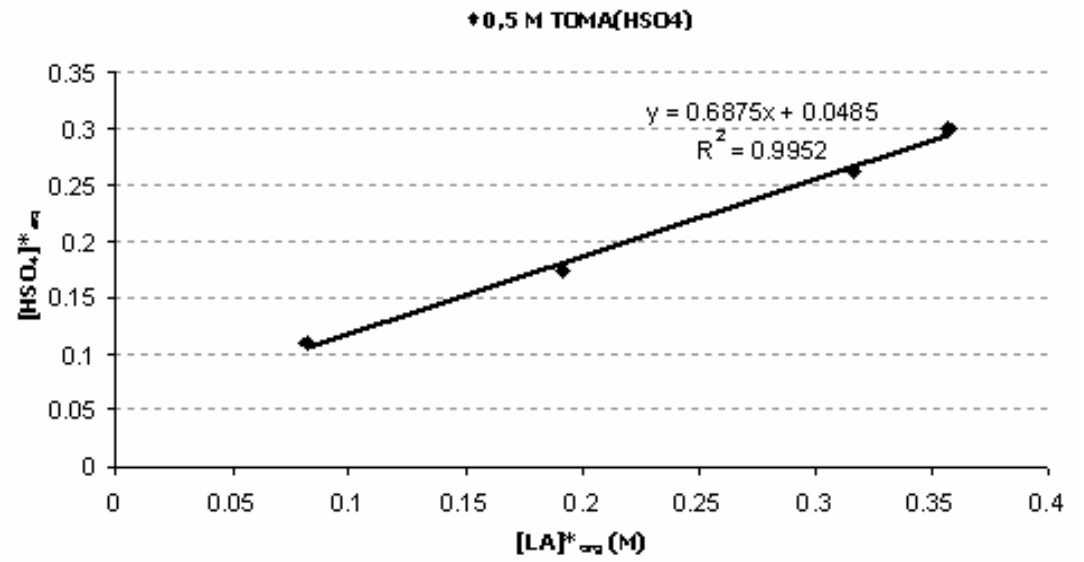


Figure 4.3.2 Variation of the amounts of lactate and hydrogensulphate ions in the extraction with 0.5 M TOMA(HSO_4) in octanol

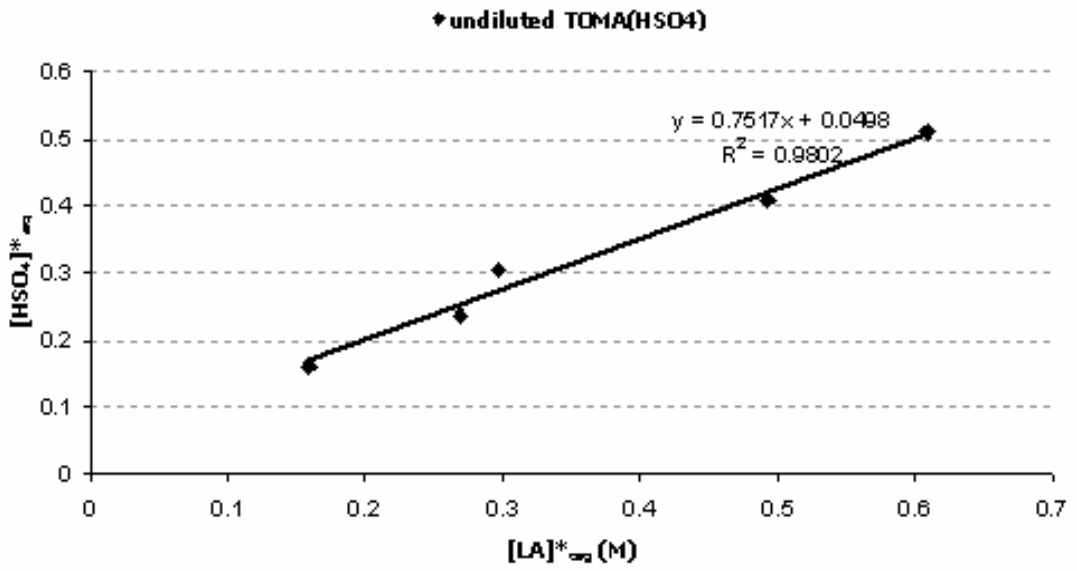


Figure 4.3.3 Variation of the amounts of lactate and hydrogensulphate ions in the extraction with undiluted TOMA(HSO_4)

4.4 Ion Exchange of TOMAC to TOMA(OH)

4.4.1 Ion Exchange Experiments to Exchange the Chloride Ion with the Hydroxide Ion

The procedure described in Section 3.3.3.2 was followed to exchange the chloride ion of TOMAC (Aliquat 336) with hydroxide ion. At the end of this procedure, despite all efforts, only 48% conversion of TOMAC to TOMA(OH) could be achieved. 0.93 M TOMA(OH) was obtained together with 1 M unconverted TOMAC. The results of the analyses performed with the ion chromatograph to determine the concentrations of chloride ions in the aqueous phase, and the calculation of the concentrations of the quaternary ammonium salts are explained in detail in Appendix B.2.

4.4.2 Extraction Experiments with TOMA(OH)

The extraction experiments with TOMA(OH) were performed with octanol used as the diluent, for three different organic phase compositions, the first one being the undiluted extractant and the other two being 0.1 M and 0.5 M solutions of TOMA(OH) in octanol, to represent the upper and lower organic phase concentration limits.

The initial aqueous phase pH was adjusted to 6 with 5 M NaOH solution and the extraction experiments were carried out on the aqueous phases at 5 different levels of lactic acid (almost 99% of which is in the dissociated form) concentrations. The results of these experiments are given in Tables D.4.1-2 and 3, and plotted in Figure 4.4.1.

The extractions with undiluted TOMA(OH) required 30 minutes of centrifuging, to clear out the cloudy aqueous phase. Other extractions with TOMA(OH) dissolved in octanol exhibited clear phase separations, pointing out that octanol has a good solvating ability for the extractant-lactate complexes.

The results of the extraction experiments with TOMA(OH) yielded surprisingly high results. When compared to the other two extractants (TOMAC and TOMA(HSO_4)) in their undiluted forms, the superiority of undiluted TOMA(OH) in the extraction of lactic acid from its aqueous solutions at pH 6, is obvious. The highest degree of extraction that could be achieved with undiluted TOMA(OH) is approximately 83% for the extraction of 0.316 M lactic acid. The degrees of extraction decrease as the initial aqueous phase lactic acid concentrations increase. But the lowest degree of extraction that was achieved in that case (for the extraction of 1.096 M lactic acid) was approximately 44%, which is still a high value. The reason why undiluted TOMA(OH) exhibited a

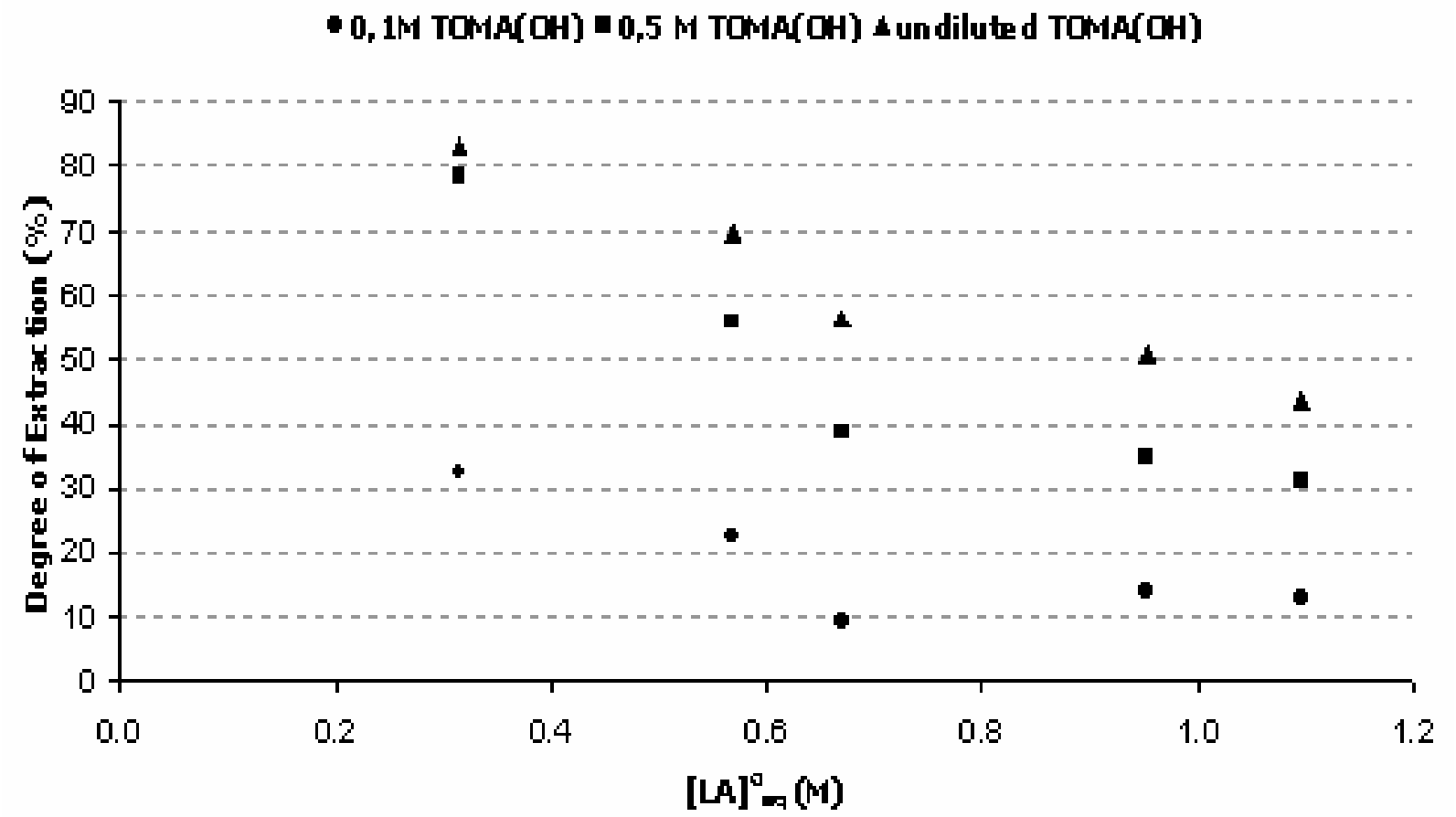


Figure 4.4.1 Variation of degree of extraction with initial lactic acid concentration for undiluted TOMA(OH) and TOMA(OH) in octanol, initial aqueous phase pH adjusted to 6

sharp decrease in the degrees of extraction (from 83 to 44%) as opposed to those of undiluted TOMA(HSO_4), which varied slightly in the range of 44-55%, is an interesting aspect of the extraction with undiluted extractants. The total concentration of the extractant is equal to 1.93 M for both salts and this amount is more than the lactic acid concentration in the aqueous phase, i.e., the extractant is in excess. In the case of undiluted TOMA(OH), 0.93 M of this total concentration belongs to the hydroxide salt and the rest is the chloride salt (TOMAC). The decrease in percent recoveries with increasing lactic acid concentration indicates that the acid was extracted preferably and mostly by TOMA(OH) and not by TOMAC and the extraction capacity of TOMAC in the presence of TOMA(OH) is low. This can be confirmed by the low concentrations of chloride ions that passed to the aqueous phase as lactic acid was extracted with TOMAC (Tables D.4.1-2 and 3). On the other hand undiluted TOMA(HSO_4) is mostly composed of the hydrogensulphate salt of Aliquat 336, which is also in excess, and the amount of the chloride salt is low. The constant trend of the degrees of extraction may be explained by the high and sustained capacity of TOMA(HSO_4) to extract the dissociated lactic acid. The contribution of TOMAC to the extraction in this case again seems to be low, from the amounts of chloride ions that was measured in the aqueous phase after extraction.

For the extraction experiments with 0.1 M and 0.5 M TOMA(OH) dissolved in octanol, the degrees of extraction were found to be much higher than those of their chloride and hydrogensulphate salt counterparts. This is partly because the extractions with 0.1 and 0.5 M TOMA(OH) included nearly the same amounts of unconverted TOMAC in the organic phase, so the total concentration of the extractants (TOMA(OH)+TOMAC) was nearly twice the total extractant concentrations in 0.1 and 0.5 M TOMAC or TOMA(SO_4). But TOMA(OH) is definitely a better extractant both in its undiluted form and when it is used in

conjunction with octanol, especially for low initial lactic acid concentrations, as can be seen from the figure below.

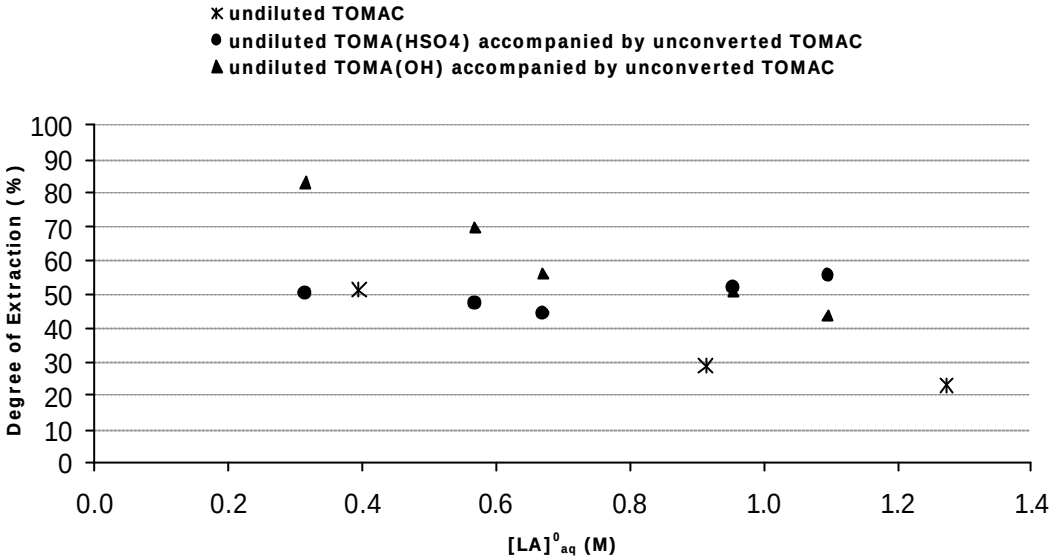


Figure 4.4.2 Comparison of the degrees of extractions of undiluted extractants, initial aqueous phase pH adjusted to 6

One major advantage of using TOMA(OH) as the extractant is the effect of the counter ion, hydroxide in this case. If TOMA(OH) could be obtained with higher yields, i.e., more TOMAC could be converted to TOMA(OH), then it would be the hydroxide counter ion that will mostly be exchanged with the lactate. The reaction between lactic acid and TOMA(OH) is:

$$HA_{(aq)} + TOMA(OH)_{(org)} \leftrightarrow TOMA^{+}(A^{-})_{(org)} + H^{+}OH^{-}_{(aq)} \qquad (4.6)$$

As can be seen from the equilibrium reaction above; as the lactate couples with the extractant, the hydroxide of the extractant passes to the aqueous side and couples with the hydrogen ions to form water. The formation of water on the aqueous side is desirable as it has no adverse effects on the fermentation media if the extraction and fermentation processes will be performed simultaneously. The reaction products of the extractions performed with TOMAC or TOMA(HSO_4) are HCl and H_2SO_4, respectively, which are strong acids that can effect the final

pH of the aqueous fermentation medium and can be harmful to the microorganism or the product itself. But no such effects are expected for the extractions with TOMA(OH) as the reaction product in this case is water.

One bottleneck to be overcome at this point is to obtain higher purity TOMA(OH) by performing a more efficient ion exchange procedure with TOMAC. The degrees of extraction that can be obtained with the hydroxide salt of tri-n-octylmethlyammonium are expected to increase as the concentration of TOMA(OH) increases. This could be done by attempting ion exchange with NaOH solutions at higher concentrations than the 2 M and 5 M NaOH solutions that were used in this study. This may not prove to be successful though, since the present study showed that increasing the NaOH concentration from 2 to 5 M didn't influenced the amount chloride exchanged with hydroxide considerably.

But with the present experimental work and the results obtained, it can be concluded that TOMA(OH) is a very promising extractant for lactic acid recovery, pointing out the great extraction potential in the quaternary ammonium salts.

Lactic acid can be recovered from the organic phase by performing back extraction with an aqueous sodium hydroxide solution as the stripping phase and it can be obtained efficiently almost in the pure form.

4.5 Equilibrium Isotherms and Distribution Coefficients

Equilibrium isotherms are plots of $[LA]^*_{org}$ vs. $[LA]^*_{aq}$ and the slopes of these curves give the distribution coefficient, K_D. The equilibrium isotherms for all the extraction experiments are plotted and given below.

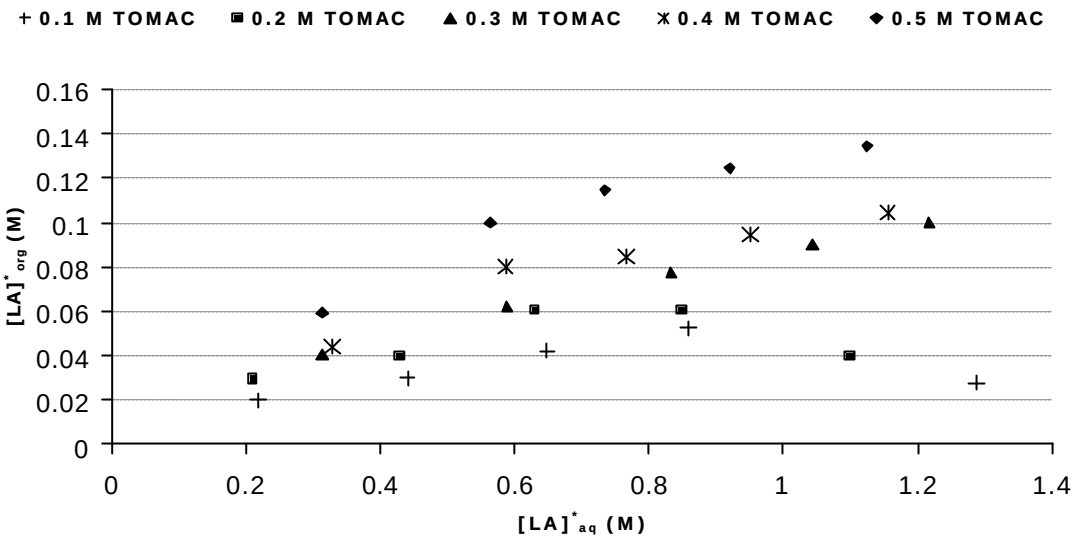


Figure 4.5.1 Equilibrium isotherms for TOMAC dissolved in oleyl alcohol, without pH adjustment (pH=2-2.5)

From Figure 4.5.1 it can be seen that the equilibrium acid concentration in the organic phase increased with increasing amine concentration. The equilibrium acid concentration in the organic phase also increased with increasing equilibrium acid concentration in the aqueous phase, for relatively high amine concentrations in the organic phase. As the concentration of the amine in the organic phase becomes lower (i.e. for 0.1 and 0.2 M TOMAC dissolved in oleyl alcohol), the organic phase concentration first increased then decreased with increasing aqueous phase concentration. This indicates that amine concentration in the organic phase is the limiting reagent in the extraction of high concentrations of acid in the aqueous phase with low concentrations of amine in the organic phase.

For the extractions with pure TOMAC, the equilibrium organic phase concentration varied almost linearly with equilibrium aqueous phase concentration and the slope of this plot yields the distribution coefficient (Figure 4.5.2).

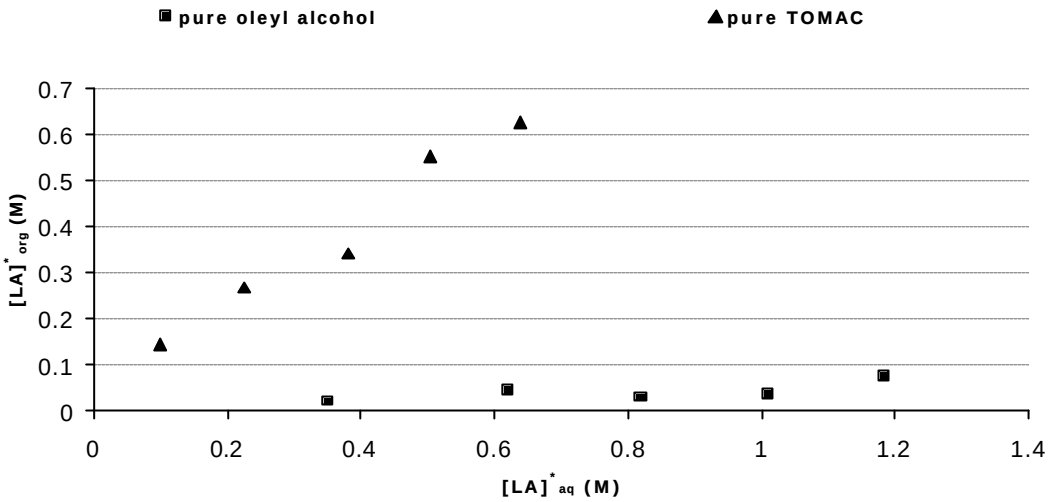


Figure 4.5.2 Equilibrium isotherms for pure TOMAC and pure oleyl alcohol, without pH adjustment (pH=2-2.5)

As the pH of the aqueous phase was increased from 2-2.5 to 4, for the extractions with TOMAC dissolved in oleyl alcohol, a similar trend was observed (Figure 4.5.3). For the extraction experiments having an initial aqueous phase pH of 6, the organic phase concentration first increased then decreased with increasing aqueous phase concentration for all concentrations of the extractant in the diluent (Figure 4.5.4). This explains the sharper decrease in the degrees of extraction in Figure 4.1.4 when initial aqueous phase lactic acid concentration was increased.

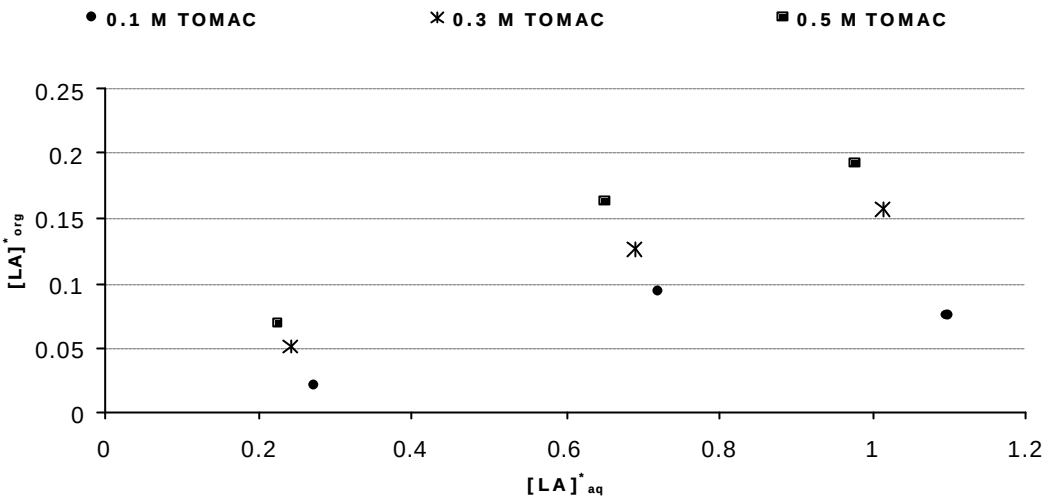


Figure 4.5.3 Equilibrium isotherms for TOMAC dissolved in oleyl alcohol, initial aqueous phase pH adjusted to 4

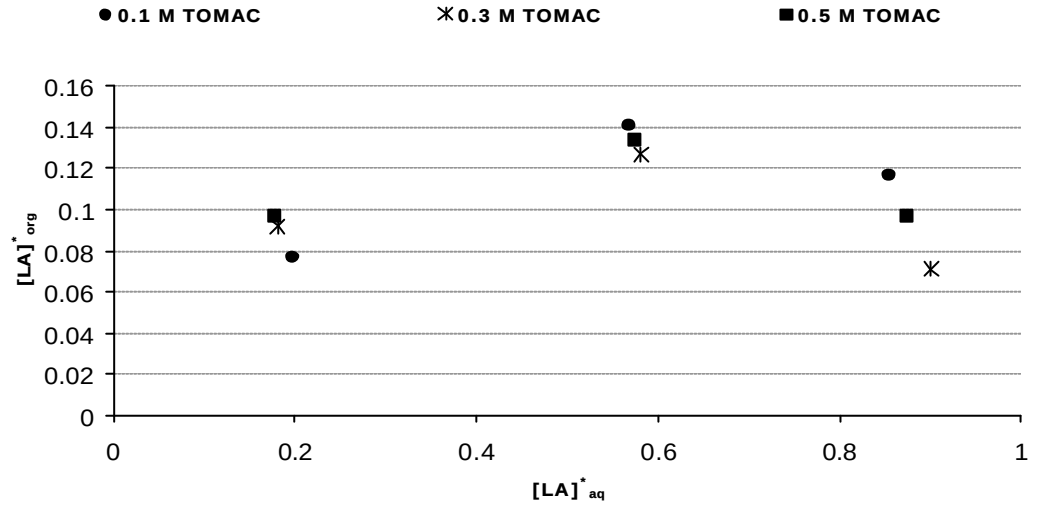


Figure 4.5.4 Equilibrium isotherms for TOMAC dissolved in oleyl alcohol, initial aqueous phase pH adjusted to 6

A similar trend was observed for extractions when TOMAC dissolved in octanol was used as the organic phase. The equilibrium isotherms for the extractions performed with aqueous phases having an initial pH of 2-2.5 and 6 are given in Figures 4.5.5 and 4.5.6, respectively.

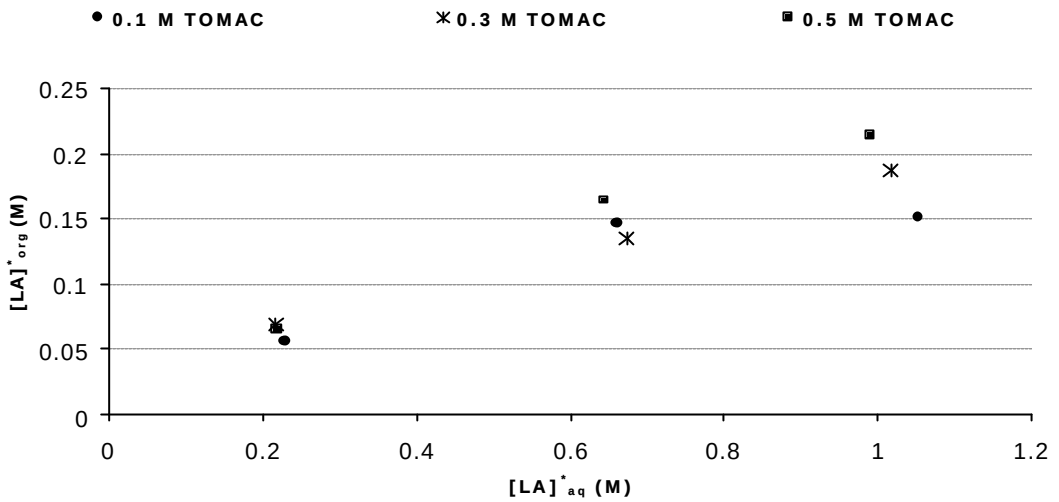


Figure 4.5.5 Equilibrium isotherms for TOMAC dissolved in octanol, without pH adjustment (pH=2-2.5)

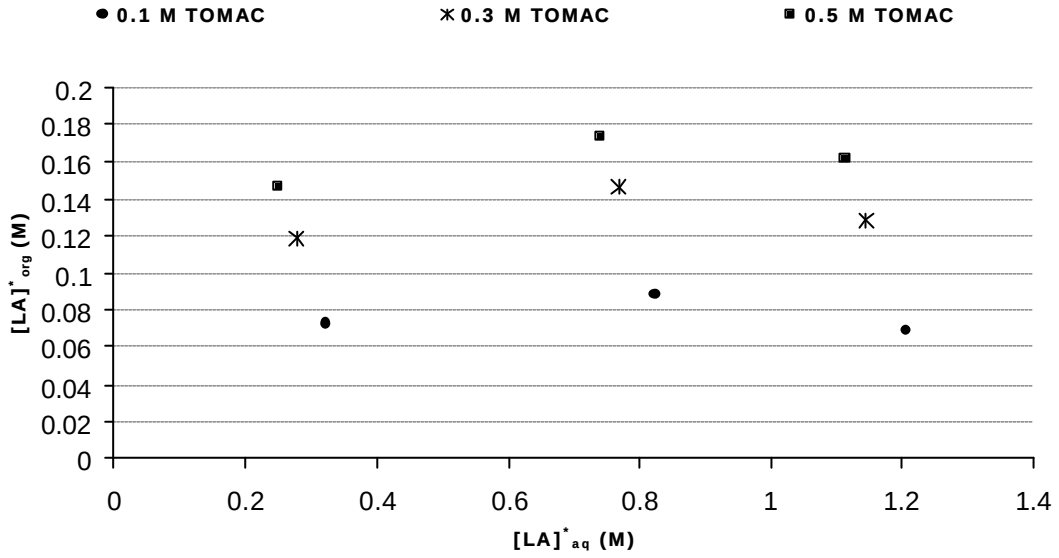


Figure 4.5.6 Equilibrium isotherms for TOMAC dissolved in octanol, initial aqueous phase pH adjusted to 6

The equilibrium isotherms for extractions with TOMA(SO_4) and TOMA(OH) are given in Figures 4.5.7 and 4.5.8 respectively. Although the equilibrium acid concentration in the organic phase tended to increase with increasing equilibrium acid concentration in the aqueous phase, for relatively high amine concentrations

in the organic phase for the extractions with TOMA(SO_4), this trend was not linear and no such relation exists for low amine concentrations. This is also valid for extractions with TOMA(OH) which have fluctuating isotherms.

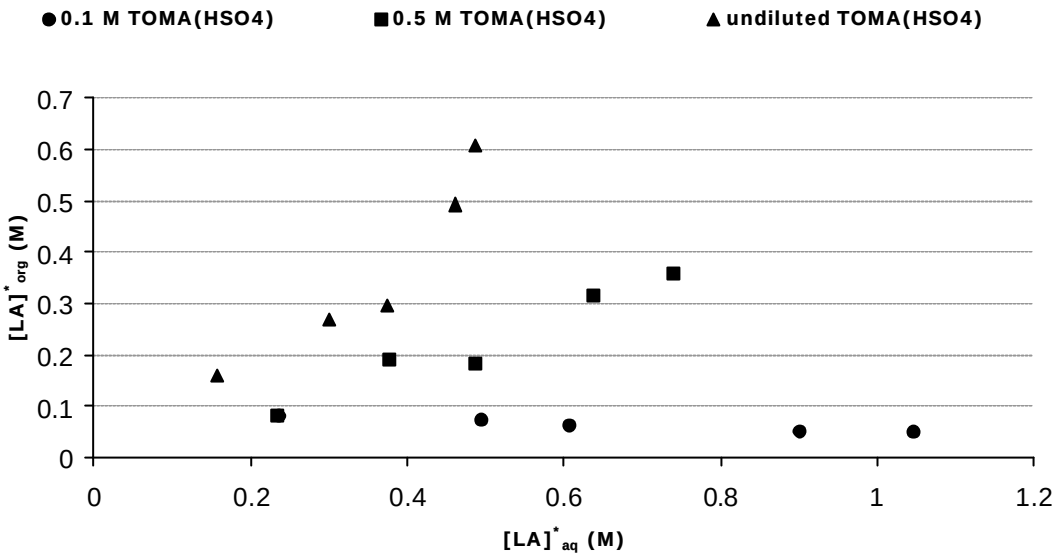


Figure 4.5.7 Equilibrium isotherms for undiluted TOMA(HSO_4) and TOMA(HSO_4) dissolved in octanol, initial aqueous phase pH adjusted to 6.

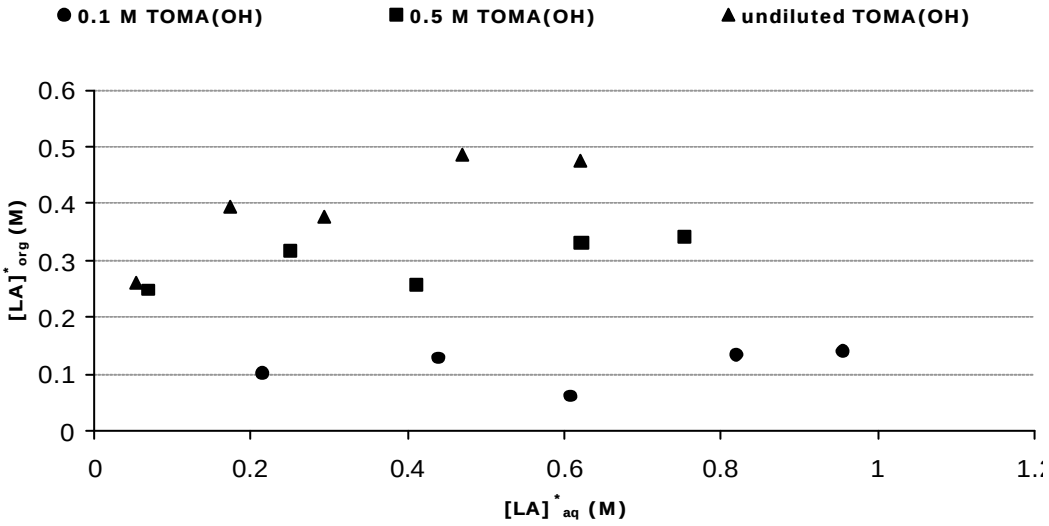


Figure 4.5.8 Equilibrium isotherms for undiluted TOMA(OH) and TOMA(OH) dissolved in octanol, initial aqueous phase pH adjusted to 6.

4.6 Loading of the Extractants

Loading values for the extraction experiments in which salts of tri-n-octylmethylammonium were used in the organic phase were calculated and tabulated in the previous sections.

Loading, Z, is a measure of the extent to which the organic phase can be loaded with lactic acid. It can be defined as the total concentration of acid (all forms) in the organic phase, divided by the total concentration of amine (all forms) in the organic phase.

$$Z = [A]_{org} / [B]_{total} \quad (4.3)$$

The calculations of the loading values were carried out according to Equation 4.3. Loading could be calculated easily for the total chloride salt concentration in the case of TOMAC without ion exchange with hydrogensulphate or hydroxide, since it is the only extractant present in the organic phase for the extractions with either pure TOMAC or its diluted solutions. But since TOMA(HSO_4) and TOMA(OH) each appear in the organic phase with some unconverted TOMAC, the contribution of the chloride, hydrogensulphate or hydroxide salts to extraction are not known exactly. So the total concentrations of the extractants in the organic phase were used to calculate the loading values in these cases rather than the individual concentrations of each ammonium salt.

According to this, 0.1 M TOMA(HSO_4) solutions were calculated to contain 0.014 M unconverted TOMAC, which add up to a total of 0.114 M extractant in the organic phase. For 0.5 M TOMA(HSO_4) solutions, the amount of unconverted TOMAC is 0.071 M and these two were added to make a total extractant concentration of 0.571 M in the organic phase containing 0.5 M TOMA(HSO_4).

The same calculations were carried out for TOMA(OH) solutions. 0.1 M TOMA(OH) solutions contained 0.108 M unconverted TOMAC, which make up to a total of 0.208 M extractant in the organic phase. The amount of unconverted TOMAC for 0.5 M TOMA(OH) solution was calculated to be 0.54 M and these two were added up to a total extractant concentration of 1.04 M in the organic phase containing 0.5 M TOMA(OH). These were used as the total extractant concentrations in the organic phase in the calculation of loading.

When the loading is greater than unity, complexes with more than one acid per amine are formed. For systems with only one amine per complex, there is no effect of total amine concentration on the loading. If there is more than one amine per complex, loading increases with increasing amine concentration at low acid concentrations (Tamada[1], 1990).

The loading value depends on the extractability of the acid (strength of the acid-base interaction) and its aqueous concentration. The stoichiometry of the overall reaction is determined by the loading ratio in the organic phase (Wasewar[1], 2002).

Loading of the extractants for most of the extractions performed in the present work was shown to be less than 1. Only those for 0.1 M TOMAC dissolved in oleyl alcohol at aqueous pH of 6, and 0.1 M TOMAC dissolved in octanol at pH 2 were greater than unity and ranged between 1.17 and 1.51. The organic phases for these sets of experiments were overloaded due to the increase in the extraction capacity of the organic phase at the specific extraction conditions.

It was reported by Wasewar et.al (Wasewar[2], 2002) that systems that include the interaction of the diluent with the complex show decreasing loading

with increasing amine concentration. This was the case for all the extractions performed in this work; loading values decreased, for the same initial aqueous phase concentrations, as the amine concentration in the diluent was increased. This may indicate that both oleyl alcohol and octanol actively participated in the extraction.

It was suggested by Wasewar et.al (Wasewar[2], 2002) that the stoichiometry of the overall reaction depends on the loading ratio. At very low loading ratios ($Z<0.5$), a (1,1) lactic acid-amine complex is formed and a plot of $Z/(1-Z)$ versus $[LA]^*_{aq}$ is a straight line whose slope gives the equilibrium complexation constant, K_E:

$$Z/(1-Z) = K_E\,[LA]^*_{aq} \qquad (4.7)$$

A sample plot of $Z/(1-Z)$ versus $[LA]^*_{aq}$ for the extraction with TOMAC dissolved in oleyl alcohol solutions, at the aqueous pH of 2-2.5 is given in Figure 4.6.1.

The equations of the trendlines are shown in the plot and the equilibrium constants are the slopes of these lines (1.174, 0.418, 0.367). This methodology can be extended to all of extractions performed to confirm the formation of (1,1) complexes at low loading values. If a straight line can not be obtained from these plots, the presence of complexes with different stoichiometries may be suspected. In the case of (2,1) complexes, for instance, the plot of $Z/(2-Z)$ versus $([LA]^*_{aq})^2$ should yield a straight line whose slope gives the equilibrium complexation constant. This is generally the case for higher loading values.

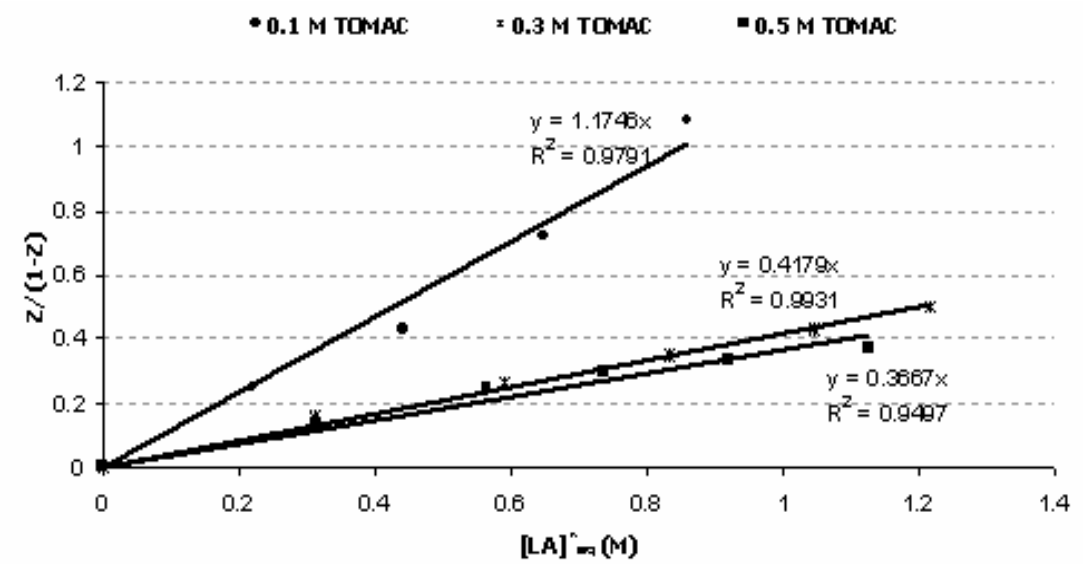


Figure 4.6.1 Plot of Z/(1-Z) versus $[LA]^*_{aq}$ for the estimation of (1,1) lactic acid-TOMAC equilibrium constants, with oleyl alcohol as the diluent

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

Equilibrium investigations on the reactive extraction of lactic acid from its aqueous solutions at different concentrations and pH values were conducted by contacting and equilibrating the aqueous phases with organic phases which contain salts of tri-n-octylmethylammonium as the extractant and oleyl alcohol or octanol as the diluent.

According to the experimental results, following conclusions were made:

1. Degrees of extraction are highest for pure extractants and decrease as the extractant is diluted with either oleyl alcohol or octanol.

2. Degrees of extraction generally decrease with increasing initial aqueous phase lactic acid concentration for all organic phases, except for pure TOMAC at pH 2-2.5, pure TOMA(HSO_4) and 0.5 M TOMA(HSO_4) in octanol at pH 6, degrees of extraction of which did not vary significantly with aqueous phase concentration.

3. Octanol has a higher solvating power than oleyl alcohol especially at lower aqueous phase pH values. Higher extraction efficiencies were obtained for TOMAC dissolved in octanol rather than oleyl alcohol. It also has better solvating power towards the acid-amine complex and no emulsion formation was observed for the extractions performed with octanol as the diluent. Whereas, a third emulsion phase occurred between the aqueous and organic phases for the extractions performed with TOMAC dissolved in oleyl alcohol at low pH values.

4. Extractions with TOMAC dissolved in oleyl alcohol and octanol were performed at different pH values (pH=2-2.5, 4 and 6) to observe the effect of pH on lactic acid recovery and it was concluded that the extractant performed better at higher pH values, when used in conjunction with a diluent, especially for the extraction of low concentration lactic acid solutions. pH 6 was identified as the optimum extraction pH for this purpose and also with the aim of harmonizing the pH of the extraction process with that of fermentation.

5. Among the different salts of tri-n-octylmethylammonium, hydroxide salt (accompanied by the unconverted chloride salt) exhibited the highest degrees of extraction. The degrees of extraction achieved with the hydrogensulphate salt were also higher than the chloride salt but lower than the hydroxide salt of tri-n-octylmethylammonium. The highest percent recovery was achieved with pure TOMA(OH) as 83% and that of 0.5 M TOMA(OH) in octanol was 78%, both for the extractions of 0.316 M

lactic acid solutions. This signifies the great potential in using hydroxide salts of the extractant (Aliquat 336) in the recovery of lactic acid from aqueous fermentation broths.

6. TOMA(OH) also has the advantage of forming water in the aqueous phase due to the transfer of its hydroxide counter ion to the aqueous phase during the anion exchange reaction with lactic acid. The formation of water on the aqueous side has no adverse effects on the fermentation media if the extraction and fermentation processes are performed simultaneously.

7. Loading of the extractants were also calculated and loading values for most of the extractions were found to be less than one, which means that mostly (1,1) complexes were formed between the acid and amine.

8. The present work showed that forward extraction of lactic acid with different salts of tri-n-octylmethylammonium is a promising alternative for its recovery. But there are many aspects of the issue that should be investigated to be able to improve and fully understand the system. By knowing the coupling mechanisms of the extractants with the lactate molecules and the amounts of counter ions exchanged with the lactate, the equilibrium behavior of the system can be explained better.

9. Forward extraction with aqueous phases with even lower lactic acid concentrations should be attempted in the future to obtain higher percent recoveries. Lactic acid can be effectively recovered from its low concentration aqueous solutions with the salts of tri-n-

octylmethylammonium, and the yields will be much higher for lower acid concentrations, even when the extraction is performed with low organic phase extractant concentrations.

10. The ability of tri-n-octylmethylammonium salts to extract other components of the real fermentation broth should also be investigated and the extraction parameters should be optimized so as to extract only the lactic acid and not the rest of the medium components.

11. Kinetic parameters for the extraction should be obtained as the next step, before designing a pilot scale extractor that will perform continuous or semi-continuous recovery of lactic acid from the fermentation broth.

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APPENDIX A

PHYSICAL PROPERTIES OF AQUEOUS LACTIC ACID SOLUTIONS

A.1 Distribution Coefficients of Lactic Acid in Other Solvents

Table A.3.1 Distribution of Lactic Acid on Hydrocarbons, Halogen Compounds and Nitro Compounds

Solvent	Temperature (^oC)	$[LA]_{aq}$ (M)	K_D
Hexane	25	5.16	<0.01
Cyclohexane	25	5.16	<0.01
Benzene	25	5.94	<0.01
Toluene	25	5.16	<0.01
Toluene	20	8.95	0.003
p-Cymene	28	1.86	0.0017
Pinene	28	1.87	0.013
Limonene	28	1.83	0.0036
Turpentine (sulfite)	28	1.87	0.01
Chloroform	28	1.87	0.01
Chloroform	20	8.84	0.011
Tetrachloromethane	25	5.97	<0.01
o-Dichlorobenzene	25	5.18	<0.01
Nitromethane	25	5.22	0.112
Nitroethane	25	5.69	0.043
Nitroethane	28	1.81	0.09

Table A.3.1 (cont.)

1-Nitropropane	25	5.86	0.031
2-Nitropropane	25	5.87	0.028
Nitrobenzene	25	5.98	0.005

Table A.3.2 Distribution of Lactic Acid on Alcohols

Solvent	**Temperature (^oC)**	**$[LA]_{aq}$ (M)**	**K_D**
1-Butanol	25	3.12	0.721
2-Methyl-1-propanol	25	3.28	0.630
2-Butanol	25	3.19	0.929
1-Pentanol	20	4.59	0.438
1-Pentanol	20	4.50	0.571
3-Methyl-1-butanol	25	4.28	0.447
3-Methyl-1-butanol	20	6.95 (%w)	0.691
3-Pentanol	26	3.18	0.493
2-Methyl-1-butanol	28	1.68	0.406
2-Methyl-2-butanol	25	3.42	0.813
Pentanols (mixed)	26	3.07	0.435
1-Hexanol	25	4.79	0.313
2-Ethyl-1-butanol	26	3.18	0.493
4-Mehyl-2-pentanol	25	0.5	0.37
4-Mehyl-2-pentanol	25	1.0	0.43
Cyclohexanol	25	3.95	0.578
Cyclohexanol	20	6.26	0.389
Benzyl alcohol	25	4.27	0.446
1-Octanol	25	5.21	0.198
2-Octanol	25	5.15	0.195
2-Ethyl-1-hexanol	28	1.98	0.147
Octylene glycol	25	4.20	0.489
Phenethyl alcohol	28	1.73	0.423
2-Phenoxyethanol	28	1.68	0.415
Pentylcyclohexanol	26	2.71	0.143

Table A.3.2 (cont.)

Phenol	26	2.56	0.740
Phenol	25	3.60	0.722
Eugenol	28	1.80	0.067

Table A.3.3 Distribution of Lactic Acid on Ethers

Solvent	**Temperature (°C)**	**$[LA]_{aq}$ (M)**	**K_D**
Ethyl ether	20	4.91	0.087
Ethyl ether	20	5.11	0.136
Isopropyl ether	25	16.21	0.029
Isopropyl ether	20	16.82 (%w)	0.0951
Ethyl butyl ether	25	5.85	0.026
Butyl ether	28	2.23	0.014
Butyl ether	25	5.97	0.009
Petyl ether	28	2.20	0.01
Ethylene glycol dibutyl ether	25	5.82	0.039
Phenyl ether	25	6.02	0.002
Furan	26	3.04	0.012
Methylal			0.4

Table A.3.4 Distribution of Lactic Acid on Ketones

Solvent	Temperature (°C)	$[LA]_{aq}$ (M)	K_D
2-Butanone	25	4.8 (%w)	0.81
3-Pentanone	25	5.04	0.164
3-Methyl-2-butanone	25	4.69	0.253
4-Methyl-2-pentanone	25	5.40	0.116
2-Heptanone	25	5.59	0.095
2-Heptanone	26	3.18	0.103
3-Heptanone	25	5.73	0.055
4-Heptanone	25	5.74	0.048
2,6 Dimethyl-3-heptanone	25	5.87	0.023
2,6 Dimethyl-3-heptanone	28	2.22	0.028
Mesityl oxide	28	1.84	0.266
Phorone	28	4.80	0.064
Cyclohexanone	25	3.85	0.524
Isophorone	25	4.27	0.419
Acetophenone	28	2.04	0.114
Acetophenone	25	5.46	0.109
Furfural	27	1.84	0.301

Table A.3.5 Distribution of Lactic Acid on Esters

Solvent	Temperature (°C)	$[LA]_{aq}$ (M)	K_D
Ethyl acetate	25	5.03	0.259
Ethyl acetate	20	6.64 (%w)	0.484
Propyl acetate	25	5.43	0.114
Butyl acetate	25	5.45	0.107
Butyl acetate	20	7.00 (%w)	0.436
Pentyl acetate	26	2.80	0.114
Amyl (fusel) acetate	26	2.15	0.091
4-Metylpentyl acetate	25	5.83	0.039
Cyclohexyl acetate	28	2.08	0.077
2-Ethoxyethyl acetate	25	4.03	0.530
2-Butoxyethyl acetate	28	2.78	0.107
2-(2 Butoxyethoxy)ethyl acetate	28	1.79	0.244
Ethyl propionate	25	5.55	0.103
Butyl lactate	25	4.26	0.569
Isobutyl lactate	28	1.61	0.537
Pentyl lactate	27	1.87	0.347
Ethyl acetoacetate	25	4.47	0.265
Diethyl carbonate	28	2.12	0.048
Tributyl phosphate	25	3.19	0.907

Table A.3.6 Distribution of Lactic Acid on Amines

Solvent	Temperature (°C)	$[LA]_{aq}$ (M)	K_D
Tributylamine	25	4.68	0.090
Aniline	25	5.33	0.108
o-Toluidine	30	6.95 (%w)	0.19

APPENDIX B

ION EXCHANGE EXPERIMENTS PERFORMED WITH PURE TOMAC

B.1 Ion Exchange with H_2SO_4

The amounts of sulphate and chloride ions present in the aqueous phase containing sulphuric acid after it is contacted with pure TOMAC are given in the table below.

Table B.1.1 Analysis results of the aqueous sulphuric acid phase after each contact with TOMAC

	$[SO_4^{-2}]_{aq}$ (mol/L)	$[Cl^-]_{aq}$ (mol/L)
1.5 M H_2SO_4	1.485	-
1st contact with H_2SO_4	0.730	1.241
2nd contact with H_2SO_4	1.464	0.094
3rd contact with H_2SO_4	1.056	0.269
4th contact with H_2SO_4	1.132	0.137
5th contact with H_2SO_4	1.158	0.074
Final contact with H_2O	0.183	0.008

1.5 M H_2SO_4 turned out to be 1.485 M from this analysis and the concentration of the sulphate ion decreased for each contact of the sulphuric acid phase with the organic phase containing pure TOMAC, due to the transfer of the sulphate ions to the organic phase. The chloride concentration of the

aqueous phase, which was initially zero, increased to the above values for each contact as the chloride ions of TOMAC are transferred from the organic phase to the aqueous phase counter-currently.

The number of moles of sulphate and chloride ions present in the aqueous phase can be found by multiplying their concentrations with the phase volume, which is equal to 100 mL (0.1 L) and which is assumed to remain constant throughout the exchange procedures.

Table B.1.2 Number of moles of the ions in the aqueous phase

	moles of SO_4^{-2} remaining in the aqueous phase	**moles of SO_4^{-2} transferred to the organic phase**	**moles of Cl^- transferred to the aqueous phase**
Before ion exchange	0.149	-	-
1st contact with H_2SO_4	0.073	0.075	0.124
2nd contact with H_2SO_4	0.146	0.002	0.009
3rd contact with H_2SO_4	0.106	0.043	0.027
4th contact with H_2SO_4	0.113	0.035	0.014
5th contact with H_2SO_4	0.116	0.033	0.007
Final contact with H_2O	0.019	-	0.001

The number of moles of sulphate that remain in the aqueous phase for each contact is subtracted from the initial number of moles of sulphate in the aqueous phase (0.149 moles) to find the number of moles of sulphate

transferred to the organic phase for that contact and listed in the second column of Table B.1.2. The summation of these values is 0.188 moles, which is equal to the total number of moles of sulphate that passed to the organic phase. The amount of sulphate re-extracted to the aqueous phase in the stage final contact with water is subtracted from this value, and the result which is equal to 0.169 moles is the final amount of sulphate in the organic phase. Dividing this with the volume of the organic phase (0.1 L) yields the concentration of TOMA(SO_4) in the organic phase as 1.69 M.

The total number of moles of chloride ions transferred to the aqueous phase can be found similarly, as 0.182 moles. Subtracting this from the initial concentration of chloride in the organic phase (0.193 M for pure TOMAC), the amount of chloride ions that remain in the organic phase is found as 0.011 moles. This is also equal to the number of moles of unconverted TOMAC in the organic phase. The concentration of residual TOMAC is 0.11 M.

The analyses and the calculations revealed that the amounts of sulphate transferred to the organic phase (0.188 moles) and the number of moles of chloride transferred to the aqueous phase (0.182 moles) are almost equal. This means that one chloride ion from TOMAC was exchanges with one counter ion. In that case, the counter ion should be hydrogensulphate (HSO_4^-) instead of sulphate (SO_4^{-2}). So TOMA(HSO_4) is produced in the organic phase, not TOMA(SO_4).

A sample chromatogram which shows the variation of chloride ion concentrations for each contact with H_2SO_4 is shown in Figure B.1. Largest peak representing the 1st contact, decreasing with increasing number of contacts.

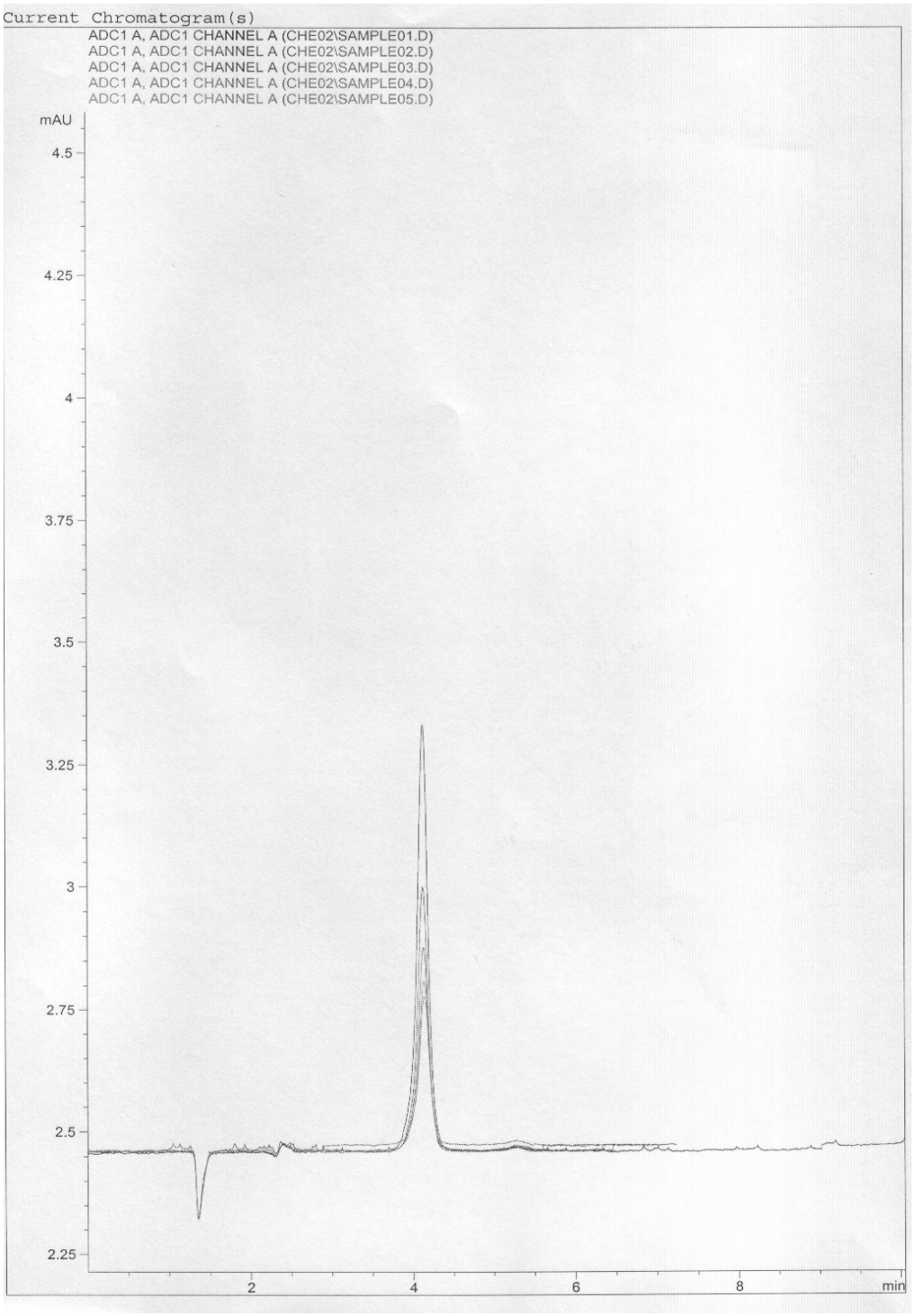

Figure B.1 Superimposed chromatograms for the concentration of chloride ion in the sulphuric acid phase after each of the five contacts of pure TOMAC with H_2SO_4

B.2 Ion Exchange with NaOH

The amounts chloride ions present in the aqueous phase containing sodium hydroxide after it is contacted with pure TOMAC are given in the table below. The initial and the remaining hydroxide ion concentrations of the aqueous phase could not be measured with the ion chromatograph since the mobile phase used in the analyses was NaOH itself. So, only the amounts of chloride ion transferred to the aqueous phase from the organic phase could be measured and the amount of TOMA(OH) formed in the organic phase was calculated to be equivalent to this amount.

The first five exchanges were done with contacting the organic phase initially containing pure TOMAC with 2 M NaOH solution. But the analyses revealed that the ion exchange that was achieved after 5 contacts was not satisfactory. The final two contacts were performed with 5 M NaOH solution, and the efforts of ion exchange were terminated after this point since minute amounts of chloride were extracted to the aqueous phase in these contacts too.

Table B.2.1 Analysis results of the aqueous sodium hydroxide phase after each contact with TOMAC

	$[Cl^-]_{aq}$ (mol/L)
1st contact with 2 M NaOH	0.270
2nd contact with 2 M NaOH	0.166
3rd contact with 2 M NaOH	0.131
4th contact with 2 M NaOH	0.110
5th contact with 2 M NaOH	0.100
1st contact with 5 M NaOH	0.080
2nd contact with 5 M NaOH	0.067

The total number of moles chloride ion that passed to the aqueous phase was found as 0.093 moles, by multiplying the above measured concentrations with the aqueous phase volume (0.1 L) and summing them up. Subtracting this value from the initial number of chloride ions present in the organic phase (0.193 moles for pure TOMAC), the number of moles of chloride remaining in the organic phase was found to be 0.1. This is equivalent to 1 M chloride ion remaining in the organic phase, i.e., 1 M unconverted TOMAC. The number of moles of the TOMA(OH) that was formed in the organic phase is equal to 0.193-0.1=0.093, which corresponds to 0.93 M TOMA(OH) in the organic phase.

APPENDIX C

ANALYSES WITH THE ION CHROMATOGRAPH AND SAMPLE CALIBRATION CURVES

The ion chromatograph was equipped with a conductivity detector, the output of which was given in terms of the conductivities of ions present in the samples in microsiemens (μs) units. The maximum measurement range of the chromatograph could be varied between 10 and 1000 μs, which determines the sensitivity of the measurements. The calibration curves for lactic acid, sulphate and chloride were derived at either 30 or 100 μs or both (sample calibration curves for lactic acid, sulphate, and chloride at 100 μs are given in Figures C.1, C.2, and C.3). The samples were injected to the chromatograph at either one of these ranges depending on their concentrations and amounts of dilution. The areas under the chromatogram peaks were calculated and automatically converted into concentration units by using the calibration curves. The pH of the NaOH mobile phase was 11.95 so lactic acid and other substances present in the samples were essentially in the ionized form.

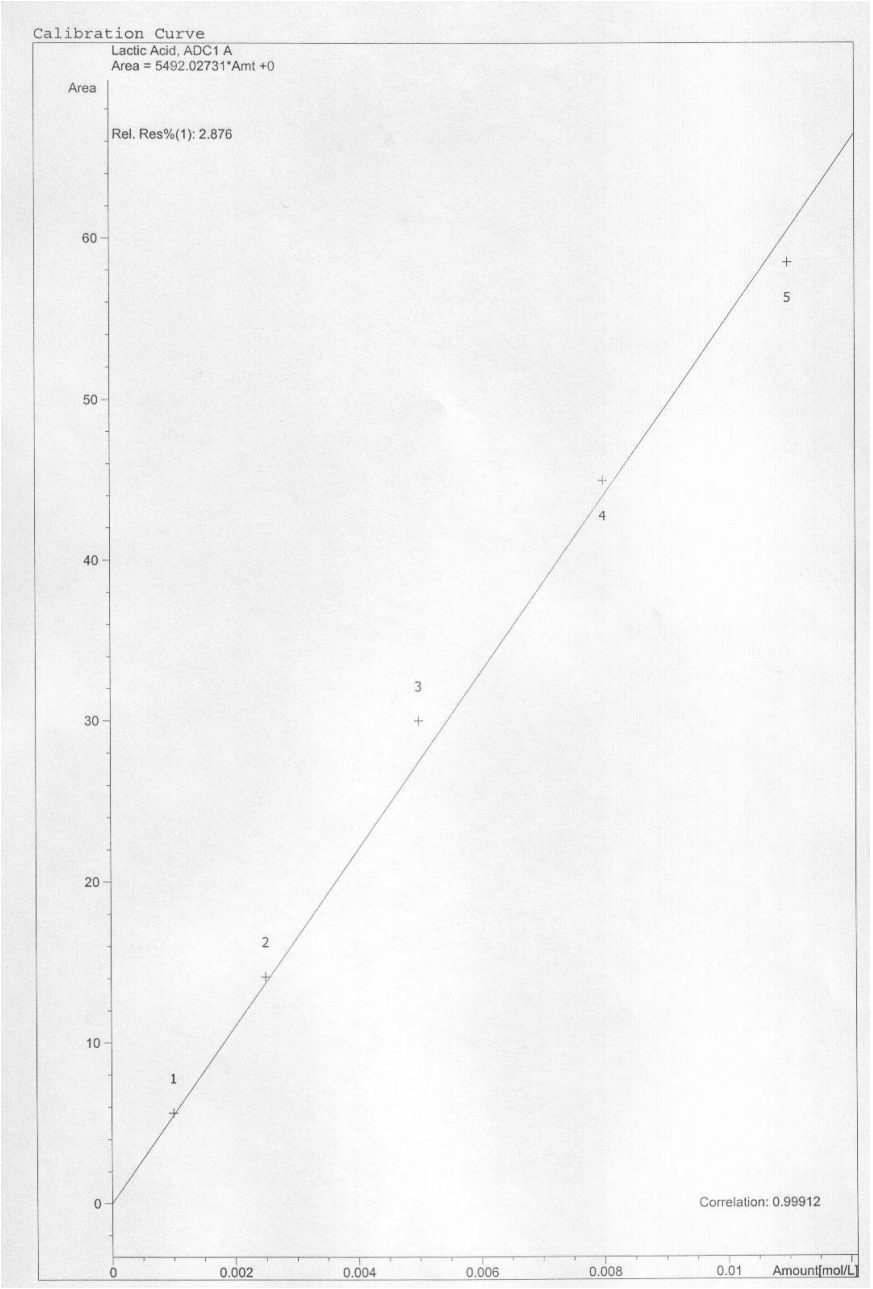

Figure C.1 Lactic acid calibration curve drawn at 100 μs range

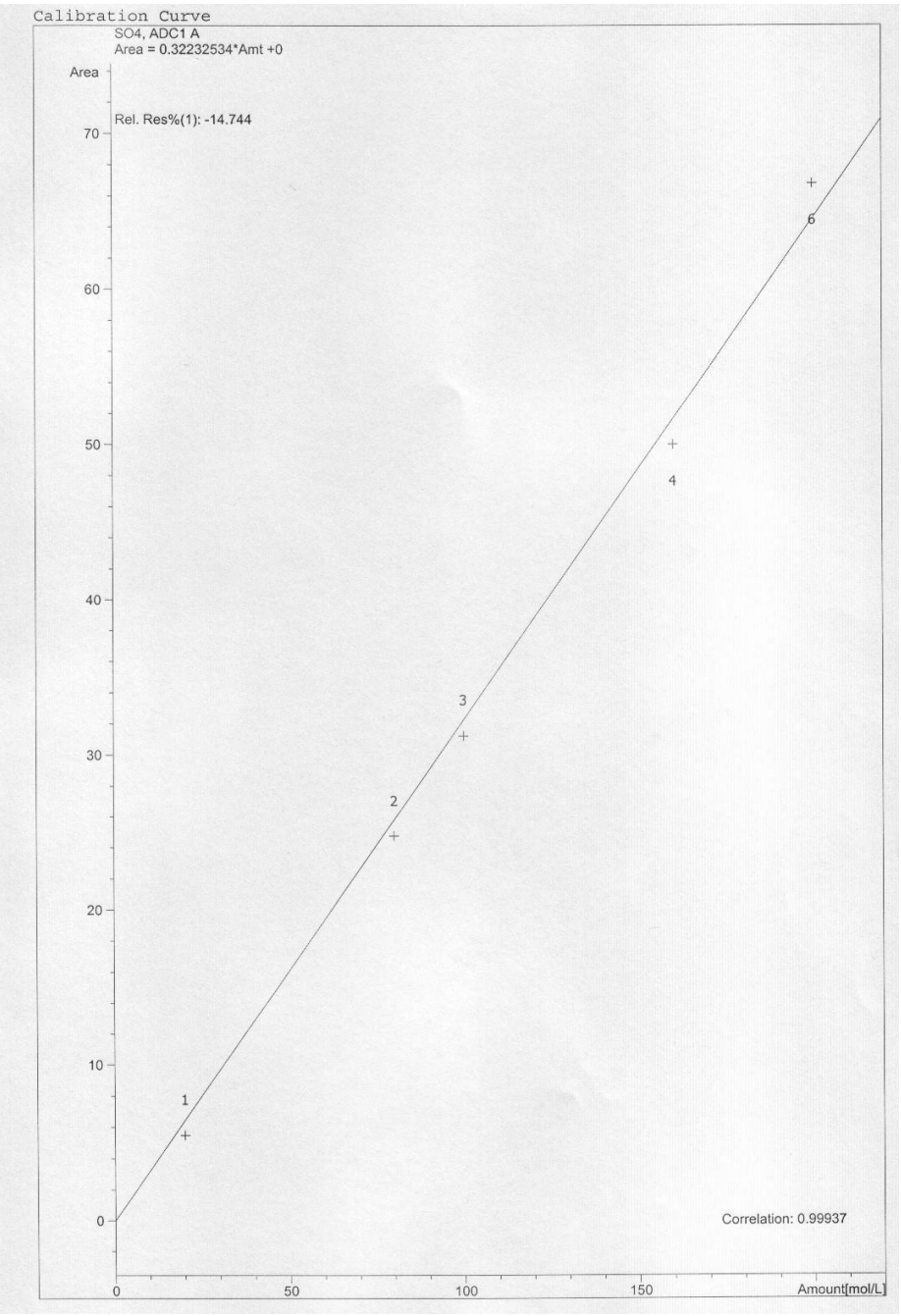

Figure C.2 Suplhate calibration curve drawn at 100 μs range

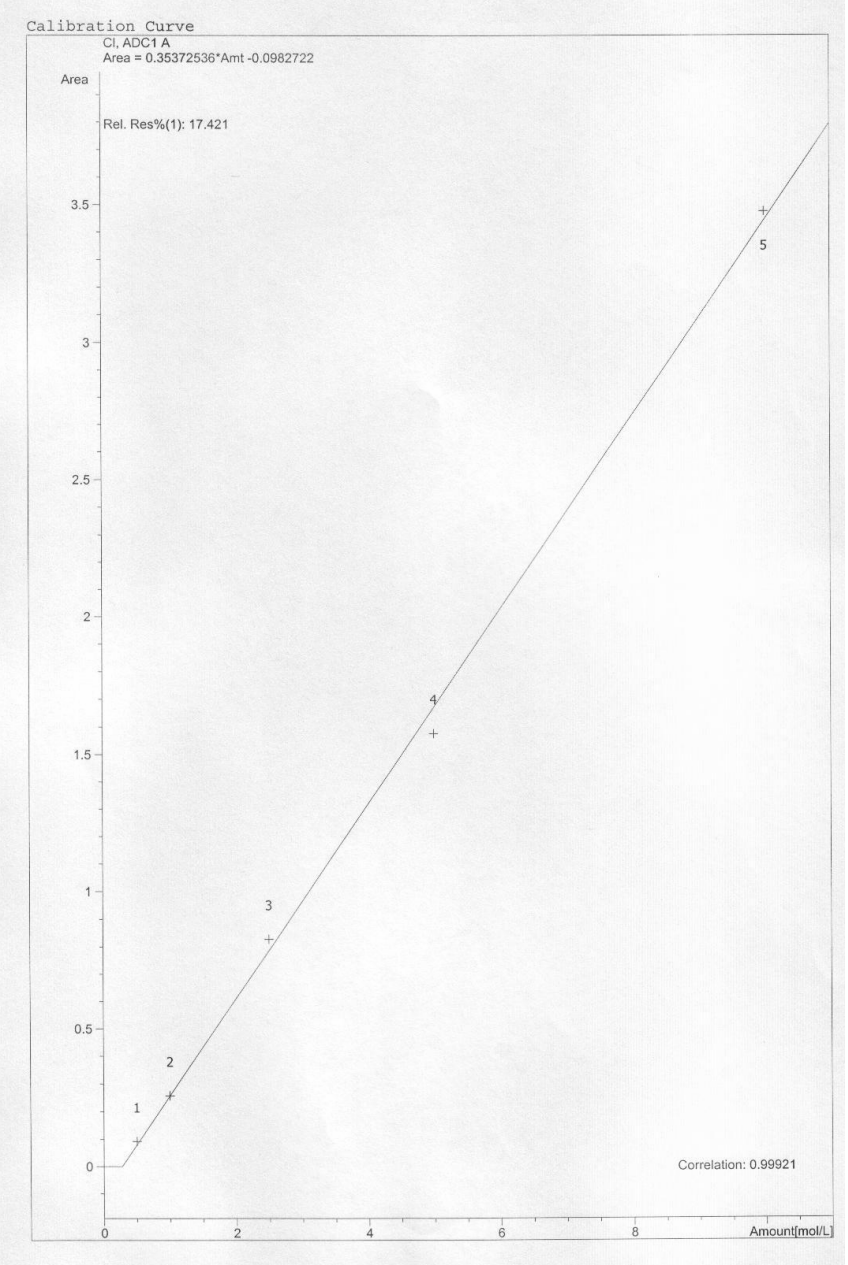

Figure C.3 Chloride calibration curve drawn at 100 μs range

APPENDIX D

RESULTS OF EXTRACTION EXPERIMENTS

D.1 Extractions with TOMAC dissolved in oleyl alcohol

D.1.1 Initial Aqueous Phase pH between 2-2.5

Table D.1.1 Extraction with 0.1 M TOMAC in oleyl alcohol, initial aqueous phase pH=2-2.5

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.240	0.220	0.02	8.33	0.091	0.2
0.470	0.440	0.03	6.38	0.068	0.3
0.690	0.648	0.042	6.09	0.065	0.42
0.910	0.858	0.052	5.71	0.061	0.52
1.317	1.289	0.028	2.13	0.022	0.28

Table D.1.2 Extraction with 0.2 M TOMAC in oleyl alcohol, initial aqueous phase pH=2-2.5

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.240	0.211	0.029	12.08	0.137	0.145
0.470	0.430	0.040	8.51	0.093	0.2
0.690	0.630	0.060	8.70	0.095	0.3
0.910	0.850	0.060	6.59	0.071	0.3
1.140	1.100	0.040	3.51	0.036	0.2

Table D.1.3 Extraction with 0.3 M TOMAC in oleyl alcohol, initial aqueous phase pH=2-2.5

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.354	0.313	0.041	11.58	0.131	0.137
0.652	0.590	0.062	9.51	0.105	0.207
0.911	0.833	0.078	8.56	0.094	0.26
1.135	1.045	0.09	7.93	0.086	0.3
1.317	1.217	0.1	7.59	0.082	0.333

Table D.1.4 Extraction with 0.4 M TOMAC in oleyl alcohol, initial aqueous phase pH=2-2.5

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.372	0.328	0.044	11.83	0.134	0.11
0.665	0.585	0.08	12.03	0.137	0.2
0.850	0.765	0.085	10	0.111	0.213
1.045	0.950	0.095	9.09	0.1	0.238
1.260	1.155	0.105	8.33	0.091	0.263

Table D.1.5 Extraction with 0.5 M TOMAC in oleyl alcohol, initial aqueous phase pH=2-2.5

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.372	0.313	0.059	15.86	0.188	0.118
0.665	0.565	0.1	15.04	0.177	0.2
0.850	0.735	0.115	13.53	0.156	0.23
1.045	0.920	0.125	11.96	0.136	0.25
1.260	1.125	0.135	10.71	0.12	0.27

Table D.1.6 Extraction with pure TOMAC, initial aqueous phase pH=2-2.5

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.244	0.1	0.144	59.02	1.44	0.075
0.493	0.225	0.268	54.36	1.191	0.138
0.723	0.382	0.341	47.16	0.893	0.176
1.055	0.504	0.551	52.23	1.093	0.285
1.264	0.638	0.626	49.53	0.981	0.324

D.1.2 Initial Aqueous Phase pH adjusted to 4

Table D.1.7 Extraction with pure oleyl alcohol, initial aqueous phase pH=2-2.5

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D
0.372	0.351	0.021	5.65	0.060
0.665	0.620	0.045	6.77	0.068
0.850	0.820	0.03	3.53	0.035
1.045	1.010	0.035	3.35	0.033
1.260	1.185	0.075	5.95	0,060

Table D.1.8 Extraction with 0.1 M TOMAC in oleyl alcohol, initial aqueous phase pH=4

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.294	0.273	0.021	7.14	0.077	0.21
0.815	0.722	0.093	11.41	0.129	0.93
1.171	1.096	0.075	6.40	0.068	0.75

Table D.1.9 Extraction with 0.3 M TOMAC in oleyl alcohol, initial aqueous phase pH=4

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.294	0.243	0.051	17.35	0.210	0.17
0.815	0.689	0.126	15.46	0.183	0.42
1.171	1.014	0.157	13.41	0.155	0.523

Table D.1.10 Extraction with 0.5 M TOMAC in oleyl alcohol, initial aqueous phase pH=4

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.294	0.225	0.069	23.50	0.307	0.138
0.815	0.652	0.163	20	0.25	0.326
1.171	0.978	0.193	16.48	0.197	0.386

Table D.1.11 Extraction with pure TOMAC, initial aqueous phase pH=4

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.382	0.146	0.236	61.78	1.616	0.122
0.935	0.472	0.463	49.52	0.981	0.239
1.327	0.806	0.521	39.26	0.646	0.269

Table D.1.12 Extraction with pure oleyl alcohol, initial aqueous phase pH=4

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D
0.382	0.354	0.028	7.33	0.079
0.935	0.850	0.085	9.09	0.1
1.327	1.233	0.094	7.08	0.076

D.1.2 Initial Aqueous Phase pH adjusted to 6

Table D.1.13 Extraction with 0.1 M TOMAC in oleyl alcohol, initial aqueous phase pH=6

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.273	0.196	0.077	28.21	0.393	0.77
0.708	0.567	0.141	19.92	0.249	1.41
0.971	0.854	0.117	12.05	0.137	1.17

Table D.1.14 Extraction with 0.3 M TOMAC in oleyl alcohol, initial aqueous phase pH=6

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.273	0.181	0.092	33.70	0.508	0.306
0.708	0.581	0.127	17.94	0.219	0.423
0.971	0.90	0.071	7.31	0.079	0.236

Table D.1.15 Extraction with 0.5 M TOMAC in oleyl alcohol, initial aqueous phase pH=6

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.273	0.176	0.097	35.53	0.551	0.194
0.708	0.574	0.134	18.93	0.233	0.268
0.971	0.874	0.097	9.99	0.111	0.194

Table D.1.16 Extraction with pure TOMAC, initial aqueous phase pH=6

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.396	0.193	0.203	51.26	1.052	0.105
0.913	0.649	0.264	28.92	0.407	0.136
1.273	0.981	0.292	22.94	0.298	0.151

Table D.1.17 Extraction with pure oleyl alcohol, initial aqueous phase pH=6

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D
0.396	0.359	0.037	9.34	0.103
0.913	0.838	0.075	8.21	0.089
1.273	1.149	0.124	9.74	0.108

D.2 Extractions with TOMAC dissolved in octanol

D.2.1 Initial Aqueous Phase pH between 2-2.5

Table D.2.1 Extraction with 0.1 M TOMAC in octanol, initial aqueous phase pH=2-2.5

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.286	0.229	0.057	19.93	0.249	0.57
0.808	0.662	0.146	18.07	0.221	1.46
1.204	1.053	0.151	12.54	0.143	1.51

Table D.2.2 Extraction with 0.3 M TOMAC in octanol, initial aqueous phase pH=2-2.5

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.286	0.217	0.069	24.13	0.318	0.23
0.808	0.673	0.135	16.71	0.201	0.45
1.204	1.017	0.187	15.53	0.184	0.623

Table D.2.3 Extraction with 0.5 M TOMAC in octanol, initial aqueous phase pH=2-2.5

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.286	0.220	0.066	23.08	0.3	0.132
0.808	0.644	0.164	20.3	0.255	0.328
1.204	0.991	0.213	17.69	0.215	0.426

Table D.2.4 Extraction with pure octanol, initial aqueous phase pH=2-2.5

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D
0.372	0.324	0.048	12.9	0.148
0.850	0.745	0.105	12.35	0.141
1.260	1.115	0.145	11.51	0.130

D.2.2 Initial Aqueous Phase pH adjusted to 6

Table D.2.5 Extraction with 0.1 M TOMAC in octanol, initial aqueous phase pH=6

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.396	0.323	0.073	18.43	0.226	0.73
0.913	0.824	0.089	9.75	0.108	0.89
1.273	1.204	0.069	5.42	0.057	0.69

Table D.2.6 Extraction with 0.3 M TOMAC in octanol, initial aqueous phase pH=6

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.396	0.277	0.119	30.05	0.429	0.396
0.913	0.767	0.146	15.99	0.19	0.486
1.273	1.145	0.128	10.05	0.112	0.426

Table D.2.7 Extraction with 0.5 M TOMAC in octanol, initial aqueous phase pH=6

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z
0.396	0.250	0.146	36.87	0.584	0.292
0.913	0.739	0.174	19.06	0.235	0.348
1.273	1.112	0.161	12.65	0.145	0.322

Table D.2.8 Extraction with pure octanol, initial aqueous phase pH=6

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D
0.396	0.376	0.02	5.05	0.053
0.913	0.877	0.036	3.94	0.041
1.273	1.212	0.061	4.79	0.050

D.3 Ion Exchange of TOMAC to TOMA(HSO_4)

D.3.1 Extraction Experiments with TOMA(HSO_4)

Table D.3.1 Extraction with 0.1 M TOMA(HSO_4) in octanol, initial aqueous phase pH=6

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z	$[Cl]^*_{aq}$ (M)	$[SO_4]^*_{aq}$ (M)
0.316	0.236	0.08	25.32	0.339	0.700	0.006	0.050
0.568	0.495	0.073	12.85	0.148	0.639	0.003	0.046
0.670	0.607	0.063	9.4	0.104	0.551	0.004	0.049
0.954	0.902	0.052	5.45	0.058	0.455	0.004	0.054
1.096	1.047	0.049	4.47	0.047	0.429	0.004	0.051

Table D.3.2 Extraction with 0.5 M TOMA(HSO_4) in octanol, initial aqueous phase pH=6

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z	$[Cl]^*_{aq}$ (M)	$[SO_4]^*_{aq}$ (M)
0.316	0.234	0.082	25.95	0.351	0.144	0.005	0.109
0.568	0.377	0.191	33.63	0.507	0.335	0.005	0.174
0.670	0.488	0.182	27.16	0.373	0.319	0.006	0.212
0.954	0.637	0.317	33.23	0.498	0.555	0.006	0.262
1.096	0.739	0.357	32.57	0.483	0.625	0.006	0.300

Table D.3.3 Extraction with undiluted TOMA(HSO_4) accompanied by unconverted TOMAC, initial aqueous phase pH=6

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z	$[Cl]^*_{aq}$ (M)	$[SO_4]^*_{aq}$ (M)
0.316	0.157	0.159	50.32	1.013	0.082	0.011	0.161
0.568	0.300	0.268	47.18	0.893	0.139	0.010	0.236
0.670	0.374	0.296	44.18	0.791	0.153	0.010	0.305
0.954	0.461	0.493	51.68	1.069	0.255	0.010	0.409
1.096	0.488	0.608	55.47	1.246	0.315	0.011	0.509

D.4 Ion Exchange of TOMAC to TOMA(OH)

D.4.1 Extraction Experiments with TOMA(OH)

Table D.4.1 Extraction with 0.1 M TOMA(OH) in octanol, initial aqueous phase pH=6

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z	$[Cl]^*_{aq}$ (M)
0.316	0.214	0.102	32.28	0.476	0.490	0.022
0.568	0.439	0.129	22.71	0.294	0.620	0.031
0.670	0.608	0.062	9.25	0.102	0.298	0.036
0.954	0.819	0.135	14.15	0.165	0.649	0.043
1.096	0.956	0.140	12.77	0.147	0.673	0.047

Table D.4.2 Extraction with 0.5 M TOMA(OH) in octanol, initial aqueous phase pH=6

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z	$[Cl]^*_{aq}$ (M)
0.316	0.068	0.248	78.48	3.636	0.238	0.014
0.568	0.250	0.318	55.99	1.274	0.306	0.032
0.670	0.412	0.258	38.51	0.627	0.248	0.048
0.954	0.622	0.332	34.8	0.534	0.319	0.068
1.096	0.754	0.342	31.2	0.454	0.329	0.081

Table D.4.3 Extraction with undiluted TOMA(OH) accompanied by unconverted TOMAC, initial aqueous phase pH=6

$[LA]^0_{aq}$ (M)	$[LA]^*_{aq}$ (M)	$[LA]^*_{org}$ (M)	Degree of extraction (%)	K_D	Z	$[Cl]^*_{aq}$ (M)
0.316	0.054	0.262	82.91	4.827	0.136	0.023
0.568	0.173	0.395	69.54	2.279	0.205	0.038
0.670	0.294	0.376	56.12	1.279	0.195	0.052
0.954	0.469	0.485	50.84	1.035	0.251	0.071
1.096	0.620	0.476	43.43	0.768	0.247	0.089

APPENDIX E

PERCENT DISSOCIATION CALCULATION FOR LACTIC ACID AT pH 4 AND 6

The dissociation of lactic acid, which is a weak acid with a pK_a value of 3.86 dissociates in water according to the following reaction.

$$HA + H_2O \leftrightarrow A^- + H_3O^+ \quad \text{(E.1)}$$

The ionization constant for this reaction is:

$$K_a = \frac{[A^-][H_3O^+]}{[HA]} \quad \text{(E.2)}$$

Taking the logarithm of both sides:

$$pK_a = pH - \log\left(\frac{[A^-]}{[HA]}\right) \quad \text{(E.3)}$$

The amounts of dissociated lactic acid for pH values of 4 and 6 can be calculated according to equation (E.3) as follows:

For pH = 4 $\Rightarrow$ $3.86 = 4 - \log\left(\frac{[A^-]}{[HA]}\right)$

$\log\left(\frac{[A^-]}{[HA]}\right) = 0.14$ $\frac{[A^-]}{[HA]} = 1.38$

From this equality the amount of dissociated acid at pH 4 [A^-] can be found as 58%.

For pH = 6 $\Rightarrow$ $3.86 = 6 - \log\left(\frac{[A^-]}{[HA]}\right)$

$\log\left(\frac{[A^-]}{[HA]}\right) = 2.14$ $\frac{[A^-]}{[HA]} = 138$

From this equality the amount of dissociated acid [A^-] at pH 6 can be found as 99%.