Resolution of Racemic Glutamic Acid Mixtures

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DEDICATION

This document is dedicated my family who have been extremely supportive to me over the last 2 years. I would especially like to recognize my grandmother Sophie Campbell who has also supported me economically over the course of my studies.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Optical isomers (enantiomers) are chiral molecules, which are commonly used in the drug and food industry. The major difference between the two D and L enantiomers is the spatial arrangement of atoms around a central carbon. During normal chemical synthesis a mixture of L and D enantiomers are formed in equally proportion (racemic mixture). Currently, the most common technique for resolution of a racemic mixture is chromatography using chiral specific columns. However, this method is limited in scale and is rather costly. This work studies the possibility for the resolution of racemic glutamic acid by crystallization. Since the solubility of the two enantiomers in water is the same, the separation is based only on the rate of crystallization by using appropriate seed crystals. Experiments are performed with a range of initial supersaturation ratios and seed concentrations. The results show that an appreciable resolution of racemic glutamic acid can be attained with the presence of L seeds in solution. At the highest seed concentration (10 g/L of L glutamic acid), the initial supersaturation of 3.60, 2.50 and 1.60 yields 24.0 %, 42.1 % and 55.5 % pure L glutamic acid crystals, respectively. Therefore as the initial supersaturation is lowered the percent yield of pure L glutamic acid increases.

ABRÉGÉ

Les isomères optiques (énantiomères) sont des molécules chirales qui sont très répandues dans l'industrie alimentaire et pharmaceutique. La différence majeure entre les deux énantiomères L et D est l'arrangement spatial des atomes autour du carbone central. Durant la synthèse chimique normale, un mélange avec une proportion égale des énantiomères L et D est formé (mélange racémique). Présentement, la technique la plus utilisée pour séparer un mélange racémique est la chromatographie utilisant une colonne chirale spécifique. Cependant, cette méthode est limitée à une petite échelle et peut être très dispendieuse. Nos travaux de recherche étudient la possibilité de séparer un mélange racémique d'acide glutamique par cristalization. En sachant que la solubilité des deux énantiomères dans l'eau est pareille, la séparation est uniquement basée sur le taux de cristalization en employant les noyaux de cristaux appropriés. Les expériences de laboratoire ont été conduits avec une variété de ratios de supersaturation et de concentrations de noyaux. Les résultats démontrent qu'une résolution appréciable du mélange racémique d'acide glutamique peut être obtenue avec la présence des noyaux L en solution. Avec la plus haute concentration de noyaux (i.e. 10 g/L de l'acide glutamique L), des ratios de supersaturation initiale de 3.60, 2.50 et 1.60 se traduisent respectivement en 24.0 %, 42.1 % and 55.5 % de cristaux de pur d'acide glutamique L. Donc, lorsque le ratio de supersaturation initiale est diminué, le pourcentage d'acide glutamique pur L augmente.

NOMENCLATURE LIST

S = Supersaturation

S^o = Initial supersaturation

S_{min} = Minimum supersaturation

T_c = Crystallization temperature

T_{sat} = Saturation temperature

t_c = Crystallization operating time

$[L_s]$ = Seed concentration of L glutamic acid

C^* = Saturation concentration at a constant temperature

C^o = Initial saturation concentration

ΔC = Concentration difference

ΔG = Critical free energy required for homogenous nucleation

$\Delta G'$ = Critical free energy required for heterogeneous nucleation

ϕ = Free energy correction factor

C = Solid mixture of L and D crystals in one lattice

σ_{max} = Maximum standard deviation for experimental results

Y_L = Percentage of L glutamic acid which has been crystallized from the initial amount present in solution.

Y_D = Percentage of D glutamic acid which has been crystallized from the initial amount present in solution.

P_L = Percentage of L glutamic acid in the crystalline solid product

r_L = Rate of L glutamic acid crystallization

A = Pre-exponential factor

E = Activation energy

R = Molar gas constant

k = Rate constant that depends on crystallization temperature and seed concentration

n = Rate order

Chapter 1 INTRODUCTION

1.1 Chirality and Enantiomer Importance

Any object that has a nonsuperimposable mirror image is defined as a chiral object, that is, in no way can the mirror image be turned or moved to cover the object. Chemically, a chiral compound consists of four different chemical groups attached to a carbon atom as shown in Figure 1.1.

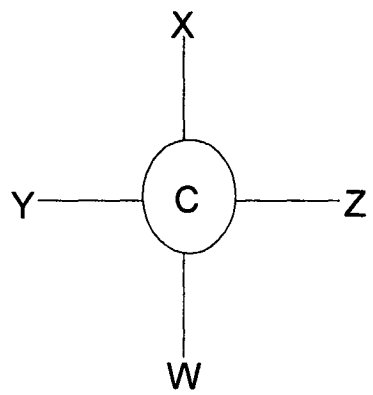


Figure 1.1: Chiral compound with 4 different chemical groups attached to the carbon center

The chemical groups (W, X, Y and Z) may consist of the same atoms as long as the bonding order of each chemical group is different. This molecule cannot be superimposed on its mirror image.

Chiral compounds are of importance, because biological reactions such as those occurring in a living organism take place in a chiral selective environment. The response of the organism to the compound depends on the fit and binding to a site on the receptor molecule. Typically, compounds used in the pharmaceutical and food industry are chiral and have a very specific function. A specific classification of chiral molecules is optical isomers, otherwise known as enantiomers, which are chiral non-superimposible mirror images; see Figure 1.2 (Zumdahl (1995)).

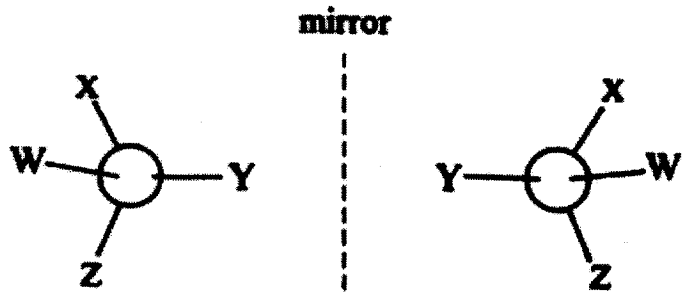


Figure 1.2: Optical isomers (enantiomers)

Each enantiomer has identical bonds to the carbon center with different atomic spatial arrangements. The physical properties such as molecular weight, melting point and solubility are identical for each enantiomer. The difference lies in the direction each enantiomer rotates plan-polarized light. The magnitude of the rotation is identical but one enantiomer will rotate light to the left (L, Levorotatory) and the other to the right (D, Dextrorotatory). The varying spatial arrangement of atoms on the carbon center results in a different response or reaction. In frequent cases only one enantiomer is useful and the other may be inert or toxic. Therefore, each enantiomer must be treated as an independent molecule and studied separately.

1.2 Enantiomer Production

Commercially important enantiomers can be synthesized either by biological or chemical techniques. Normally, pure enantiomers are produced with biological agents, such as bacteria, yeast and genetically altered micro organisms. An industrial example of fermentation involves the production of L-Lysine from carbohydrates of raw sugar or beet molasses with a pair of E-coli mutants as described by Kauffman (2004).

In most cases chemical synthesis is the manufacturing process of choice, where large quantities can be produced at economical costs. A drawback of this method is that an equal mixture of the D and L enantiomers is formed. This mixture of 50 % L and 50 % D is called a racemic mixture. Thus, the development of techniques to separate a racemic mixture into its pure

enantiomer components is needed. The resolution of enantiomers is not conventional because of their identical physical properties. Chromatography on chiral stationary phases is a current approach to the resolution of several amino acid and pharmaceuticals. Antal *et al* (2000), gives examples for the resolution of phenyalanine, asparagine, glutamic acid and ibuprofen with a chiral teicoplanin – based column. Alternatively, crystallization is a viable option for some molecules and is detailed in Section 1.3.

1.3 Crystallization Process

Crystallization provides several advantages for the separation of racemic mixtures. It is more straightforward and economical than current approaches and can produce high purity products in a single stage, instead of a multi-stage process. Crystallization kinetics depends on many factors, the most important of which is the departure from the saturation condition. Other factors include the presence of impurities, seed crystals and agitation. The addition of the desired enantiomer crystals has the potential of improving the rate of crystallization and attracting solute molecules from solution to adhere and combine with the growing crystal lattice. The added crystal surface minimizes the energy required for nucleation and growth as described in Section 2.1.2.

There are three common methods used for creating the departure from equilibrium (i.e. creating supersaturation):

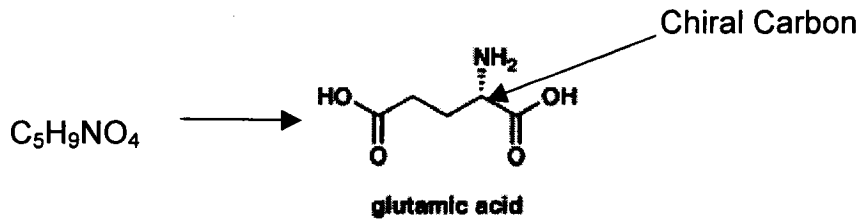
1) Evaporation of solvent, this method is employed when there is minimal solubility increase with an increase in temperature. For example the crystallization of NaCl is achieved by heating the saturated solution of water and salt above 100 °C. It is critical for this method that the solute is stable at an elevated temperature. This is not a method of choice for most biochemicals.

2) Addition of a precipitating agent, which is miscible in the original solvent and does not dissolve the solute. This results in the precipitation of the solute from the mixture of solvents. This method is usually applied to the crystallization of compounds that have relatively high solubilities at room temperature as described by Orella & Kirwan (1989).

3) Temperature reduction lowers the solubility, causing solute crystallization. This method is commonly used when there is a significant change in solubility with temperature.

1.4 Glutamic Acid

Glutamic acid is the selected compound for this project. It has an industrial importance in the food industry where it is a component of a flavour-enhancing additive (MSG – monosodium glutamate). The L glutamic acid enantiomer is used as the food additive; the D enantiomer is avoided because it is tasteless and sometimes causes adverse reactions (Kauffman (2004)). The structure of glutamic acid and the location of the chiral carbon are shown below:



glutamic acid

The chemical synthesis of glutamic acid yields L and D crystals in equal proportion, forming a racemic mixture. When crystallized the enantiomers are mechanically separable, that is each one crystallizes into its own lattice. This type of racemic mixture is called a conglomerate system, which is described in further detail in Section 2.2.

The method to induce crystallization or to create a departure from saturation for glutamic acid in this research project is based on its physical properties. The solubility of glutamic acid is significantly influenced by temperature; therefore the evaporation of solvent method is not suitable.

The addition of a precipitating agent is also not appropriate because the solubility of glutamic acid at room temperature is fairly low at 8.64 g/L. Therefore, the method of choice is temperature reduction and is detailed in section 2.1.1.

1.5 Scope and Objectives

This research focuses on characterizing the rate of crystallization of L and D glutamic acid from a racemic mixture solution. The specific objectives to be addressed are:

1) To design a robust and repeatable experimental set-up to perform solubility and crystallization experiments.

2) To develop a protocol for crystallizing L glutamic acid from a racemic mixture solution and retaining D glutamic acid.

3) To determine conditions of initial supersaturation (S^o), crystallization temperature (T_c), crystallization time (t_c) and L glutamic acid seed concentration ($[L_s]$) which produce the highest purity and yield of L glutamic acid crystals.

4) To develop a rate law that relates the rate of supersaturation decay or rate of crystallization to supersaturation. This equation can be used for modelling the crystallization process.

Chapter 2 BACKGROUND – LITERATURE REVIEW

In this Chapter, basic definitions and concepts required for crystallization are explored. Background information required to use crystallization as a separation process for racemic mixtures is presented.

2.1 Basic Definitions Required for Crystallization

Crystallization is a process which presents a phase change from an initial liquid solution to a thermodynamic equilibrium between liquid solution and solute crystals. The final equilibrium mixture of solid and liquid is conventionally separated by simple filtration or centrifugation. A solution is a homogenous mixture of two or three components, where the solvent is the component in abundance and the solutes are the components in lesser amounts. The three types of solutions important for crystallization are: unsaturated, saturated and supersaturated. Theoretically, the common feature of each solution is that there are no solid crystals present. An unsaturated solution contains a certain amount of solute dissolved in a solvent. However a greater amount of solute can be added and the solvent can dissolve the added crystals. A saturated solution contains the maximum amount of solute that the solvent can dissolve. A supersaturated solution retains a greater amount of dissolved solute than the saturation amount as explained in 2.1.1.

2.1.1 Supersaturation by Temperature Reduction

Typically, the amount of solute which can dissolve in a solvent increases with temperature. For illustrative purposes, Figure 2.1 demonstrates schematically a typical solubility curve for solute as temperature increases.

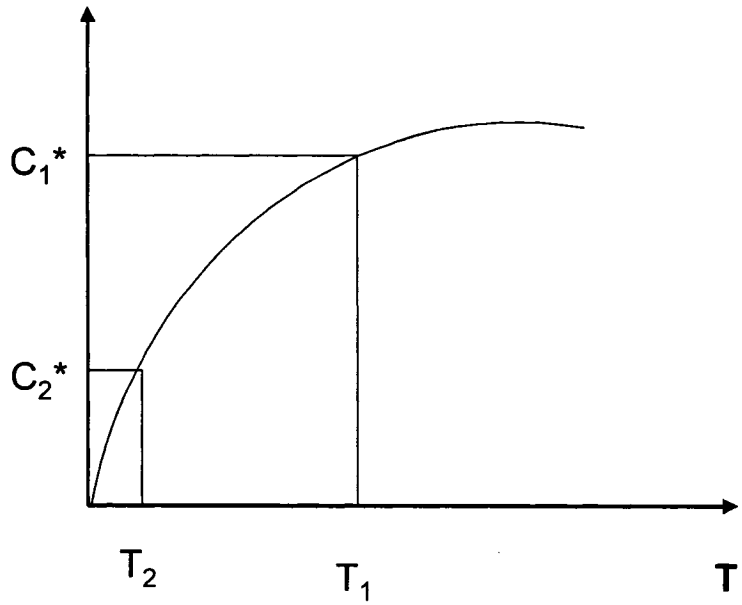


Figure 2.1: Solubility as a function of temperature

At T_1 and T_2, the saturation concentrations are C_1^* and C_2^* respectively, it is clear that T_1 is greater than T_2 and C_1^* is greater than C_2^*. The solution obtained upon instantaneous cooling from T_1 to T_2 where crystals do not have time to form is defined as a supersaturated solution, which is described in Figure 2.2.

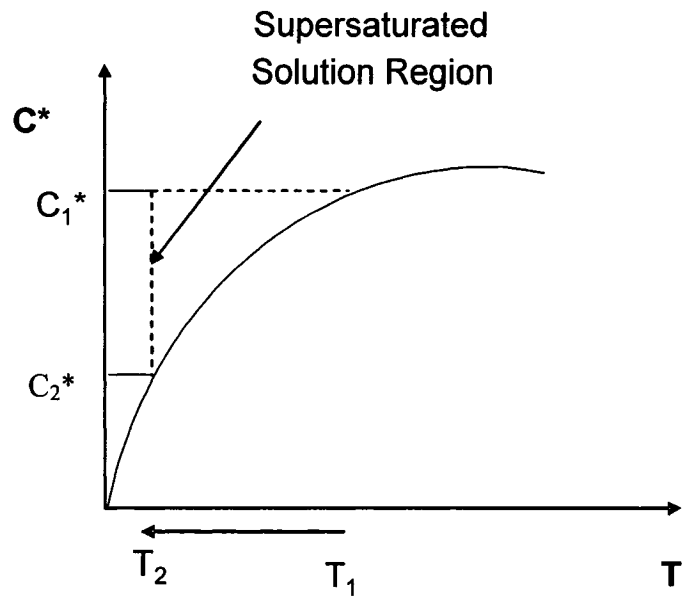


Figure 2.2: Supersaturated solution region based on temperature reduction

In practical application it is difficult to attain a supersaturated solution at C_1^* because any slight agitation or disturbance may reduce the concentration towards C_2^*. The concentration approaches C_2^* by the removal or precipitation of solute from solution. This is the process of crystallization and the key parameters used to control the rate of crystallization are:

$$\Delta C = C_1^* - C_2^* \qquad (2.1)$$

where ΔC is the concentration difference which drives the crystallization.

$$S = C/C_2^* \qquad (2.2)$$

where S is the supersaturation which is equal to a transient concentration (C) divided by the saturated concentration at temperature T_2. The maximum or initial supersaturation occurs when C is the same as C_1^* and the minimum supersaturation occurs when C is equal to C_2^*, which implies that the supersaturation is equal to unity. Figure 2.3 demonstrates the variation of supersaturation during a typical crystallization process

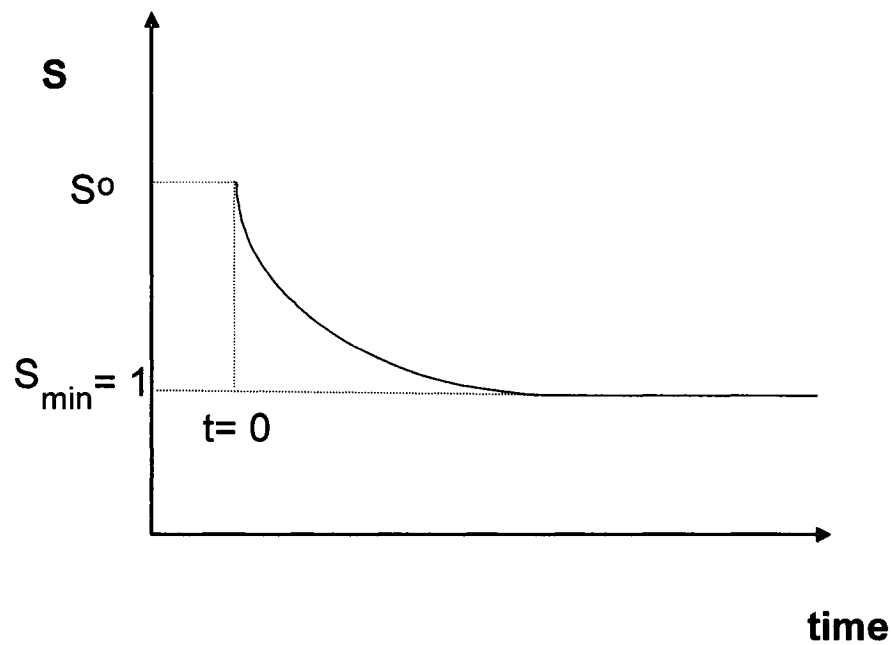


Figure 2.3: Identification of supersaturation variation as a function of time

The rate of decrease in supersaturation depends on physical properties of the solute, temperature, concentration, speed of agitation and the addition of inoculation material as described by Mullin (2001).

2.1.2 General Nucleation

Before the occurrence of crystal growth, small solute crystals, embryos or nuclei have to be present in suspension. Currently, there is no one general agreement to how these nuclei are created. For the purpose of this thesis the classification given by Mullin (2001) of the nucleation process is used and can be seen in Figure 2.4.

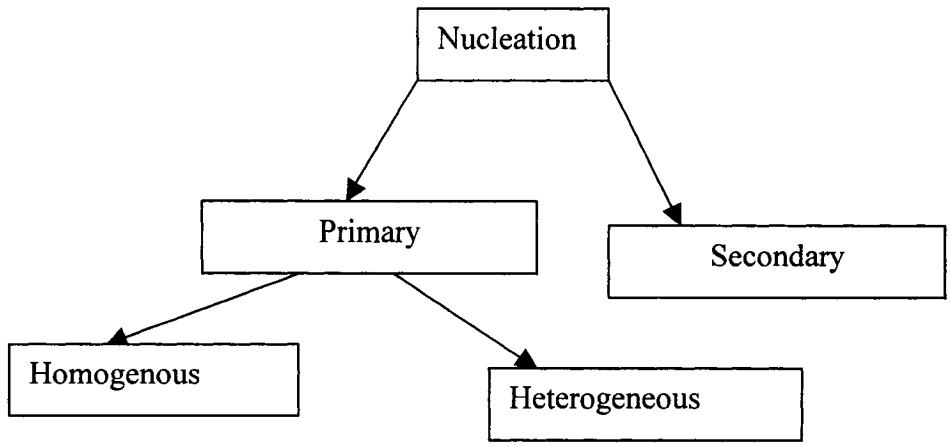


Figure 2.4: Classification of the nucleation process

Primary nucleation can be homogenous where nuclei are formed spontaneously in the absence of external stimuli or heterogeneously where impurities or foreign particles induce nuclei formation. Secondary nucleation is usually initiated by the inoculation of particles or seeds of the solute material to solution. It is quite difficult to identify perfectly if the nucleation occurring is primary or secondary.

2.1.2.1 Primary Homogenous Nucleation

The solid nuclei, embryos or seed crystals which act as crystallization centers must reach a minimum size for a nucleus to stabilize and to allow the

possibility for crystal growth. Any nuclei radius below this critical value continuously redissolves in solution. Therefore molecules must coagulate together and must orient into a fixed lattice structure. The mechanism to how this is achieved is unclear but it is likely based on a series of bimolecular collisions. For example, two molecules collide and then a third and a fourth combine to the growing nuclei. It is extremely unlikely that the critical nucleus is formed by several molecules combining in one stage. For this mechanism to occur a certain amount of energy is needed. The critical free energy ΔG is the energy requirement for the formation of stable nuclei and the initiation crystal growth.

2.1.2.2 Primary Heterogeneous Nucleation

The critical energy required to form a stable nucleus can be lowered by the presence of possible impurities or foreign bodies in solution. The foreign bodies must be active to lower the energy requirement. The lowered energy requirement is represented by Equation 2.3.

$$\Delta G' = \phi \Delta G \qquad (2.3)$$

where $\Delta G'$ is the critical free energy requirement for heterogeneous nucleation and ϕ is the energy correction factor, which must be less than or equal to 1. The magnitude of this correction factor depends on the interaction or wetting between the impurity particles and the crystalline solute. If there is no affinity between the impurity particles and the crystalline solute it can be stated that ϕ is equal to 1, i.e. impurities or foreign particles have no effect on nucleation kinetics. In this present thesis, Section 4.1.3 demonstrates this case where glass beads are added to solution and nucleation has not improved. If the solid impurities have a partial affinity with the crystalline solute the value of ϕ is between 0 and 1. The final possibility is if foreign particles demonstrate complete affinity where ϕ is equal to 0. No energy is required to create a stable nucleus or to promote crystal growth.

2.1.2.3 Secondary Nucleation

Typically, this phase of nucleation is induced by inoculating a solution with particles or seeds of the material to be crystallized. The size and amount of crystals added is important where a large amount of seeds provide a greater probability for contact and collision required for critical nucleus formation. If the concentration of seeds is too small the nucleation and growth of solute crystals will not occur.

2.2 Separation of Racemic Mixtures by Crystallization

Ternary solubility diagrams can be used to depict two types of racemic mixtures. The two common classifications for racemic mixtures are: conglomerates and racemic compounds. This Section explains each of these systems from a theoretical background utilizing ternary solubility diagram. The typical manner for ternary solubility diagram presentation is in molar composition. Appendix 1 gives a brief review of the use of ternary solubility diagrams.

2.2.1 Conglomerate Classification

A conglomerate system occurs when the D and L enantiomers crystallize separately, that is each enantiomer has it own distinct crystal structure. Thus when a conglomerate mixture crystallizes, the suspension contains a mixture of two types of crystals, the first type for the L enantiomer and the other for the D enantiomer. These crystals in suspension can be mechanically separated. This system represents 10 – 15 % of all racemic mixtures. Asparagine, threonine and glutamic acid are examples of conglomerates, described by Jacques *et al* (1981). The ternary solubility diagram for a general conglomerate system is illustrated in Figure 2.5.

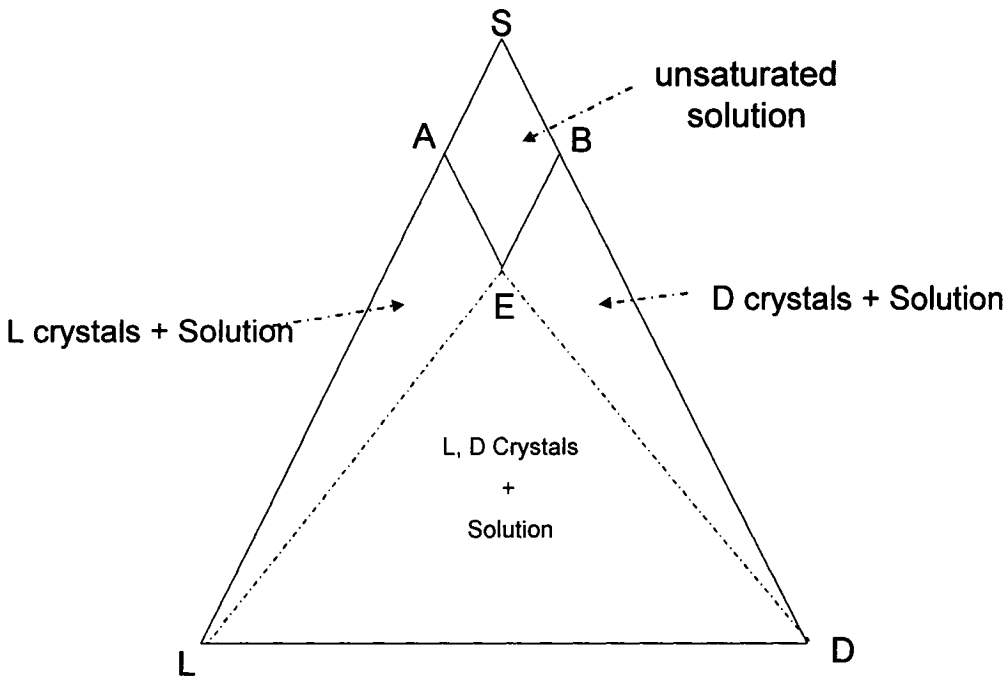


Figure 2.5: Schematic diagram of conglomerate ternary solubility diagram

This ternary solubility diagram shows the solubility lines (AEB) and phases that exist at a given temperature. There are four regions: 1) unsaturated solution, 2) D crystals and solution, (3) L crystals and solution and (4) L and D crystals and solution. In the above triangle the side SL represents the composition of L and solvent. Similarly, SD represents the composition of D and solvent and LD represents the mixture of L and D enantiomers. Point A represents the solubility of L, point B represents the solubility of D and point E is the solubility of the racemic mixture, all at a given temperature. A vertical line connected from the solvent apex (S) to the base of the triangle (LD), represents the locus of possible points having a 50 % L – 50 % D composition (all possible racemic mixtures).

The effect of temperature on ternary solubility lines is of prime importance for crystallization and it is demonstrated in Figure 2.6.

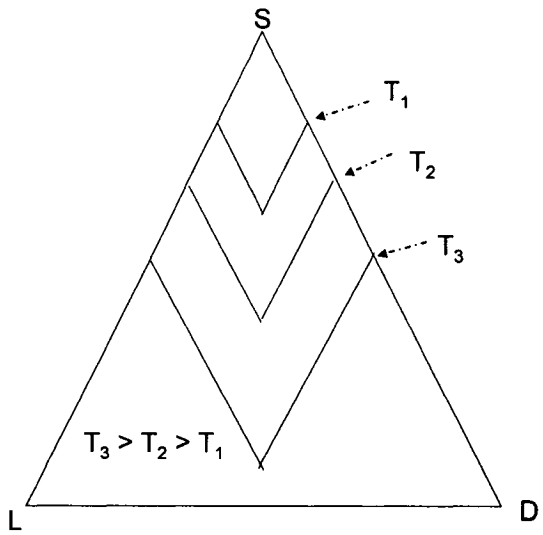


Figure 2.6: Effect of temperature on solubility lines for a conglomerate system

A decrease in solution temperature shifts the solubility line upwards, resulting in a decrease in D and L concentrations. The consequence of this temperature reduction is a solution in the state of supersaturation. It is possible to have a supersaturated solution without crystal formation. However, for all practical purposes it is difficult to have so few disturbances that crystallization is prevented. Knowledge of ternary solubility lines at different temperatures is of prime importance for identification of the state of solution (i.e.: saturated solution, unsaturated solution and supersaturated solution). The following description demonstrates how a conglomerate mixture can be separated by crystallization, (i.e. type of resolution process).

2.2.1.1 Conglomerate Resolution

The resolution of a conglomerate system can be achieved by two direct methods: 1) Spontaneous Resolution and 2) Resolution by Entrainment. Each resolution is explained with suitable ternary solubility diagrams demonstrating the process changes.

Both resolutions begin with the preparation of a racemic saturated solution at a high temperature T_2. An equal amount of the L and D enantiomers are dissolved in solution. The composition of this solution is at point P in Figure 2.7;

point P is on the solubility line at T_2 at the center of the diagram as the racemic mixture composition.

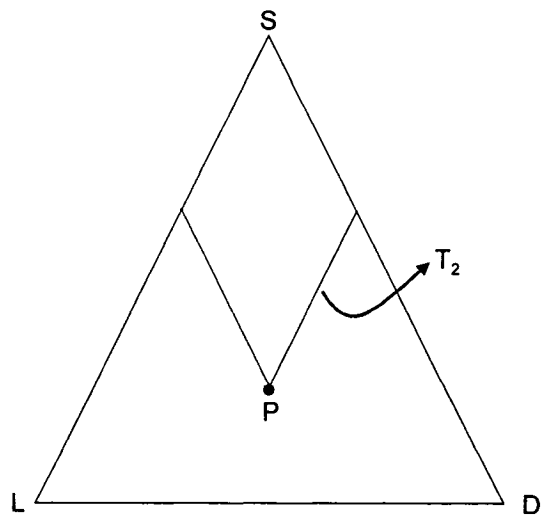


Figure 2.7: Initial saturated solution at composition P and temperatureT_2

This is followed by the reduction of temperature from T_2 to T_c as shown in Figure 2.8.

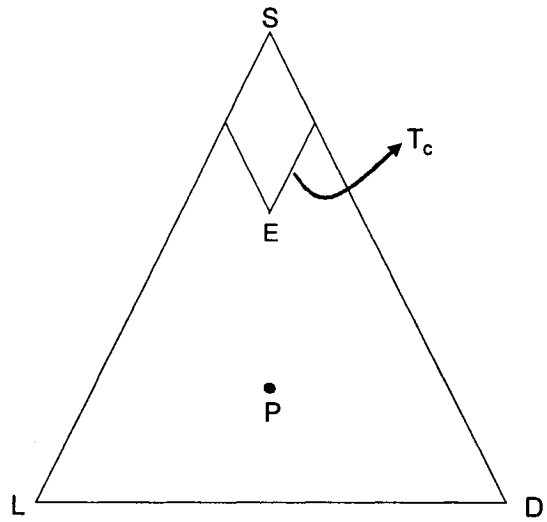


Figure 2.8: Supersaturated solution at composition P and temperatureT_c

The molar composition at point P has not changed but the solubility lines have shifted upwards. The solution is supersaturated with respect to both enantiomers,

where the extent of supersaturation is identical for each enantiomer. Therefore, the slightest agitation or disturbance may initiate nucleation and growth of each enantiomer. From a thermodynamics perspective the composition of solution eventually changes to the point E, the racemic mixture composition at T_c (crystallization temperature). The trajectory followed to reach point E determines the type of resolution.

A spontaneous resolution allows the racemic mixture solution to naturally crystallize with no crystal seed addition. Figure 2.9 demonstrates that a vertical line represents the trajectory followed to attain the equilibrium composition at T_c. This method has L and D enantiomers crystallizing from solution at the same rate. The time required to reach point E with no seed inoculation can be an extremely long period of time.

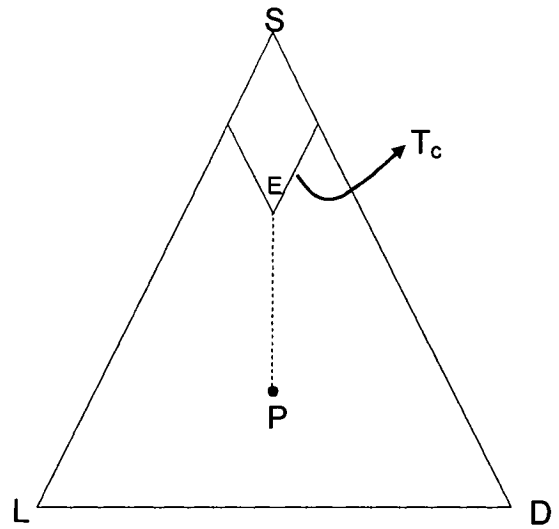


Figure 2.9: Crystallization trajectory with no seed inoculation (spontaneous resolution)

The resolution by entrainment utilizes the identical conditions except that seed crystals of the desired enantiomer are added to the saturated solution. This inoculation not only speeds up the kinetics of the system but also changes the trajectory to which point E is reached. The crystal surface of the desired enantiomer minimizes energy required for nucleation and encourages adhesion of the desired enantiomer from solution to the growing nucleus. Figure 2.10 describes the resultant curve where an ideal amount of L seeds are added to

favour the crystallization of L enantiomer. Along this trajectory the optical rotation is initially zero at point P, followed by a maximum positive rotation at point O (the first instance of D crystals) and finally a zero rotation at the point E. Typically, the optimal condition to halt the entrainment process is at point O to maximize the crystals of the L enantiomer and avoid any D crystals. The segment between P and O is linear and if extended it goes directly through the L apex. This demonstrates that only the L enantiomer is crystallizing.

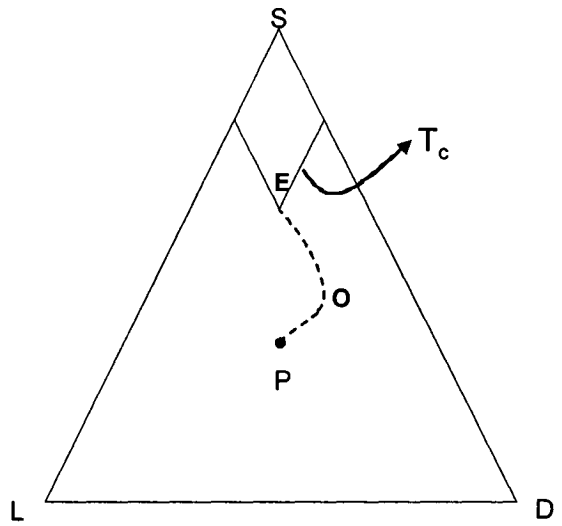


Figure 2.10: Crystallization trajectory with L enantiomer seeding (resolution by entrainment)

2.2.2 Racemic Compound Classification

The racemic compounds are more complicated classification of racemic mixture. During crystallization there is the formation of a definite double compound C. This compound consists of a solid mixture of L and D crystals where a separation is not physically possible. That is the L and D enantiomers form together in one crystal lattice. This system represents nearly 80 % of all racemic mixtures. Figure 2.11 demonstrates the 6 phases for a racemic compound system: (1) unsaturated solution, (2) D crystals and solution, (3) L crystals and solution, (4) C crystals and solution, (5) D and C crystals and

solution, (6) L and C crystals and solution. The solution is represented by a liquid mixture of all constituents (Jacques *et al* (1981)).

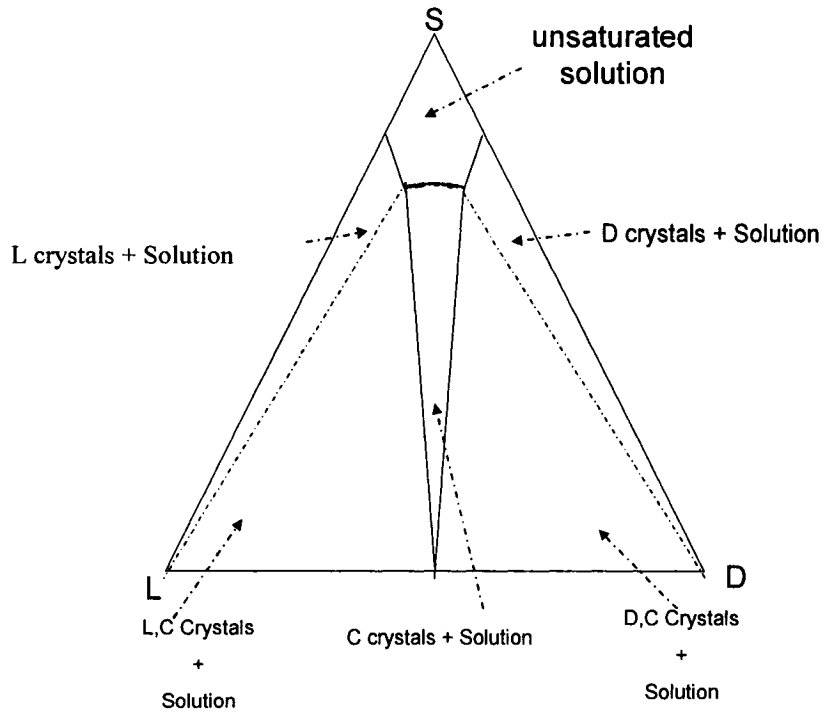


Figure 2.11: Schematic diagram of racemic compound ternary solubility diagram

Figure 2.12 shows the effect of temperature on the solubility lines for a racemic compound system. As temperature rises, the solubility lines shift downwards as is seen for the conglomerate system. However, a slightly different transition is followed, where a conglomerate system evolves at an elevated temperature. The double compound C is only stable at relatively low temperatures and if the temperature is too high, the compound C breaks up and a conglomerate system may evolve. However, it is difficult to raise the temperature without significant solvent evaporation.

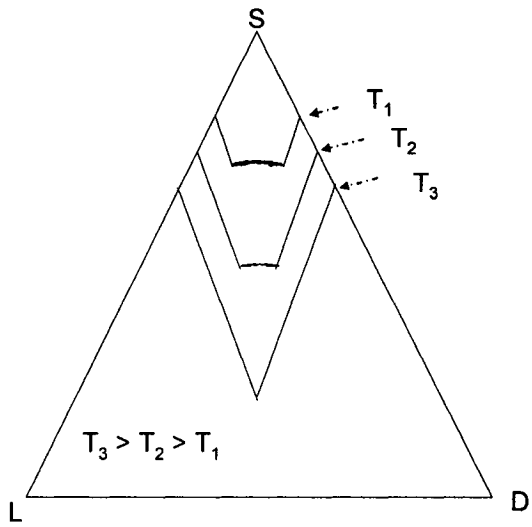


Figure 2.12: Effect of temperature on solubility lines for a racemic compound system

Direct resolution is not an applicable separation approach for the racemic compound system because a solid mixture is formed. The resolution usually involves the addition of a chiral reagent to the racemic mixture solution. This forms two diastereoisomer salts which have different solubilities. The different physical properties allow conventional separation techniques including direct resolutions. A detailed description for the selection of appropriate chiral reagent is given by Collet (1999).

2.3 Literature on Resolution of Conglomerates

The following Section details the general history of racemic mixture separations by direct crystallization methods. It also cites specific work which deals with glutamic acid resolutions.

2.3.1 Resolution of Racemic Mixtures

The detailed list of work below explains common methods for resolutions of conglomerate systems. The following examples include salts, amino acids and pharmaceuticals.

The first resolution of a racemic mixture was achieved by Pasteur in 1848, summarized by Secor (1962). He showed that direct spontaneous resolution (Section 2.2.1.1) of sodium ammonium DL-tartrate is possible. Below 27 °C it is possible to mechanically separate the crystals of L and D enantiomers. The visual appearance of each enantiomer could be distinguished with use of a microscope and careful sorting is done. He stated that 27 °C is a transition temperature; any higher temperature would present the crystallization of a racemic compound which could not be separated into its pure enantiomer components. The manual sorting is an extremely tedious effort that is not commonly employed.

The work of Gernez in 1867, Ostromisslensky in 1908 and Gero in 1953 are also summarized by Secor (1962). Gernez work showed the first example of direct resolution by an entrainment process (Section 2.2.1.1). There are no quantitative numbers given, but the results demonstrated that the addition of sodium ammonium L tartrate seeds to a racemic mixture solution promoted the crystallization of the sodium ammonium L tartrate enantiomer.

Ostromisslensky used a similar seeding approach to induce crystallization of the same salt. However, he did not add crystals of either pure enantiomer to the solution. He attempted to add crystals of an optically active substance which would preferentially cause the deposition of one enantiomer. It turned out that the addition of L-asparagine to solution caused the crystallization of sodium ammonium L-tartrate. This demonstrates that the seed addition is not totally dependent on the molecule and has a relation to the crystalline structure.

Gero demonstrated the crystallization of a partially resolved DL adrenaline mixture which initially consisted of 55 % L adrenaline and 45 % D adrenaline, i.e. not a racemic mixture. The technique used to induce crystallization was partial neutralization at constant temperature with no seed addition. The precipitated solid consisted of 85 % L adrenaline and 15 % D adrenaline.

The resolution of DL-praziquantel was achieved by Lim (1995). Praziquantel is a drug used to treat parasitic infections. However, only the L enantiomer is desired because the D enantiomer acts as a toxin. The separation

is achieved by coupling a continuous chromatography process with a multi-stage crystallization process. The results show that a great degree of separation of racemic praziquantel can be achieved by continuous chromatography. However it is not possible to attain a product with purity greater than 97.5 % L. Thus, the raffinate stream from the chromatography process was sent to the multi-stage crystallization process to obtain pure L- praziquantel. Supersaturation was induced by solvent evaporation and crystallization was achieved by the addition of a few milligrams of pure L-praziquantel to the L - enriched solution. The final results yielded pure L-praziquantel with 80 % recovery.

2.3.2 History for the Resolution of Glutamic Acid

This Section details several works which address the resolution of racemic glutamic acid. It identifies the attributes and obstacles for each work and demonstrates how L glutamic acid was resolved. A common feature of the work described below involves the addition of a mineral acid or an equivalent base to increase the initial amount of glutamic acid which the solvent can dissolve.

The work of Ogawa and Akashi (1960) demonstrates that the L glutamic acid enantiomer could be isolated from a mixture of DL glutamic acid dissolved in aqueous HCL solution. The work used partial neutralization and temperature reduction as separate techniques to induce crystallization or supersaturation. The first example of this work used partial neutralization where a 10 % HCL solution is saturated with DL glutamic acid at 30 °C. The pH of this solution ranged from 1.5 – 2.5. The addition of NaOH to the solution increased the pH to the isoelectric point for glutamic acid at 3.2. At the isoelectric point glutamic acid has its lowest solubility at a fixed temperature. Concurrently with the NaOH addition; 3.5 g of L glutamic acid seeds were inoculated to the solution. This mixture is stirred for 5 hours at 30 °C and then filtered. The resulting solid was 99 % pure L glutamic acid and the yield was 8 % from the total L glutamic acid in the initial solution. The second example of this work utilized a saturated solution of DL glutamic acid in 6.5 % aqueous HCL at 45 °C. This solution was seeded with 1.5 g of L glutamic acid crystals and was cooled to 30 °C in 3 hour with constant

stirring. The resulting crop was 6 % pure L glutamic acid crystals from the total amount initially present. A positive feature of this work is that pure L crystals were attained, unfortunately in long periods of time and in small quantities.

The work of Purvis, (1961) improves significantly the time required to isolate pure L glutamic acid. The major difference with this work is that a greater concentration difference is used and L glutamic acid.HCL crystals are inoculated to solution. The procedure for attainment of pure L glutamic acid begins with the preparation of saturated DL glutamic acid at 60 °C in aqueous 10 % HCL solution. This is followed by the reduction of the temperature to 30 °C and inoculation of solution with L glutamic acid.HCL crystals. The crystallization time at 30 °C is between 1 to 60 minutes depending on experimental conditions. The resulting product is filtered and pure L glutamic acid.HCL crystals are obtained. The L glutamic acid.HCL crystals are then dissolved in water and neutralized with the addition of 2. 5 N NaOH and crystallized in an ice box for 24 hours. The final product consists of pure L glutamic acid crystals. Therefore, this work uses cooling as the primary technique to separate the D and L glutamic acid.HCL enantiomers from each other. The crystallization operating time is approximately 30 minutes. However, partial neutralization and cooling in an ice box is a required purification step to remove the HCL from the solid crystals of L glutamic acid.HCL. The best results of this work give 99 % pure L glutamic acid crystals with 32 % yield of available L glutamic acid from original solution.

Balmat, (1953) took a slightly different approach to the resolution of racemic glutamic acid. He resolved DL glutamic acid in aqueous sodium hydroxide and found that an ideal amount of NaOH was 0.72 to 0.74 moles per mole of DL glutamic acid. Below this range high purity L glutamic acid crystals were attained but the yield was too low. A specific condition for his work had 0.738 moles NaOH/1 mole DL glutamic acid and 27.8 % pure L glutamic acid crystals were obtained in 15 minutes. This method used a suitable cooling and neutralization scheme similar to that of Purvis to attain pure L glutamic acid crystals.

The expected contribution of this master research is to attain a high yield of pure L glutamic acid crystals without the adjustment of solution pH. That is there is no addition of mineral acid or bases to increase solubility. Additionally, this work will illustrate how initial supersaturation and crystallization temperature influence the quality of a resolution. The rapid cooling of solution (Section 3.3.2) is of prime importance for improving yield of the desired crystals. The rationale used for the glutamic acid resolution can be used for any conglomerate system.

Chapter 3 MATERIAL AND METHODS

In this Chapter the experimental design and procedures for the solubility and crystallization experiments are described. It makes reference to the chemicals and equipment needed for practical operation. It also explains the analytical method (HPLC) used to quantify experimental results.

3.1 Materials

Both L and D glutamic acid was purchased from Fisher Scientific and their physical properties are given in Table 3.1. The physical properties were attained from the product specification data sheet and were identical for the L and D enantiomers.

Table 3.1: Physical properties for L and D glutamic acid – from Fisher Scientific

Appearance	*Molecular Wt*	Melting Pt	Purity %	Solubility (25 °C)
White powder	147.13	205 °C	› 99	8.64 g/L (aq. sol)

The only difference, is in their optical rotation, where L glutamic acid has a + 32 ° rotation and D glutamic acid has a - 32 °. To ensure no moisture content the glutamic acid powder was dried in a 60 °C oven for 8 hours. The amount of moisture removed for L glutamic acid is 0.3 % of the total weight and 0.8% for D glutamic acid. This demonstrates that the powder attained from Fisher had minimal moisture content.

The dried glutamic acid crystals were not modified or screened and used directly in the solubility and crystallization experiments. The crystal size distribution was determined for L glutamic acid because it was the seed material used. The crystal size distribution was determined using a Hydro 2000S particle size analyzer in conjunction with the Mastersizer 2000 software. This particle size analyzer operates by sending a laser beam through a suspension of glutamic acid in methanol. Glutamic acid is practically insoluble in this organic solvent and the particles scatter the light beam in many directions. There are 40

photodiode detectors located at different positions which measure the light intensity. This intensity at each position is mathematically related to a particular particle size. Figure 3.1 shows the crystal size distribution for L glutamic acid averaged over 4 runs.

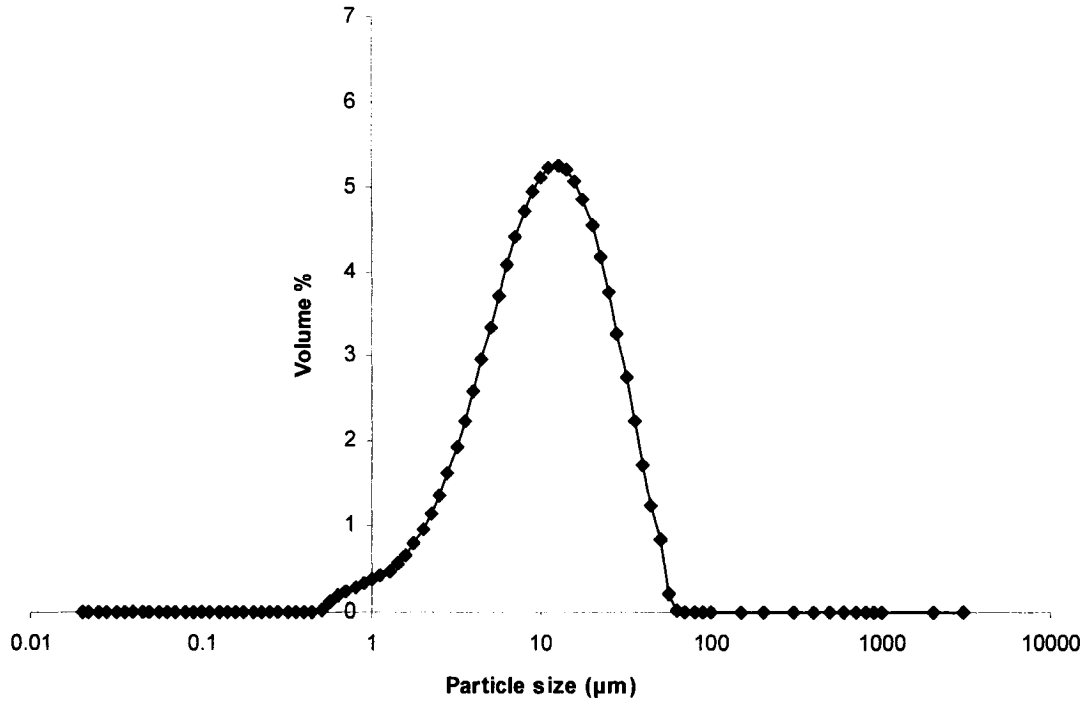


Figure 3.1: Particle size distribution for L glutamic acid crystals used as seeds for crystallization.

The median particle size was 10.0 µm which represents 5.2 % (volume %) of the total particles present. The determination of this distribution demonstrated that the L glutamic acid crystals which were added as seeds had a highly reproducible particle size distribution.

The water used in all experimental and analytical work was deionized and the techniques and instruments used for its purification are given in Appendix 2.

3.2 Separation and analysis of L and D enantiomers

Samples containing the two isomers were analyzed using an Agilent 1100 series HPLC (high performance liquid chromatography) equipped with a UV absorbance detector and an automatic sampler. The separation of the

enantiomers was accomplished using a specialized chirobotic T column from Advanced Separation Technologies Inc. The column was packed with a suitable chiral material, teicoplanin, which contains eight chiral carbon centers. This allowed for the separation and identification of each enantiomer because each had different binding affinities to the chiral packing. More specifically, the hydrogen bonding and ionic interactions were lower for the L enantiomer to the teicoplanin than was the D enantiomer to the teicoplanin. Figure 3.2, is a typical chromatograph, which demonstrates the separation of the L and D glutamic acid enantiomers with the above column. There was a void volume peak due to water around three minutes.

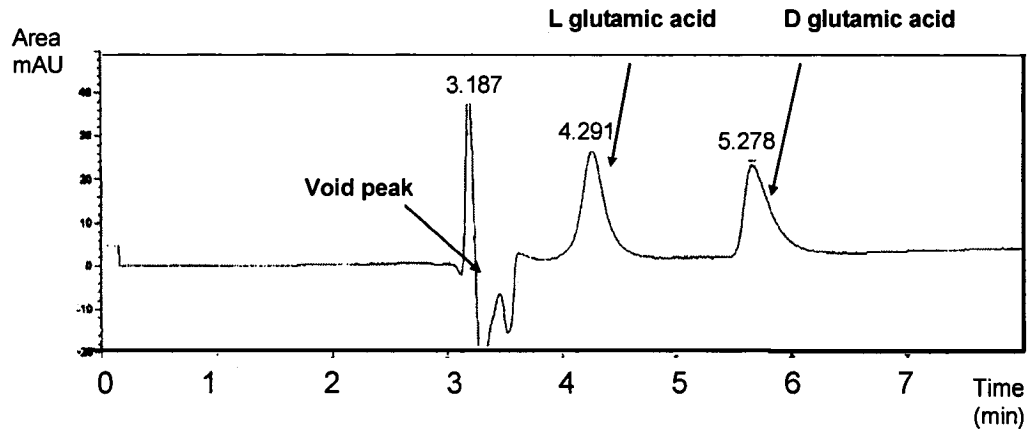


Figure 3.2: HPLC chromatograph for glutamic acid enantiomer separation and identification

The HPLC analysis conditions for glutamic acid were determined by taking into account the physical properties of glutamic acid and trial and error. For example, glutamic acid is an acidic amino acid that requires the use of a buffered solution and an organic solvent for measurement. The buffered solution suppresses the additional carboxylic group on the amino acid (lower pH), and the organic solvent increases the residence time so that the glutamic acid peaks do not overlap the void peaks. The control of temperature was at 20 °C because it shifted the L and D peaks further away from the void peaks without broadening of

the glutamic acid peaks. The most appropriate conditions for the identification and separation of glutamic acid enantiomers are shown in Table 3.2

Table 3.2: Conditions required for glutamic acid separation and identification

Solvent Composition	80 % Methanol (HPLC grade) and 20 % Ammonium Acetate buffer (pH= 4.08)
Flow rate	0.9 ml/min
Temperature	20 °C
Sample Injection Volume	2 μl
UV Absorbance Wavelength	210 NM

The buffered solution was prepared with the titration of a suitable amount of acetic acid to a dilute 10 mmol solution of ammonium acetate. The buffered solution is filtered to ensure that no solid particulates travel through the chiral column.

3.2.1 HPLC Calibration

The calibration was performed in a concentration range of 0.5 g/L to 3 g/L of glutamic acid. The selection of this range was based on two factors:
1) Identification of the lowest concentration where glutamic acid can be accurately measured, i.e. the peak area of glutamic acid which was significantly larger than the chromatographs baseline noise.
2) Determination of the maximum concentration where there was no glutamic acid crystallization.

The lower limit was found by dissolving known small amounts of glutamic acid in water and identifying the resulting peaks. The lowest detectable concentration of glutamic acid is 0.5 g/L. The upper limit was based on the solubility value at the analysis temperature of 20 °C, which was 7.17 g/L for each enantiomer. To ensure no chance of crystallization during the analysis it

was decided to set the maximum concentration to 3 g/L. Figure 3.3 and 3.4 represent the calibration curves for L and D glutamic acid, respectively.

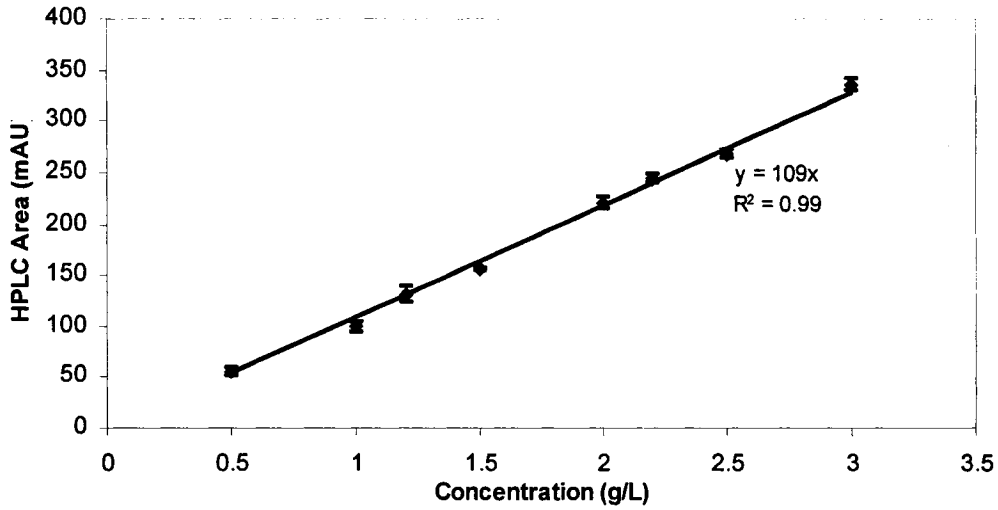


Figure 3.3 HPLC calibration for L glutamic acid at a analytical temperature of 20 °C

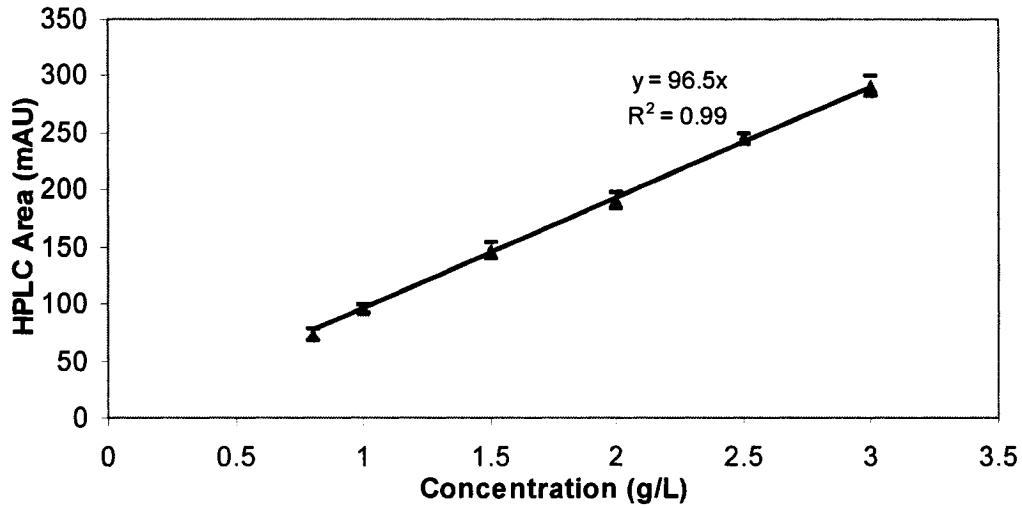


Figure 3.4 HPLC calibration for D glutamic acid at an analytical temperature of 20 °C

Figure 3.3 and 3.4 show a linear relationship between HPLC area and concentration for each glutamic acid enantiomer. Good reproducibility was demonstrated for both enantiomers by the standard deviation error bars above and below the average points. The major difference lies in the slope of the line, where slope of L glutamic acid is 109 (mAU/ (g/L)) and slope of D glutamic acid is 96.5 (mAU/ (g/L)). These two slopes were expected to be identical because the physical properties of each enantiomer were the same. It is likely that the slope difference was due to the different binding energy between the individual enantiomer and the chiral stationary phase. There is a greater binding energy for the D enantiomer because its peak elutes second. Although, this binding energy difference causes the desired separation, it also causes the D enantiomer to be more strongly bound to the stationary phase. Thus delaying its elution time and resulting in a smaller and broadened peak, as shown in Figure 3.2. This trailing or broadening was due to the slower mass transfer between the mobile phase and the stationary phase. The calibration for each enantiomer was verified for each new experiment performed by preparing a standard of suitable concentration.

3.3 Experimental Equipment

The two experimental apparatuses designed for this research were for solubility and crystallization experiments. The details of each of the experiments are given below.

3.3.1 Solubility Experiment

Experiments were carried to determine the solubility of pure L glutamic acid, pure D glutamic acid and a racemic mixture with water as the solvent. The temperature range evaluated was from 20 to 70 ^{o}C, the results of these experiments are shown in Section 4.1.1. Two experimental methods for solubility experiments were used as described by Mullin (2001):

1) The undersaturated solution approach where the solubility determination is based on the dissolution process. Solid particles of L, D or the racemic mixture in 10 % excess were added to water at room temperature. The excess amount added is an approximate value which was based on solubility data of Henry (1963). The mixture was then heated up to the desired solubility temperature where the mixture of solute and solvent was allowed to equilibrate for 1 to 2 days. At the end of the equilibrium period there were still undissolved crystals in suspension.

2) The oversaturated solution approach involves a process of crystallization rather than dissolution. A suitable amount of the solute was added to water and the mixture was heated to a temperature above the target equilibrium temperature to dissolve all crystals. The temperature was then lowered to the equilibrium temperature and crystallization of the solute from solution occurred. This mixture is allowed to equilibrate for 1 to 2 days or until the concentration of solution remains the same.

Experiments at low temperatures for both methods agreed quite well; however the majority of solubility experiments were carried out using the undersaturated solution approach. This method is simple and minimizes water evaporation.

The apparatus for solubility experiments involves a 250 ml round bottom flask which was mixed with a teflon stirrer that is attached to an rpm controlled motor with a glass rod. The round bottom flask is immersed in a constant temperature water bath. Figure 3.5 is a schematic diagram of the solubility set-up.

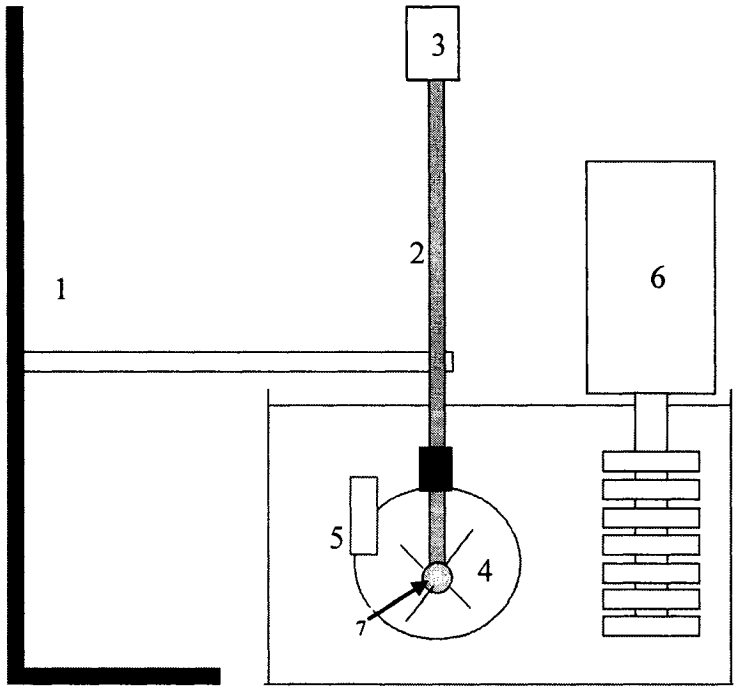


Figure 3.5: Solubility experiment set-up, (1) stand and clamp, (2) glass rod, (3) rpm controlled motor, (4) round bottom flask, (5) sample input port, (6) water bath temperature control, (7) teflon stirrer

A typical experiment starts by introducing the desired amount of glutamic acid to a clean and dry round bottom flask, which was clamped in the water bath. The glutamic acid was added to the round bottom flask by washing through a solid funnel with the appropriate amount of deionized water. The funnel was placed in the sample input port and the addition was done at room temperature. It is important that no glutamic acid accumulates or adheres on the wall of the flask. It is necessary to lubricate the section of the glass rod which contacts the round bottom flask to avoid frictional stresses. Typical vacuum grease is used as the lubricant. The rpm controlled motor was then turned on and the stirrer began to rotate at 200 rpm. This speed did not present significant frictional stresses and was used for extended times. The temperature was then heated to the desired equilibrium temperature with the water bath temperature control.

The sampling was done by transferring 10 ml of solution which was filtered and diluted to prevent crystallization at room temperature, with the use a syringe and pipette. The magnitude of dilution was determined based on the range of concentration which can be adequately detected with the HPLC, (see Section 3.2.1). The samples were taken over a two day period to assure the solution had reached equilibrium. All samples were then analyzed to see how long it took for a stable concentration to be attained. The assumption of ideal mixing is reasonable due to the mixing speed (200 rpm) of the stirrer and the relatively small 250 ml volume of the round bottom flask. Typically 15 hours are required for glutamic acid to reach a stable concentration.

3.3.2 Crystallization Experiment

A typical crystallization experiment consisted of two steps: (1) Preparation of the saturated solution and (2) Crystallization from the supersaturated solution. In the first step, a saturated solution was prepared at a high temperature. The initiation of crystallization occurred by rapidly reducing the saturated solution temperature to that of the crystallization temperature. The behaviour was attempted to assimilate closely with instantaneous temperature reduction as seen in Figure 3.6. This was achieved by cooling the solution in a small 10 ml test tube with a large surface area to volume ratio.

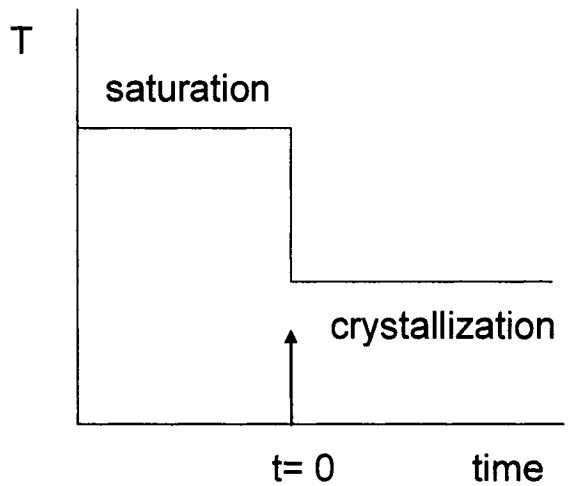


Figure 3.6: Instantaneous temperature reduction

The set-up for crystallization experiments consist of a 500 ml Erlenmeyer flask, several 10 ml test tubes, 2 temperature controlled water baths and a mechanical shaker. The crystallization set-up is depicted in Figure 3.7.

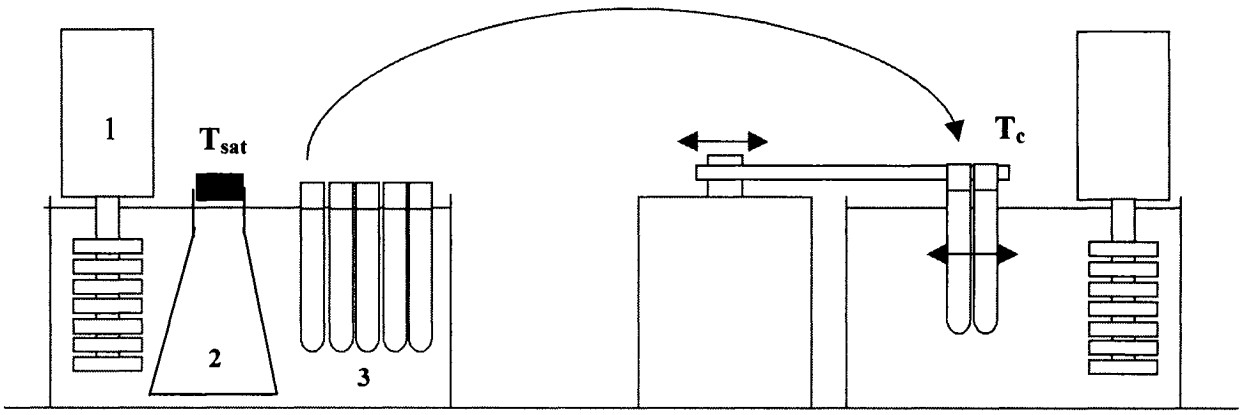


Figure 3.7: Crystallization experiment set-up, (1) high temperature saturation bath, (2) 500 ml Erlenmeyer flask, (3) 10 ml test tubes, (4) mechanical shaker, (5) low temperature crystallization bath

The experiment began with the preparation of a saturated solution of racemic glutamic acid at a given temperature. The solution was prepared by adding an equal amount of L and D glutamic acid with a suitable amount of water to an Erlenmeyer flask. This mixture was dissolved on a hot plate with a magnetic stirrer at a temperature above the saturation temperature to speed up the time required for complete dissolution. Once there were no crystals the flask was weighed and corrected for water which evaporated during the heating of solution. The flask was then clamped in the high temperature saturation bath which had a temperature equal to the saturation temperature for the specific concentration of glutamic acid. The flask was allowed to settle at the saturation temperature for 1 hour. A thermocouple was used to ensure that the temperature of the solution was at the saturation temperature. Once the temperature was reached the contents of the Erlenmeyer flask were transferred to several small 10 ml test tubes with a 10 ml syringe. The test tube rack was submerged in the water bath to ensure no temperature gradients along the test tube axis. At this stage there were several test tubes which represented an independent

crystallization system. Each test tube was a saturated solution which was maintained at the saturation temperature.

The first test tube was sampled to confirm that the initial saturated concentration was accurate. Each of the other test tubes were taken from the high temperature saturation bath and inoculated with L glutamic acid seeds ($[L_s]$ were 2 g/L, 7 g/L and 10 g/L) and then shaken in the low temperature crystallization bath. A stop watch was started once the mechanical shaker was turned on and the watch was stopped once the desired crystallization time had been reached. After suitable processing each test tube was sampled by pipetting a volume which was filtered and diluted in a 10 or 25 ml volumetric flask. The choice of processing conditions such as initial supersaturation (S^o), L glutamic acid seed concentration $[L_s]$ and crystallization time (t_c) are explained in Chapter 4. Based on the experimental results it was revealed that a robust and reproducible experimental procedure has been established, Section 4.2.1.

This experimental set-up has several advantages, the cooling time required to reach the crystallization temperature (t_c) was quite fast because of the large test tube surface area to volume ratio. The time required to reach 25 °C is at most 1 minute. Additionally, if an experimental error occurs (contamination, spillage or breakage) it only affects the results of one particular test tube system. The limitation of the set-up is that the maximum amount of seeds to be added to the test tube is 10 g/L of L glutamic acid. This is the maximum amount of seeds which could be added quickly without spilling. Also the mechanical shaker had a maximum time of operation of 1 hour; where any longer time will cause extreme heating and possible mechanical failures. This limit was a result of a modification to the mechanical shaker where an arm was attached to hold the test tube in place.

Chapter 4 RESULTS & DISCUSSION

This Chapter is divided into two main parts: The first part gives results which describe experiments with solutions consisting of water and one of the individual enantiomers (D or L). The solubility results of each enantiomer were used to confirm literature data. The crystallization rates for each enantiomer can be used for the design of resolution experiments because the addition of one enantiomer to solution does not affect the other enantiomers solubility. The second part describes the results of the actual resolution experiments where L glutamic acid is crystallized preferentially from a racemic mixture solution. The experimental conditions which present the highest yield of pure L glutamic acid crystals are given. Additionally, this Chapter relates experimental trends to mechanisms of nucleation and growth for crystallization.

4.1 Preliminary Results

4.1.1 Solubility Results

This Section demonstrates the results for solubility experiments with both of the pure enantiomers dissolved in water as a function of temperature. The experimental solubility data for L and D glutamic acid are the same and confirm the data of a literature reference Henry (1963), as shown in Figure 4.1.

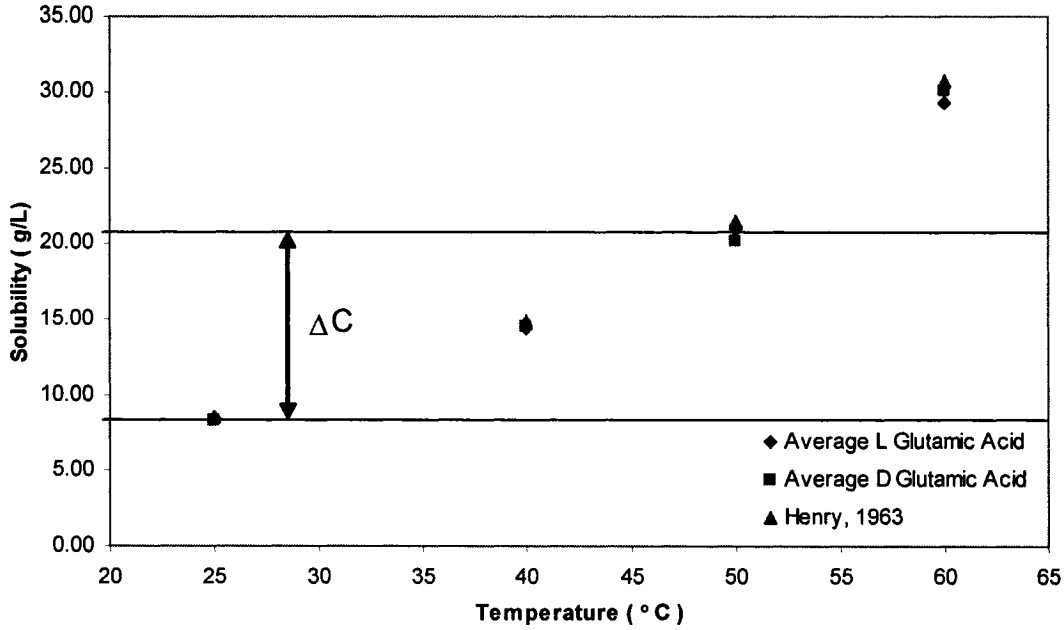


Figure 4.1: L and D glutamic acid solubility data as a function of temperature for experimental and literature data – constant pH (3.2-3.3) and maximum standard deviation of 7 %.

These results show that the glutamic acid solubility increases with temperature and that it is a moderately soluble amino acid at a pH of 3.2 - 3.3, which is its isoelectric point. The low solubility of glutamic acid is explained by its molecular structure and chemical properties. The ability of water to dissolve an amino acid can be predicted by the polarity of the amino acid side chain. The side chain group consists of a two-chain alkyl group plus a carboxylic acid. The alkyl group increases the non-polar behaviour, whereas the carboxylic acid increases side chain polarity, (Zumdahl (1995)). Most amino acids have a solubility of 30 g/L at 25 °C which is more than triple that of glutamic acid.

The main use of such solubility data is to establish an appropriate concentration driving force to induce supersaturation from solution for a crystallization experiment, as described in Section 2.1. In the first step of the crystallization process, the solute is dissolved at a high temperature to form a saturated solution. A suitable driving force is shown in Figure 4.1 starting with a saturated solution at 50 °C cooled to 25 °C. The saturated

concentration of L glutamic acid at 50 °C is 21 g/L and 8.64 g/L at 25 °C, resulting in a concentration difference (ΔC) of 12.34 g/L. The initial supersaturation S^o for this example is equivalent to 2.50, Equation 2.2 in Section 2.1.

Knowledge of accurate solubility data for a range of temperatures is critical, otherwise when preparing a solution there may be undesired crystals initially present. For example, if the reference solubility data is 30 g/L at 60 °C but in actuality it is only 27 g/L, this would result in 3 g/L of crystals in solution. The crystals would consist of a mixture the L and D enatiomers and thus when inducing crystallization the solution would crystallize racemically, even if pure enantiomer crystals were added as seeds. Therefore, there would be no possibility for the desired enantiomer to crystallize preferentially.

4.1.2 L Glutamic Crystallization with No Seed Addition

The following are the results of the initial experiments used to determine the kinetics or rate of crystallization for glutamic acid. In this experiment the time required for the crystallization of L glutamic acid from solution with no seeds is determined. A saturated solution of L glutamic acid at a temperature of 50 °C (initial concentration C^o = 20 g/L) is cooled to a crystallization temperature (T_c) of 25 °C. There is no D glutamic acid present in this experiment. Figure 4.2 shows there is no crystallization in the first 60 minutes of operation. This is the maximum time of operation for the shaker as mentioned in Section 3.3.2.

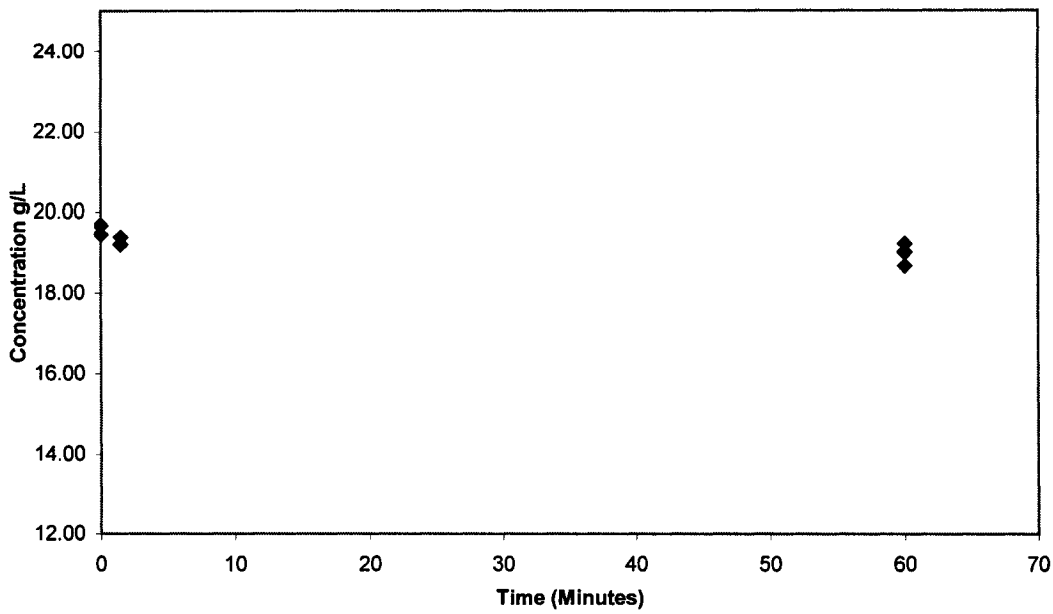


Figure 4.2: L glutamic acid crystallization at 25 °C from a 20 g/L saturated solution of L glutamic acid with no seed inoculation.

However, when the solution is left with no agitation for 10 hours there is significant crystallization as the concentration of L glutamic acid reduced from 20 g/L to 13 g/L. The key observation from these results is that there is not significant crystal growth in the first hour; and in turn there is minimal homogenous nucleation, where any crystal surface formed is redissolved in water before it could agglomerate to a critical radius.

4.1.3 Effect of Glass Bead Addition on Crystallization Rates

This Section demonstrates how the kinetics of glutamic acid crystallization is influenced by the addition of glass beads of 1 mm spherical diameter. The objective of this experiment is to determine if any material can induce heterogeneous nucleation. The conditions of this experiment are at an initial saturated concentration (C^o) of 21.6 g/L and crystallization temperature of 25 °C. The concentrations of the inert glass beads added to solution are 4, 8 and 20 g/L. Figure 4.3 demonstrates the

variation of the concentration of L glutamic acid with time and different glass bead concentrations.

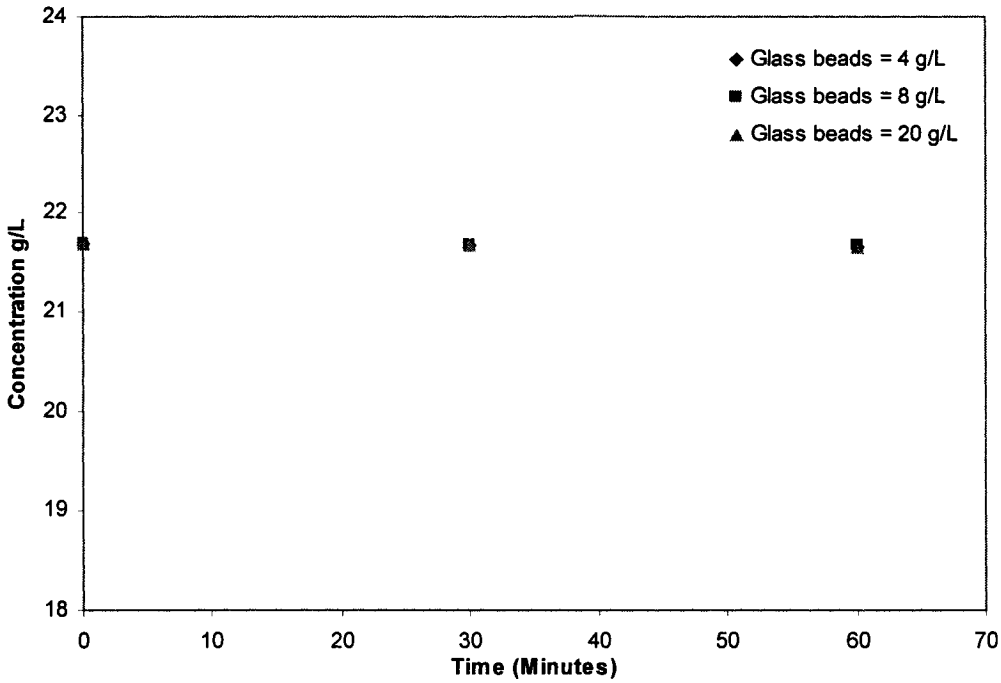


Figure 4.3: Effect of glass bead addition on the crystallization of L glutamic acid with an initial concentration C° = 21.6 g/L and a crystallization temperature T_c = 25 °C

Regardless of the bead concentration, there is no improvement in crystallization rate as compared to that of the experiments with no seeds inoculations, see Section 4.1.2. The initial concentration does not change over the first hour of operation. In the subsequent Sections it is shown that the crystalline structure of the pure enantiomer added as seeds is of prime importance for the enantiomer separation.

4.1.4 Crystallization of L Glutamic Acid – With L Seeds

In Section 4.1.2 and 4.1.3 it is shown that primary nucleation (homogenous or heterogeneous) for L glutamic acid is extremely slow. Therefore seed crystals of the L glutamic acid are introduced to solution to increase the rate by obtaining crystals within a reasonable period of time. Although in this experiment only L glutamic acid is used, similar

experiments with the D enantiomer show that the results are applicable to D glutamic acid with D glutamic acid seeds. In addition from these results an estimate of the crystallization rate for L glutamic acid can be obtained and used for resolution experiments.

Figure 4.4 shows the results of the experiment where a 2 g/L seed concentration of L glutamic acid is added to a 22 g/L saturated solution of L glutamic acid. The solution is shaken at varying speeds in a 25 °C crystallization bath, as described in Section 3.3.2.

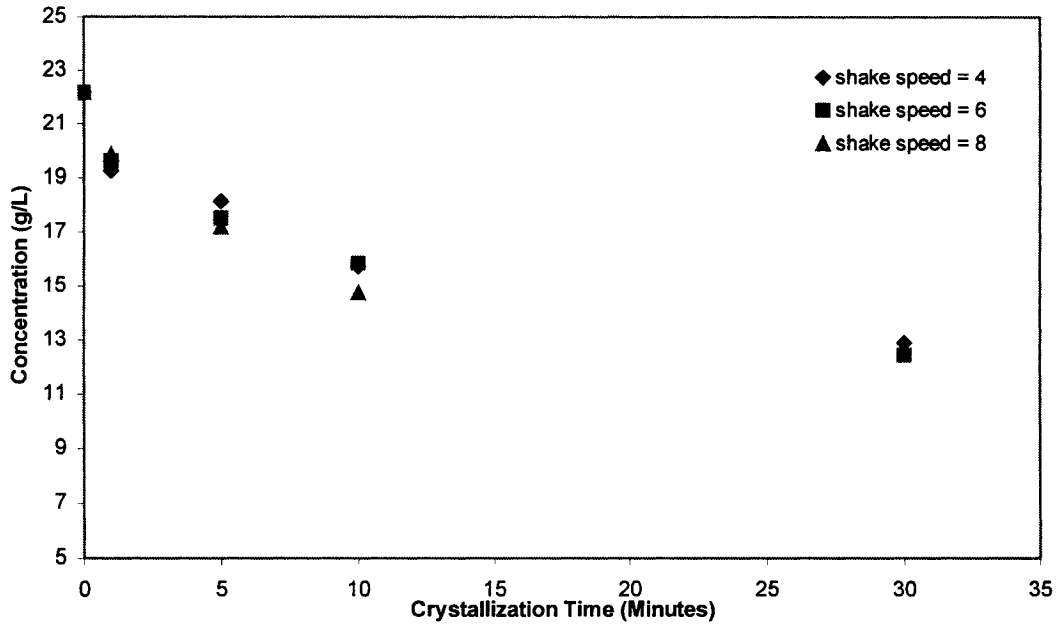


Figure 4.4: L glutamic acid crystallization at 25 °C from a 22 g/L saturated solution of L glutamic acid with the addition of L glutamic acid seeds with a concentration of 2 g/L.

The results demonstrate that a significant amount of L glutamic acid crystallizes in the first hour of operation with minimal influence of shaking speed. The crystallization is much faster than the case without seed additions (shown in Figure 4.2) because the L glutamic acid seeds present a crystal surface suspension which promotes nucleation at a lower energy level. Primary nucleation is bypassed and secondary nucleation is encouraged due to presence of solute material. The exact mechanism for crystal growth is unknown; however the seed crystals attract solute

molecules from solution to adhere and agglomerate with the growing crystal lattice (Section 2.1.2). The addition of seeds reduces the crystallization time and will likely promote L glutamic acid to crystallize preferentially from a racemic mixture, as is detailed below.

4.2 Resolution Experiments

This Section describes a detailed analysis of the crystallization and separation of L glutamic acid from a racemic mixture solution. In each experiment the initial solution contains an equal amount of L and D glutamic acid dissolved in water. Several conditions for experimentation are studied to find the conditions which present a suitable resolution. The parameters studied are: Initial concentration C^o or initial supersaturation S^o, crystallization temperature T_c, crystallization time t_c and L glutamic acid seed concentration $[L_s]$.

The two parameters used to quantify the quality of a resolution are L glutamic acid yield (Y_L) and L glutamic acid purity (P_L). The yield is the percentage of L glutamic acid which crystallized from the initial amount present in solution. The purity is the percentage of L glutamic acid in the crystalline solid product. If only L glutamic acid is present in the crystalline product the purity is 100 %. Equations 4.1 and 4.2 give the appropriate definition for yield and purity. Similar equations and notations are used for the calculation of yield and purity for the D enantiomer.

$$Y_L\ \% = \frac{[(S^o - S)]}{[(S^o - 1)]}\ \text{X}\ 100 \qquad (4.1)$$

$$P_L\ \% = \frac{[(Y_L)]}{[(Y_L + Y_D)]}\ \text{X}\ 100 \qquad (4.2)$$

The main experiments used to determine conditions which favour L glutamic acid separation are given in Table 4.1. In these experiments the

crystallization rates of L and D glutamic acid are evaluated at various initial solution concentrations, seed concentrations and crystallization temperatures.

The effect of seed concentration is analyzed at a fixed initial solution concentration and crystallization temperature. Experiments 1a, 1b and 1c compare the rate of concentration decay for an initial concentration of 31.0 g/L and experiments 2a, 2b and 2c compare the rate for an initial concentration of 21.6 g/L. These experiments evaluate the variation of 2, 7 and 10 g/L of seeds at a crystallization temperature of 25 °C. Experiment 3 determines the rate of crystallization of L and D glutamic acid at the lowest initial concentration (14.0 g/L) and a 25 °C crystallization temperature. Only the highest seed concentration for L glutamic acid (10 g/L) is added to solution because of the small concentration difference (driving force). The other seed concentrations would present a very low crystallization rate. Experiment 4 evaluates the effect of changing crystallization temperature, where the other conditions remain fixed. The conditions of experiment 4 are identical to 2c except that the crystallization temperature is 35 °C.

Table 4.1: Resolution experiments at different conditions: (C°, S°, T_c and $[L_s]$)

Experiment #	C° (g/L)	S°	T_c °C	$[L_s]$ (g/L)
1a	31.0	3.60	25	2
1b	31.0	3.60	25	7
1c	31.0	3.60	25	10
2a	21.6	2.50	25	2
2b	21.6	2.50	25	7
2c	21.6	2.50	25	10
3	14.0	1.60	25	10
4	21.6	2.50	35	10

4.2.1 Reproducibility

The reproducibility of the experiments is investigated prior to quantitative analysis, to demonstrate that the experiments are repeatable. For each resolution experiment there are at least 3 replicates performed at each condition. Table 4.2 shows the largest L glutamic acid concentration standard deviation (σ_{max}) obtained for each experiment. Table 4.3 gives the largest D glutamic acid concentration standard deviation for each experiment. The largest standard deviation for L glutamic acid is 6.10 % and the largest standard deviation for D glutamic acid is 5.50 %. It is clear from these results that regardless of the initial concentration C°, crystallization temperature and seed concentration [L_s] that the experimental technique and analytical method is robust and reproducible. Therefore, from this point forward experimental results will report the average values.

Table 4.2: Maximum standard deviation (%) of L glutamic acid concentration for each experiment

Experiment #	σ_{max} %
1a	3.60
1b	5.20
1c	6.10
2a	1.80
2b	2.10
2c	2.20
3	0.90
4	1.70

Table 4.3: Maximum standard deviation (%) of D glutamic acid concentration for each experiment

Experiment #	σ_{max} %
1a	4.0
1b	3.50
1c	2.70
2a	4.40
2b	5.50
2c	3.40
3	1.20
4	2.50

4.2.2 Qualitative Analysis of Results

The results in the experimental series 1, 2 and 3 are averaged and plotted against time to elucidate the trends for the effect of seed concentration and initial saturation concentration. The results are shown in pairs of figures for L and D glutamic acid concentration decays. Figures 4.5 & 4.6 are the results for experiments1a, 1b and 1c; Figures 4.7 & 4.8 are the results for experiments 2a, 2b and 2c; Figures 4.9 & 4.10 are the results for experiment 3.

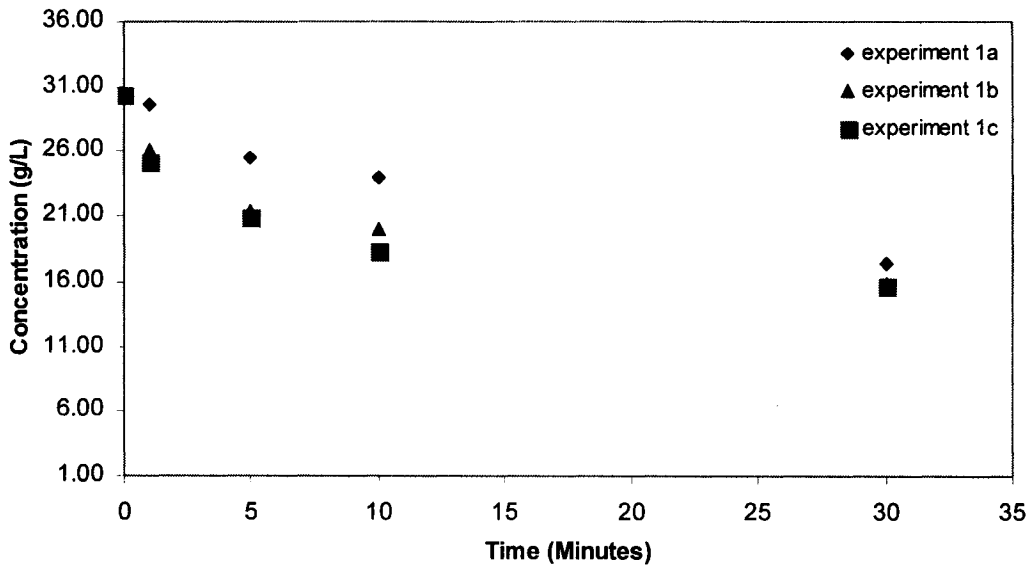


Figure 4.5: Effect of seed concentration on the amount of dissolved L glutamic acid from a racemic mixture solution with initial concentration of 31 g/L and T_c = 25 °C (experiment series 1)

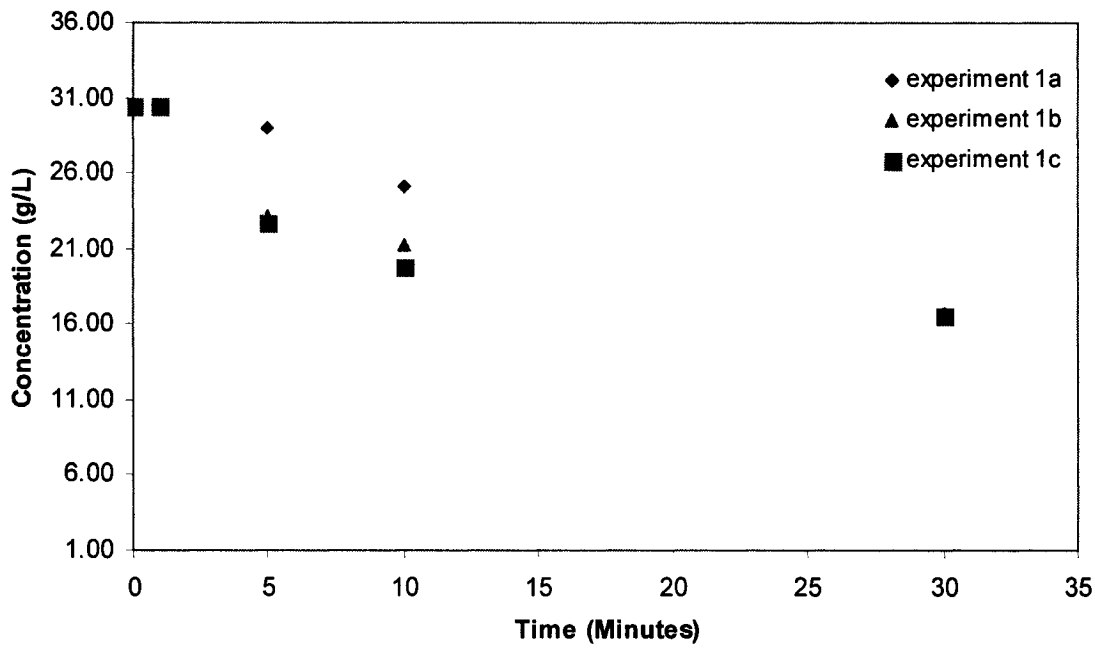


Figure 4.6: Effect of seed concentration on the amount of dissolved D glutamic acid from a racemic mixture solution with an initial concentration of 31 g/L and T_c= 25 °C, (experiment series 1)

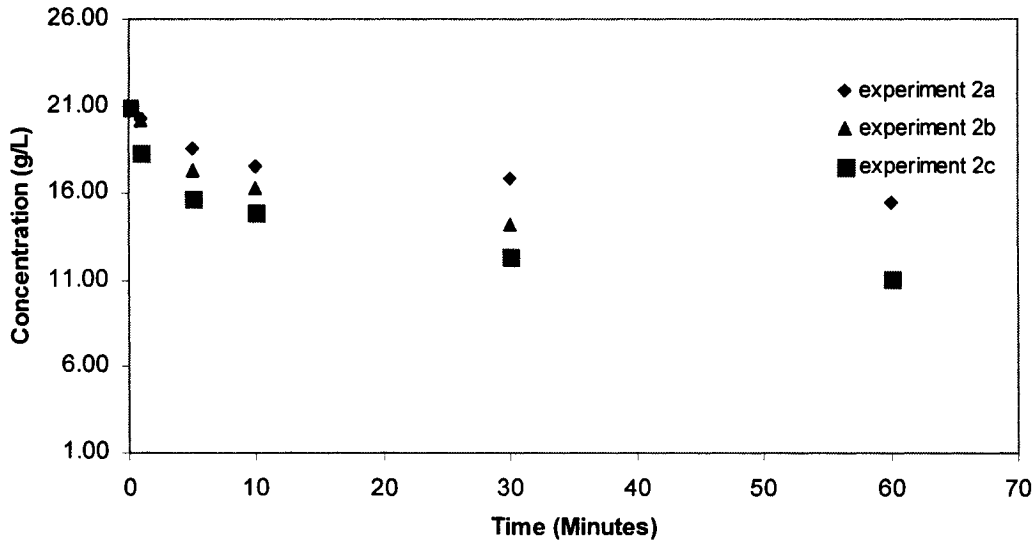


Figure 4.7: Effect of seed concentration on the amount of dissolved L glutamic acid from a racemic mixture solution with an initial concentration of 21.6 g/L and T_c= 25 °C, (experiment series 2)

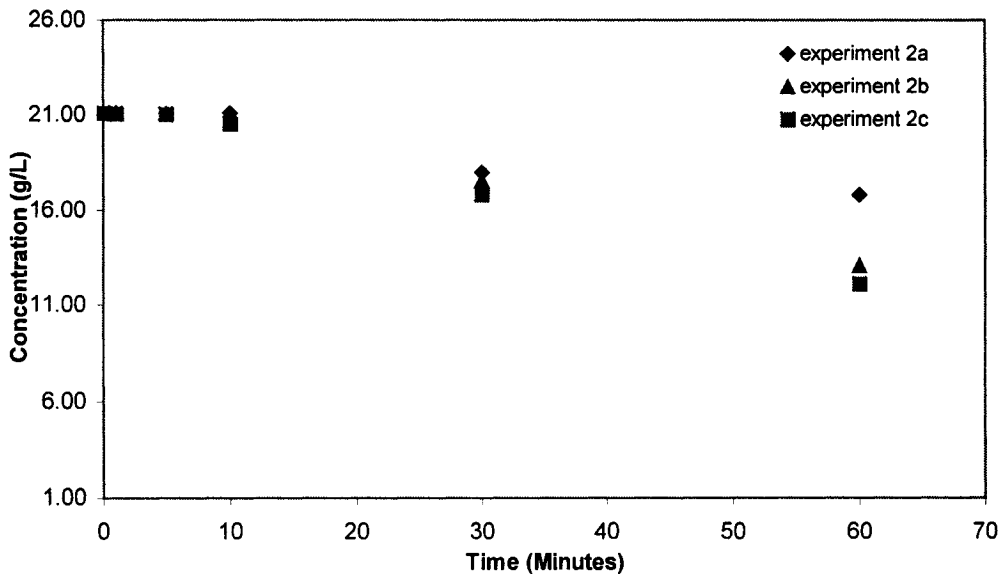


Figure 4.8: Effect of seed concentration on the amount of dissolved D glutamic acid from a racemic mixture solution with an initial concentration of 21.6 g/L and T_c= 25 °C, (experiment series 2)

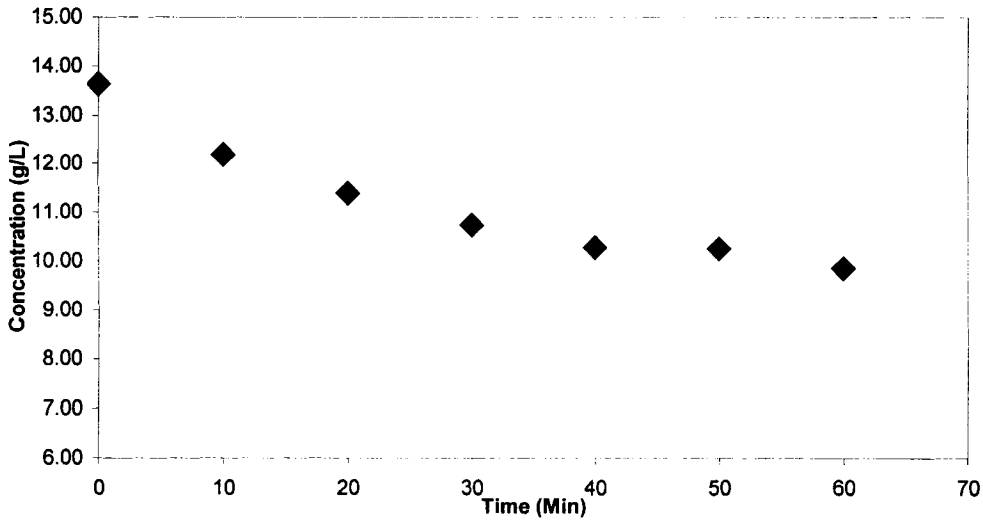


Figure 4.9: Effect of 10 g/L seed concentration on the amount of dissolved L glutamic acid from a racemic mixture solution with an initial concentration of 13.80 g/L and T_c = 25 °C (experiment 3)

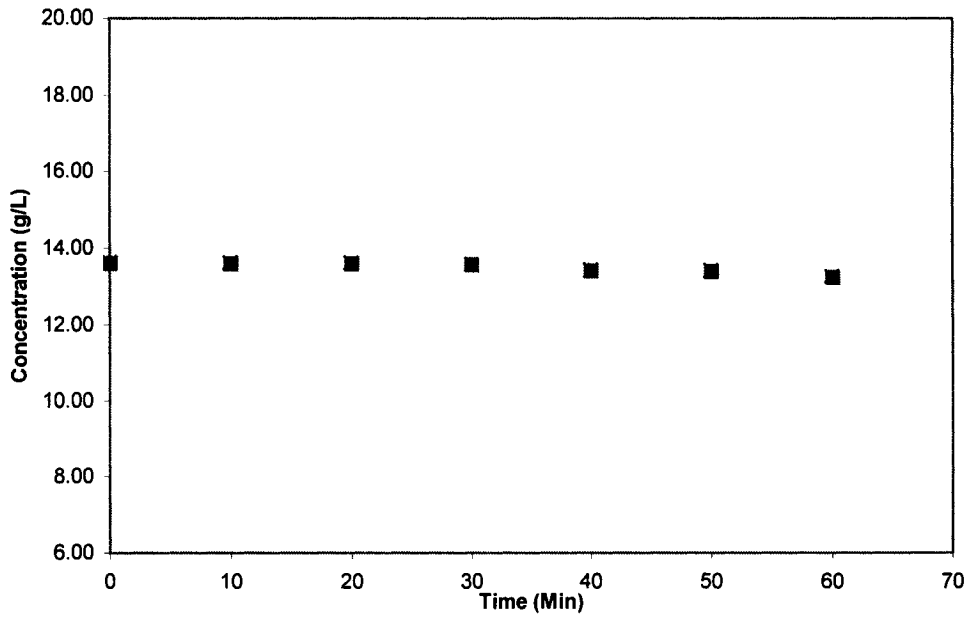


Figure 4.10: Effect of 10 g/L seed concentration on the amount of dissolved D glutamic acid from a racemic mixture solution with an initial concentration of 13.80 g/L and T_c = 25 °C (experiment 3)

The first observation is the expected result that at a given initial concentration C° increasing the seed concentration [L_s] increases the rate of L glutamic acid crystallization. This effect is particularly significant in the initial period of the crystallization. For example, in Figure 4.5 within the first minute, when the seed concentrations are 2 g/L and 10 g/L the corresponding L glutamic acid concentrations are 30 g/L and 25 g/L, respectively. As crystallization progresses, the concentration of L glutamic acid eventually converges to the saturation condition at the crystallization temperature, irrespective of the seed concentration. A similar trend for the effect of seed concentration on L glutamic acid concentration can be seen in Figure 4.7 for the second experimental series. In contrast, the effect of seed concentration on the rate of D glutamic acid crystallization is not as significant at early processing times. There is an initial lag period where there is no D glutamic acid crystallization, as shown in Figure 4.6 and 4.8. As with L glutamic acid, eventually the D glutamic acid concentration will converge to the saturation concentration at the crystallization temperature.

The second observation is that at a given seed concentration an increase in initial concentration (increase in concentration driving force) results in a faster rate of crystallization. This can be seen by comparing the decrease in the concentration of L glutamic acid at the highest seed concentration of 10 g/L. Figure 4.5, 4.7 and 4.9 show that the slope of the initial rate is steepest for the case with the highest initial concentration (Figure 4.5). The same trend holds true for the D glutamic acid concentration reduction, however there is the initial lag period where no crystallization occurs.

The next series of Figures (4.11, 4.12 and 4.13) show the results of the experiments in terms of supersaturation for the 10 g/L seed concentration (experiments 1c, 2c and 3). The areas of interest denoted in these figures mark the time interval where there is an actual resolution (separation).

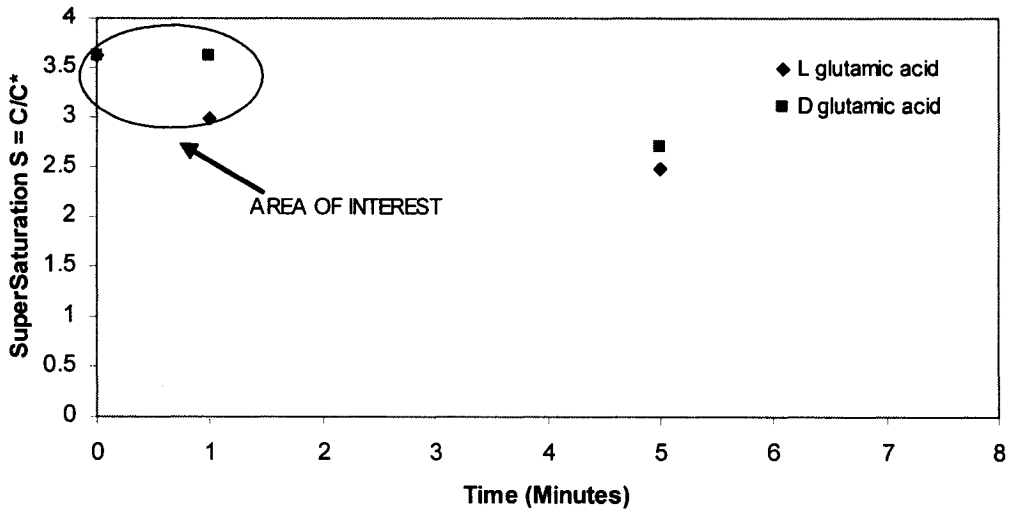


Figure 4.11 : L and D glutamic acid supersaturation comparison at S^{o} = 3.60 and $[L_s]$ = 10 g/L (experiment 1c)

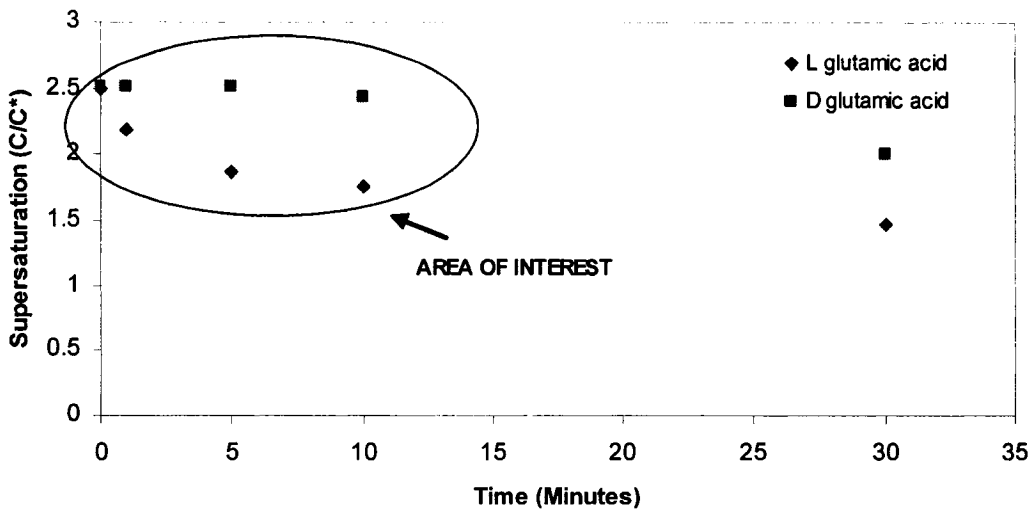


Figure 4.12 : L and D glutamic acid supersaturation comparison at S^{o} = 2.50 and $[L_s]$ = 10 g/L (experiment 2c)

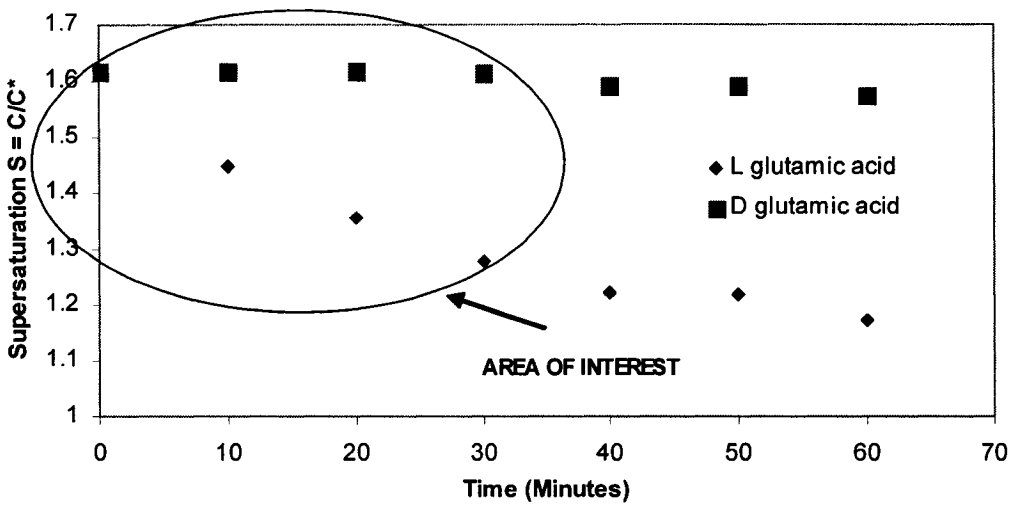


Figure 4.13 : L and D glutamic acid supersaturation comparison at S° =1.60 and $[L_s]$ = 10 g/L (experiment 3)

In all cases as initial supersaturation is decreased the crystallization rates slow down. This can be seen by determining the time in each of the above figures where L glutamic acid crystallizes out of solution, whereas D glutamic acid is retained. The initial supersaturations of 3.60, 2.50 and 1.60 present very little D glutamic acid crystallization before 1, 10 and 30 minutes, respectively. Any time longer would present a significant amount of D glutamic acid crystals especially for the initial supersaturations of 3.60 and 2.50.

4.2.2 Quantitative Analysis of Average Results

This Section quantitatively compares the experimental data for the first three experimental series in Table 4.1. The results of these experiments can be used to identify conditions giving the highest yield and purity of L glutamic acid as defined in Equations 4.1 and 4.2. Tables 4.4, 4.5 and 4.6 summarize the experimental results for experimental series 1, 2 and 3, respectively.

Table 4.4: Effect of seed concentration [L_s] at a fixed initial supersaturation S^o = 3.60 on L glutamic acid yield and purity (experiment series 1)

[L_s]→	**2 g/L**		**7 g/L**		**10 g/L**	
Time (min)	Y_L %	P_L %	Y_L %	P_L %	Y_L %	P_L %
1	3.30	99.50	19.30	99.90	24.00	99.90
5	22.50	77.40	40.90	55.30	43.00	55.00
10	29.50	55.00	47.50	53.10	55.40	53.30
30	59.10	48.70	66.40	51.20	67.30	51.60

Table 4.5: Effect of seed concentration [L_s] at a fixed initial supersaturation S^o = 2.50 on L glutamic acid yield and purity (experiment series 2)

[L_s]→	**2 g/L**		**7 g/L**		**10 g/L**	
Time (min)	Y_L %	P_L %	Y_L %	P_L %	Y_L %	P_L %
1	5.80	99.50	6.30	99.10	21.10	99.20
5	19.40	99.50	29.20	99.80	42.00	99.20
10	27.70	98.50	37.80	93.20	48.60	91.40
30	33.00	57.20	54.30	65.80	68.60	67.00
60	44.00	56.40	70.00	52.40	28.50	52.40

Table 4.6: Effect of [L_s] = 10 g/L at a fixed initial supersaturation S^o = 1.60 on L glutamic acid yield and purity (experiment 3)

[L_s] = 10 g/L		
Time (min)	Y_L %	P_L %
10	28.00	99.40
20	43.00	99.60
30	55.50	99.30
40	64.40	94.40
50	64.90	93.90
60	72.50	91.00

Regardless of the initial supersaturation for a particular solution, the highest yield of pure L glutamic crystals occurs at the highest seed concentration ([Ls] = 10 g/L). This can be observed from Table 4.4 where the yield of pure crystals increases from 3.30 % to 24 %, when the seed concentration varies from 2 to 10 g/L. In this table, the optimal conditions are shaded in grey. Therefore, the discussion is confined to the effect of varying initial supersaturation at the high seed concentration. For this seed concentration the results at an initial supersaturation of 3.60 give pure L glutamic acid crystals with a yield of 24 % in 1 minute. At an initial supersaturation of 2.50 the yield of pure L glutamic acid crystals increases to 42 %, but the time required is 5 minutes. Lastly, at an initial supersaturation of 1.60 the yield of pure crystals increases to 55.50 % in 30 minutes.

These results can be explained by considering that the rates of crystallization increase with a greater amount of glutamic acid dissolved in solution, by providing a greater concentration driving force for nucleation and growth. However, the high rates of crystallization apply both for the L and D enantiomers, minimizing the purity. In order to obtain high purity L crystals with a good yield the initial supersaturation should be maintained at a low value, thus minimizing the rate of heterogeneous nucleation for the D enantiomer and maximizing secondary nucleation for the L enantiomer.

4.2.3 Ternary Solubility Diagram

Ternary solubility diagrams can depict the molar composition at the crystallization temperature and the transient crystallization trajectory as mentioned in Section 2.2.1. Such a diagram is a useful tool for analyzing the resolution of racemic glutamic acid because it provides detailed data for all 3 components during the crystallization process. However, since the solution contains small amounts of glutamic acid the data is bunched in the upper solvent apex, as shown in Figure 4.14.

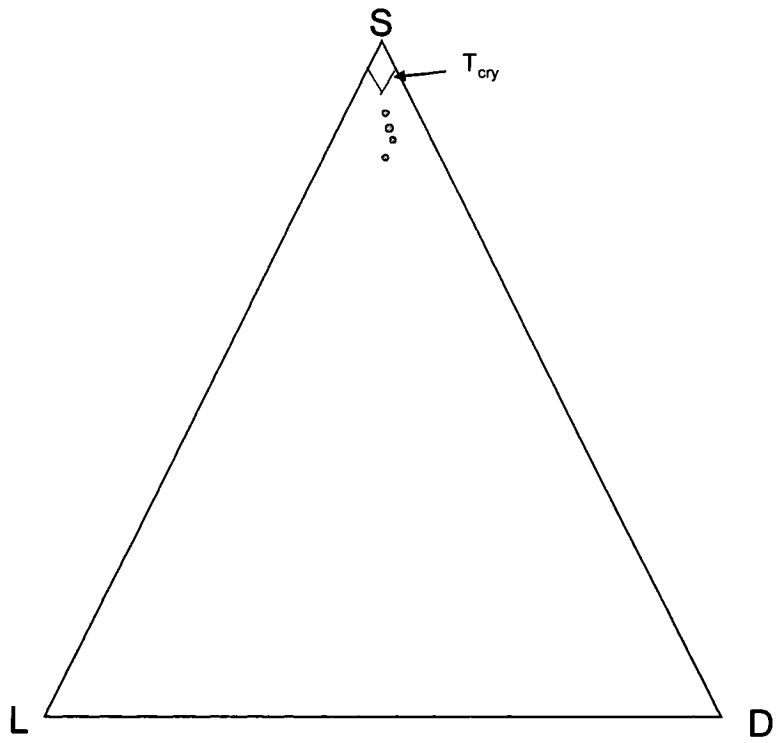


Figure 4.14 : Bunching of molar composition data points in the upper apex of a ternary solubility diagram

The crystallization time that yields the greatest amount of pure L glutamic acid crystals can be found by drawing a straight line from the L apex through the initial racemic composition, as shown in Figure 4.15. Based on a material balance, all points on this line represent a constant solvent to D glutamic acid composition ratio. The L glutamic acid composition varies from 100 % at the L apex to 0 % at the SD side of the triangle.

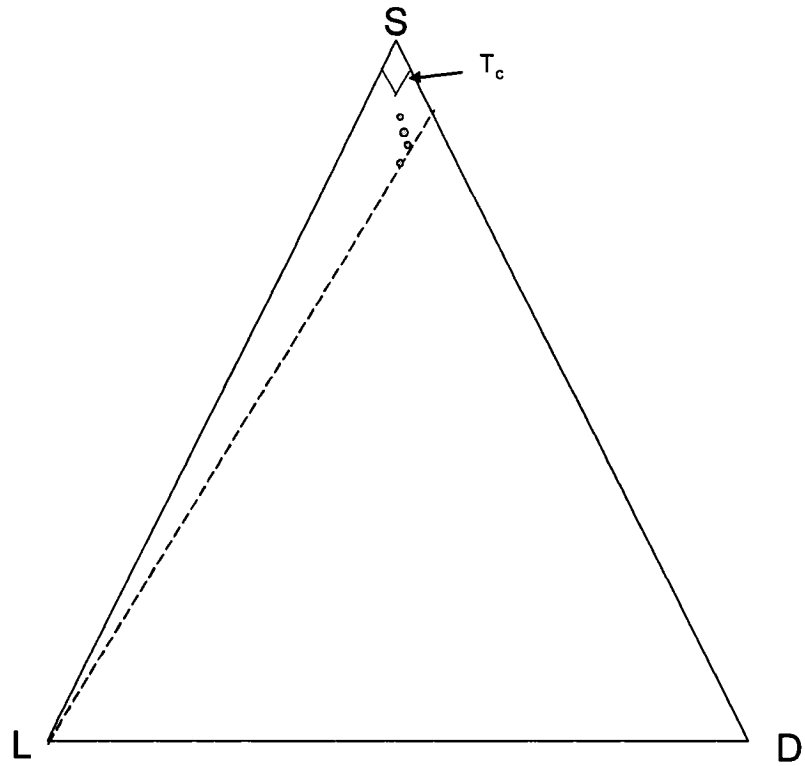


Figure 4.15: Demonstration of straight line through L apex to determine the longest time where L glutamic acid crystallizes alone

Additionally, the point at which D glutamic acid begins to crystallize can be seen when the transient trajectory begins to curve. Eventually for all cases as time increases the final composition will be that of the racemic mixture and T_c. It would take a significantly longer period of time to reach the racemic composition at the lowest initial supersaturation.

The interpretation of data from Figure 4.14 and 4.15 is quite difficult because of the extremely dilute molar concentration of glutamic acid in solution. Therefore, to see clearly the crystallization trajectories the scales in the ternary solubility diagrams are magnified. Figure 4.16, 4.17 and 4.18 are the ternary solubility diagrams for the initial supersaturation of 3.60, 2.50 and 1.60. These figures represent the results for experiment 1c, 2c and 3, respectively (L glutamic acid seed concentration of 10 g/L).

Each characterizes the trajectory from the initial racemic composition to the termination of crystallization at 25 °C.

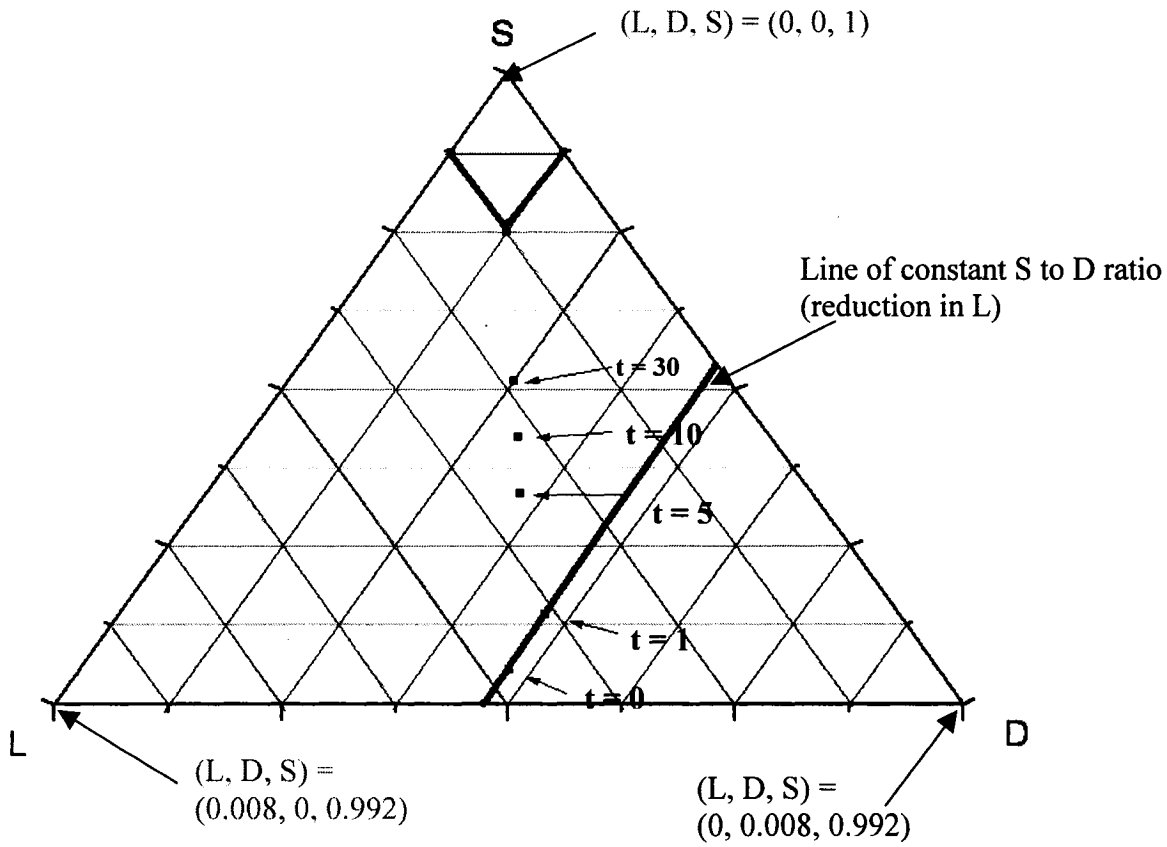


Figure 4.16: Ternary solubility diagram for the resolution conditions of S^{o} = 3.60, T_c= 25 °C & [L_s] = 10 g/L (experiment 1c)

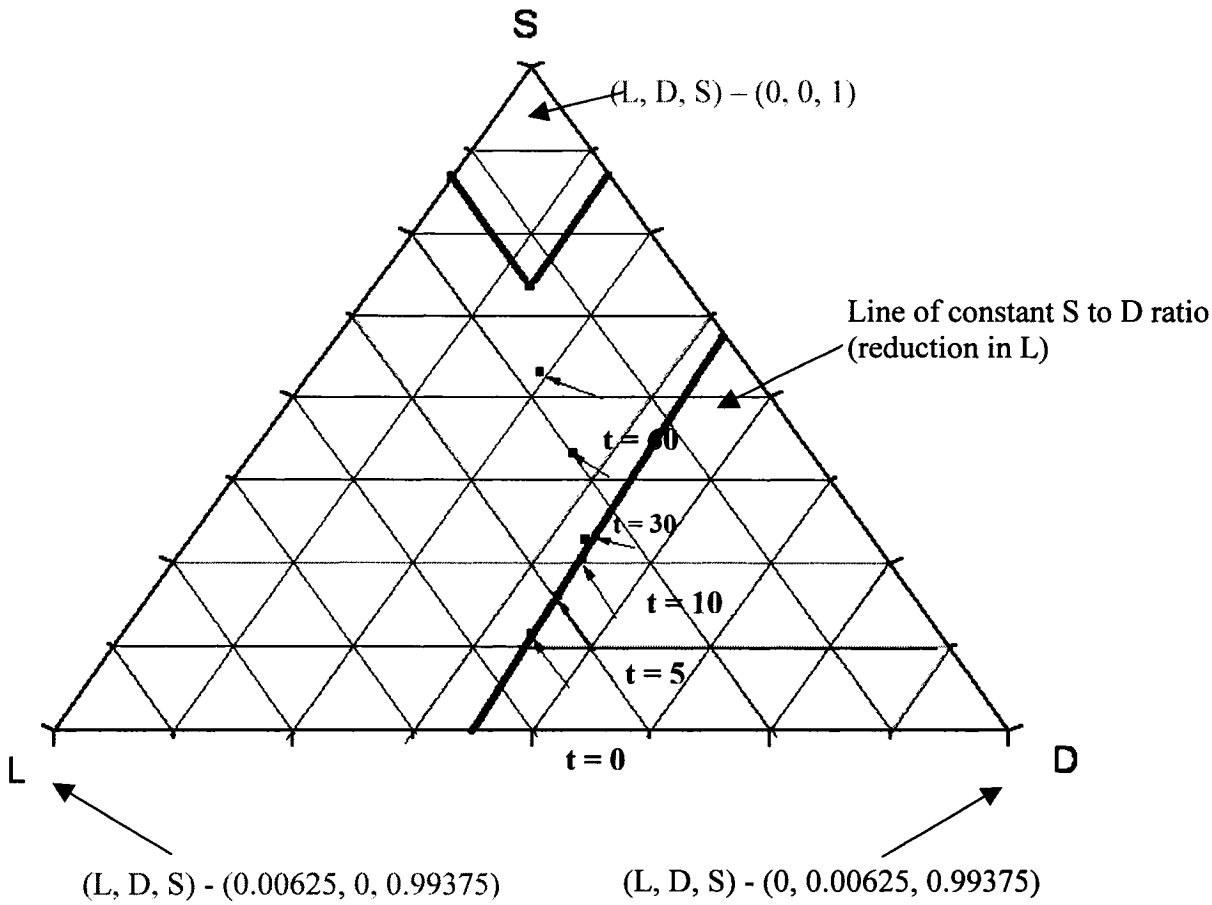


Figure 4.17: Ternary solubility diagram for the resolution conditions of S° = 2.50, T_c= 25 °C & $[L_s]$ = 10 g/L (experiment 2c)

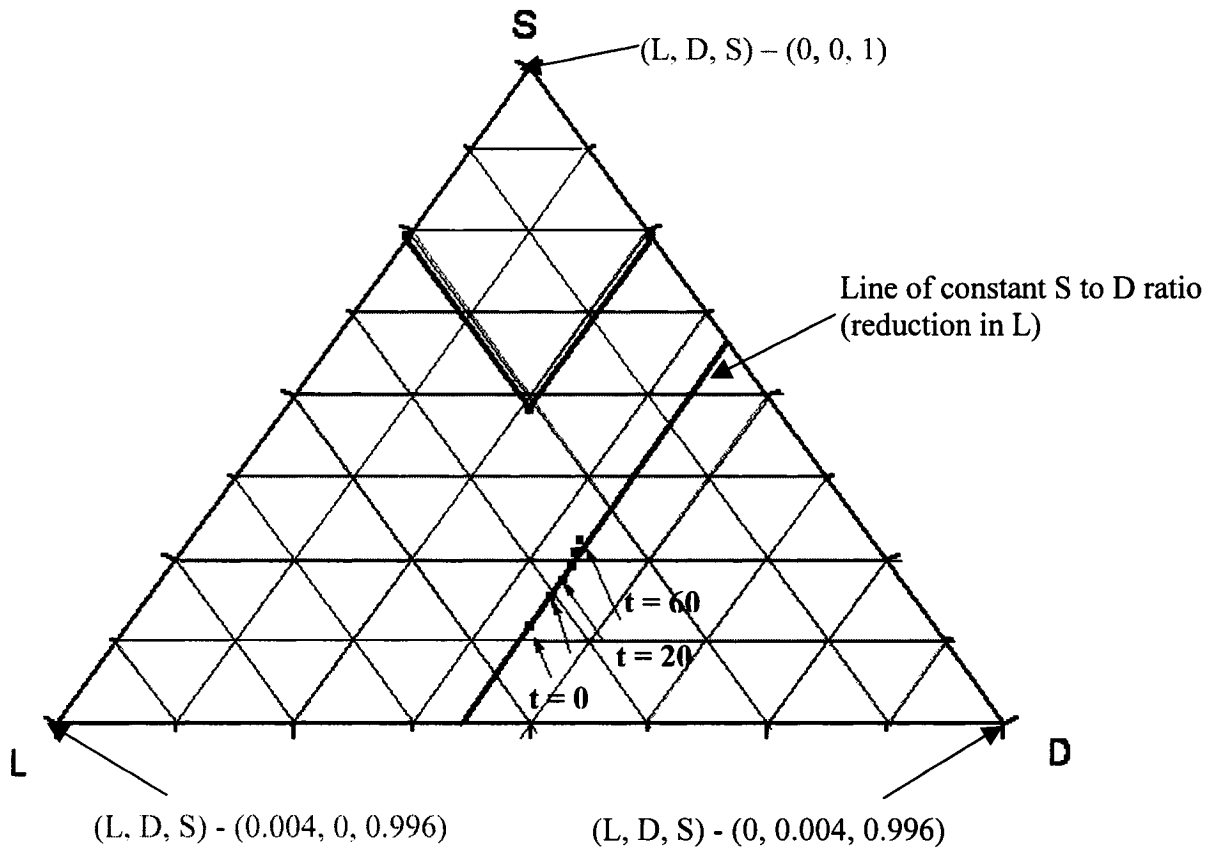


Figure 4.18: Ternary solubility diagram for the resolution conditions of S° = 1.60, T_c= 25 °C & [L_s] = 10 g/L (experiment 3)

Figure 4.16 at an initial supersaturation of 3.60, shows that L glutamic acid crystallizes preferentially between 0 and 1 minute. Figure 4.17 at an initial supersaturation of 2.50 demonstrates that L preferentially crystallizes over a period of 10 minutes. Figure 4.18 demonstrates that L glutamic acid crystallizes preferentially in 30 minutes for an initial supersaturation of 1.60. The time in each of these cases is found by the last point on the line from the L apex to the SD side of the triangle.

4.2.4 Rate Law Determination for Variation in Initial Supersaturation

The objective of this Section is to determine a rate law for the crystallization of L glutamic acid in the presence of seeds at a

crystallization temperature of 25 °C. The experimental data of experiments 1c, 2c and 3 are used for determine the rate law parameters. The highest seed concentration of 10 g/L is used to determine these parameters because the results indicate that the highest yield of pure L crystals is attained at this condition. A rate law of the following form is assumed, stating that the rate is dependent only on the supersaturation value:

$$r_L = -kS^n \qquad (4.1)$$

where:

r_L = rate of decrease in the supersaturation (min^{-1})

k = constant that depends on temperature and seed concentration -10 g/L in this case (min^{-1})

n = rate order

The material balance for the solute gives:

$$dS/dt = -kS^n \qquad (4.2)$$

The constant k and n can be determined using two methods: 1) The integral method and 2) the differential method.

1) The integration of equation 4.2 gives expression 4.3 where the constants k and n are empirically found by fitting a line through $\left((s^{(1-n)} - S^{o(1-n)})/(1-n)\right)$ versus time for all data points in experiments 1c, 2c and 3 where there are insignificant amounts of D glutamic acid crystallizing out of solution.

$$\left((s^{(1-n)} - S^{o(1-n)})/(1-n)\right) = -kt \qquad (4.3)$$

Figure 4.19 shows that there is a good linear fit for the data, where an R^2 of 0.995 is attained. The value of k is identified by the slope of the

trend line which has a k value equal to 1.6 X 10^{-3} (min^{-1}) and this is determined with a corresponding n value of 5.3.

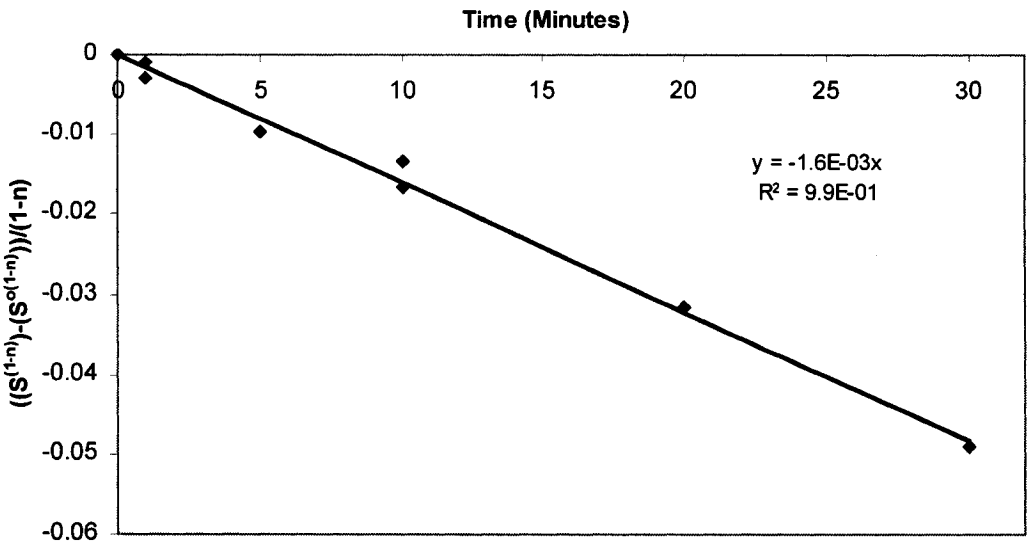


Figure 4.19: Determination of rate law constants k and n based on integral method

2) The differential method is used to confirm the rate law parameters determined with the integral method. Equation 4.2 is linearized by taking the logarithm of both sides yielding Equation 4.4.

$$\ln(-dS/dt) = (\ln k) + n\ln(S) \qquad (4.4)$$

The application of this equation involves the determination of the differentials (dS/dt) for each data interval and is plotted against the average supersaturation in that interval. As mentioned above only data intervals with minimal D glutamic acid crystallization are used. Figure 4.20 shows the experimental data linear fit for ln(-dS/dt) plotted against ln(S). The R^2 of 0.94 demonstrates a fairly good fit with a k and n value being 1.5 X 10^{-3} (min^{-1}) and 5.2, respectively.

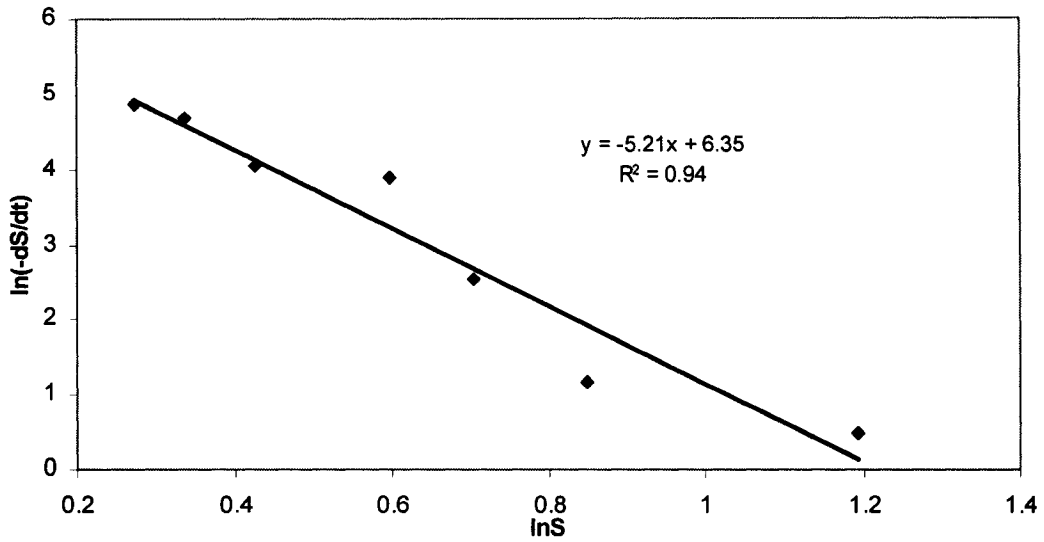


Figure 4.20: Determination of rate law constants k and n based on differential method

The results show that regardless of the method used to determine the rate law parameters that there is a good agreement with experimental results. The differential method gives a slightly poorer fit because of the inexact estimation of the derivative (dS/dt). The rate law is useful for the design of crystallization experiments at varying supersaturations, which have a constant crystallization temperature and seed concentration.

4.2.5 Effect of Changing Crystallization Temperature

In this Section the experiments on the effect of crystallization temperature on the rate of crystallization of L glutamic acid are discussed. The results of experiment 2c and 4 are compared because of their identical conditions, expect for the crystallization temperature. Experiment 2c has a crystallization temperature of 25 °C and a saturation temperature of 60 °C. Experiment 4 has a crystallization temperature of 35 °C and a saturation temperature of 70 °C. The increase in saturation temperature is a requirement to ensure the initial supersaturation is identical for both experiments (S° = 2.50). A crystallization temperature higher than 35 °C would imply that the saturated solution temperature would be above 70 °C with a much higher evaporation rate.

Figure 4.21 shows that the rate of supersaturation decay for L glutamic acid has not been significantly affected by the increase of crystallization temperature by 10 °C. This minimal influence can be justified by the following analysis:

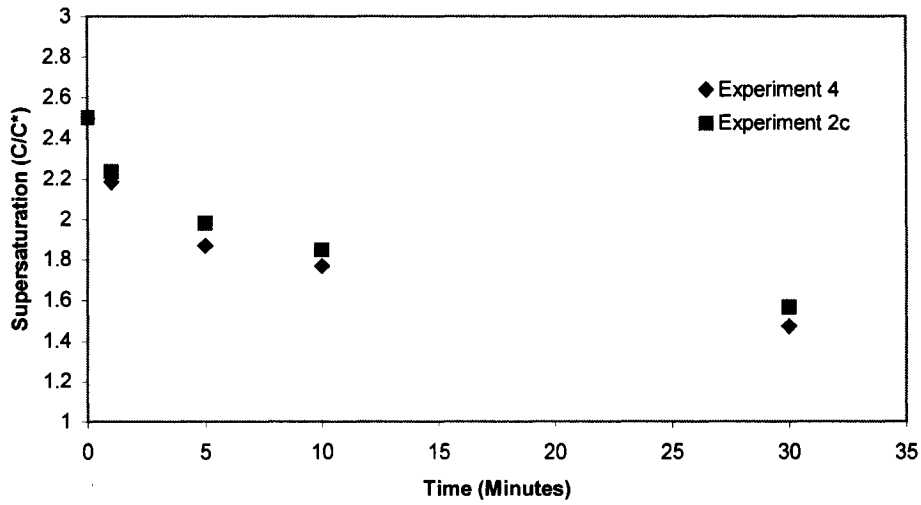


Figure 4.21: Effect of crystallization temperature on the rate of crystallization for L glutamic acid with an S° = 2.50 (experiment 2c and 4)

The variation of constant k with temperature in the rate law can be described by an Arrhenius type relation as shown in Equation 4.5:

$$k = A\exp^{(-E/RT)} \qquad (4.5)$$

where:

A is the pre-exponential constant

E is the activation energy

R is the molar gas constant

The work of Orella (1990), gives a common activation energy E for most amino acids to be 10000 J/mol. From this information the ratio of $K_{25}{}^{\circ}C/K_{35}{}^{\circ}C$ is calculated and found to be 0.9, indicating a small change in the rate of L glutamic acid crystallization with the adjustment of temperature.

Chapter 5 CONCLUSIONS & RECOMMENDATIONS

5.1 Conclusions

The following conclusions correspond to the objectives given in the Introduction. The experimental results show a maximum standard deviation of 7 % for solubility experiments and 6.10 % for crystallization experiments. This demonstrates that the successful design of robust apparatuses for solubility and crystallization experiments was achieved. An important feature of the crystallization experiments was that test tubes decreased significantly the time required for the saturated solution to reach the crystallization temperature. The large surface area to volume ratio promotes rapid heat transfer.

The experimental solubility data was in good agreement for each enantiomer with a literature reference (Henry (1963)) for a temperature range of 20 °C to 70 °C.

Preliminary crystallization experiments with only L glutamic acid dissolved in solution showed that primary nucleation is extremely slow. There was no crystal formation in the first hour of operation for both homogenous and heterogeneous (glass bead addition) experiments. Therefore, L glutamic acid seed crystals were added to solution to bypass primary nucleation and promote secondary nucleation. This addition improved kinetics where crystallization was occurring in a reasonable period of time.

Resolution experiments of racemic mixtures were performed to determine conditions that give the greatest yield of pure L glutamic acid crystals. Table 5.1 shows that the yield of pure crystals at a crystallization temperature of 25 °C and the seed concentration was 10 g/L. The lowest initial supersaturation provided the greatest recovery of pure L glutamic acid (greater than 55 %).

Table 5.1: Resolution Results at $[L_s]$ = 10 g/L, T_c = 25 °C

Experiment	S^o	Time (Minutes)	L yield %	L Purity %
1c	3.60	1	24.00	99.90
2c	2.50	10	42.10	99.20
3	1.60	30	55.50	99.30

A rate law describing the crystallization of L glutamic acid was found by the integral method. The law was determined for experiments 1c, 2c and 3 where the highest seed concentration was used. The specific rate law is shown in Equation 5.1 and the differential method confirmed that the rate law parameters were in fact accurate. The rate of L glutamic acid crystallization is related directly to the change in supersaturation of the solution at constant temperature and seed concentration. Therefore, regardless of the starting initial supersaturation the rate law parameters are acceptable for preliminary design of crystallization experiments for glutamic acid.

$$dS/dt = -1.6X10^{-3} * S^{5.3} \qquad (5.1)$$

Lastly, it has been demonstrated that varying the crystallization temperature by 10 °C had minimal affect on the rate of crystallization of L glutamic acid. This is due to the low activation energy for amino acids.

This research provides a framework and rationale for the resolution of any conglomerate system. Knowledge of solubility data is a prime importance in determining the appropriate method to induce crystallization. The effect of initial supersaturation, seed material, seed concentration and crystallization temperature on crystallization rate can be used for determination of appropriate conditions for the attainment of the desired pure enantiomers. The major contribution of this research was that it is possible to resolve racemic mixtures of glutamic acid without the

addition of mineral acids and bases and still get a pure product and this with a high yield.

5.2 Recommendations

The resolution of racemic glutamic acid mixtures can be improved by making the process continuous or cyclical. A requirement for this is knowledge of batch experimental results as were determined in this thesis. The following description demonstrates how the resolution by entrainment can be made continuous, (Jacques *et al.* (1981)). Figure 5.1 is a schematic diagram for the concentration profiles of L and D glutamic acid during the continuous entrainment process.

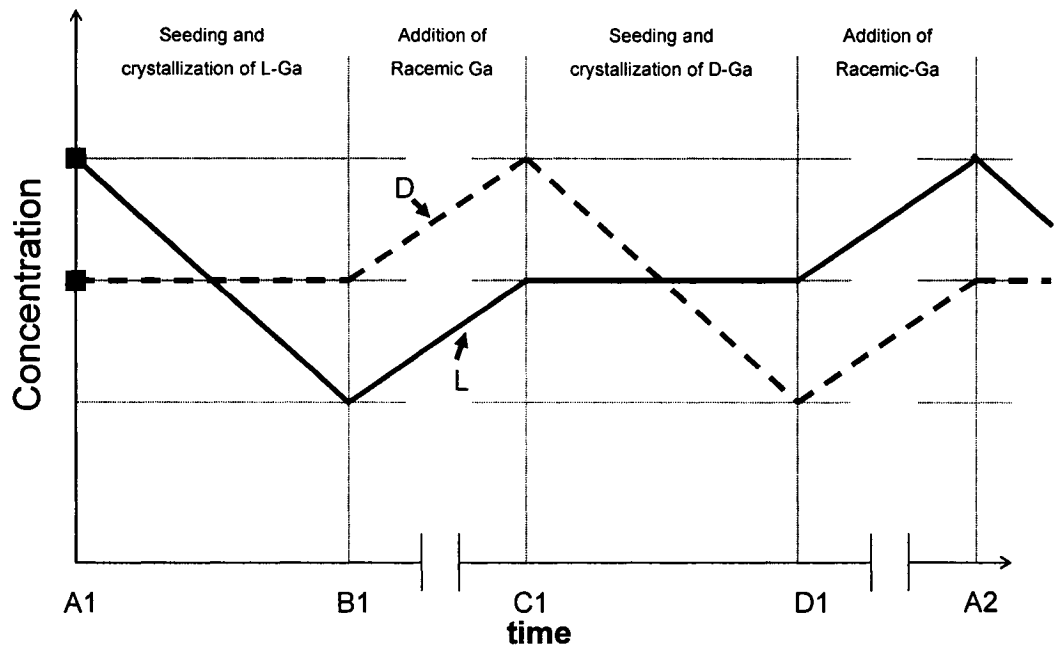


Figure 5.1: Cyclical or continuous entrainment process for racemic glutamic acid resolution

The continuous process begins by preparing a racemic mixture of glutamic acid at a high temperature. Then a slight excess of L glutamic acid crystals are dissolved in the original solution (t = A1). This is followed by seeding with L glutamic acid crystals and allowing the solution to crystallize at a low temperature in the time interval (A1 – B1). The crystallization is stopped at B1 where the recovery of two times the excess amount of L glutamic acid added is attained. Also as seen from the above figure there would be no D crystallization in this interval.

After the removal of L crystals an equivalent amount of racemic glutamic acid is added to solution, so that the D enantiomer would be in excess. This occurs in the time interval (B1 – C1). The solution at C1 is seeded with D crystals and is allowed to crystallize until D1, where the D crystals are collected. This process can be repeated where for each stage two times the initial amount of the enantiomer in excess is recovered.

An additional recommendation for the resolution of racemic glutamic acid or any other moderately soluble compound would be to improve the durability of the mechanical shaker. This would allow the experiments to run for extremely long periods of time without the chance of mechanical failures. This gives flexibility, where experiments at extremely low initial supersaturations can be performed and high yields of pure crystals can be attained.

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APPENDIX

Appendix 1: Ternary Solubility Diagram Rules

The following techniques are standard for reading and interpreting ternary solubility diagrams. Figure A.1 is a schematic diagram of a ternary solubility diagram. There are three components on this diagram:

1) Solvent (S)
2) D – enantiomer
3) L – enantiomer

where each apex represents the maximum composition for a given component. For example from Figure A.1 point y has the following molar compositions (L, D, S) → (20 %, 50 %, 30 %) and point x has the following mole compositions (L, D, S) → (40 %, 10 %, 50 %). Familiarity with use of the diagonal lines in this diagram is mandatory to identify changes in compositions of each component.

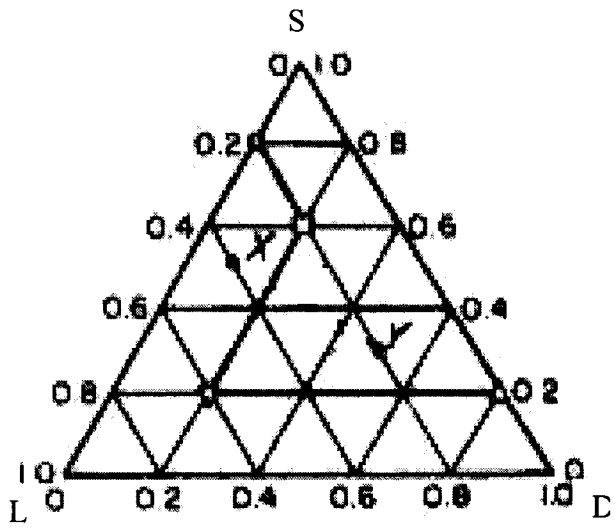

Figure A.1: Schematic diagram of ternary phase diagram lines

An example of how the composition of a particular ternary system varies is described in Figure A.2. For this particular case the starting point is at point M and its mole composition is (70% L, 30% D, and 0% S). Once Solvent is added so that the mixture will become 20% solvent, the

mixture will be at point P (56 % L, 20 % D, 24 % S). This point is identified by the intersection of the line MS and the 20% solvent axis. I.e. the ratio of L to D remains constant along the SM line.

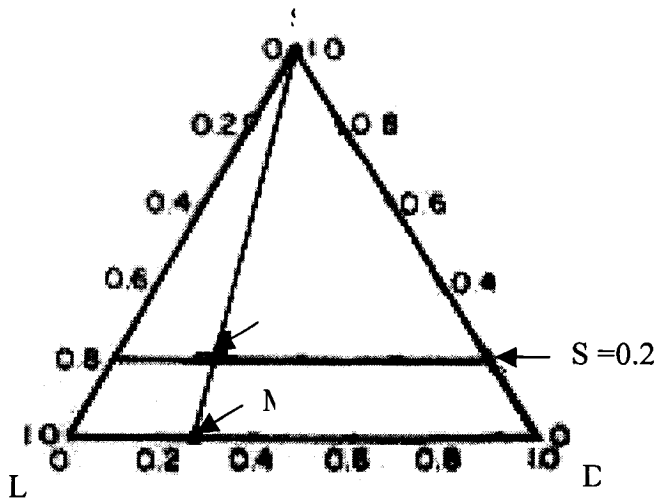

Figure A.2: Effect of solvent addition to ternary molar composition

Appendix 2

The first step in the water purification process is to convert tap water to distilled water. This is done by heating tap water in a glassware tank. The steam from this heating is condensed into the purified distilled water. This process is inexpensive but only 5 % of feed water is converted to purified distilled water.

The distilled water is further processed to remove additional dissolved solids and gases through a deionization process. The Easypure RF deionzer from Barnstead consists of 4 resins cartridges and a microporous filter. The cartridges are either packed with cation or anion exchange beads. The cation exchange beads are charged with hydrogen and the anion exchange beads are charged with hydroxyl groups. The removal of dissolved NaCL from water illustrates how this deionizer operates. The Na^+ ion is exchanged with a hydrogen atom on the cation exchange bead, resulting in the formation of HCL. The HCL then contacts the anion exchange bead where the CL^- replaces a hydroxyl group,

resulting in the formation of H_20. Eventually, the resin cartridges must be replaced because they become entirely packed with impurities.

During the deionization process there is a tendency for stagnant water to form in the cartridges. This facilitates bacteria growth which must be removed to attain purified deionized water. A 0.2 micron pore size membrane at the outlet of the deionizer assures the removal of bacteria and carbon fines. The above procedure for purifying water was used to assure the water quality required for experimentation or analysis.