This is to certify that the

thesis entitled

CHARACTERIZATION OF ION EXCHANGE RESINS FOR THE IMMOBILIZATION OF ESCHERICHIA COLI

presented by

Leonard Matthew Czupski

has been accepted towards fulfillment
of the requirements for

M.S. degree in Chemical Engineering

Eric A Grulke

Major professor

Date August 8, 1989

CHARACTERIZATION OF ION EXCHANGE RESINS FOR THE IMMOBILIZATION OF *ESCHERICHIA COLI*

By

Leonard Matthew Czupski

A THESIS

in

partial fulfillment

of the

requirements

of

MASTERS OF SCIENCE

Department of Chemical Engineering

Michigan State University

1989

ABSTRACT

CHARACTERIZATION OF ION EXCHANGE RESINS FOR THE IMMOBILIZATION OF ESCHERICHIA COLI

By

Leonard Matthew Czupski

Adsorption of bacteria is a very easy and mild technique of immobilization. Though studies show that adsorption on to ion exchange resins is an applicable technique none, define the resin characteristics needed for high cell loading. Escherichia coli was used as a test organism since common organism for product expression genetic engineering. Viable cells were suspended in a non growth solution at favorable living conditions, 37°C and pH 7.0 - 7.5, and contacted with the Cl^- form of ion exchanger. The geometric characteristics found to be favorable are high external surface areas with small porosity and small percentage of crosslinking. Charge characteristics favor a Type I strong base anion exchanger. Adsorption of bacteria follow a Langmuir isotherm with the mechanism being a second order irreversible reaction of cells with open "sites" on the resin. The mass transport follows normal transport phenomena for constant surface composition.

ACKNOWLEDGEMENTS

I would like to thank Dr. Eric A. Grulke and Dr. Patrick J. Oriel support and expertise shared with me during this project. Allen Greenberg of Dow Chemical Company for his expertise on ion exchange and the use of the ion exchange resins studied. The use of Dr. Dennis J. Miller's mercury porosimeter and the Composite Center's scanning electron microscope is greatly appreciated. As is Doug Degaetano of the Center for Electron Optics, Michigan State University, for the preparation of the immobilized resins and the photographs taken of the immobilized resins.The financial assistance from the State of Michigan's Research Excellence Fund and from the Department of Chemical Engineering was greatly appreciated.

A special thanks to my wife **Trish**, for her patience during this study.

TABLE OF CONTENTS

LIST OF TABLES

TABLE OF FIGURES

CHAPTER I: INTRODUCTION

The simple observation of algae covered rocks in a stream suggests that immobilization of cells has occurred as long as the combination of solids, cells and water.

Industrially the use of immobilized cells has been used since 1823 where Acetobacter was adsorbed onto wood chips for the "quick" vinegar process. Waste water treatment is another process in which immobilized cells are used as a part of the process.

The use of immobilized cells in industrial fermentations is a recent phenomena. The first article appeared in 1960 (Hattori and Furuska, 1960) and the first application for continuous production was with L-aspartic acid. (Chibata et al., 1974; Sato et al., 1975)

The methods of cell immobilization are similar to methods used for enzyme immobilization. A definition for immobilized cells was given at the first Enzyme Engineering Conference in 1971. Immobilized cells are: "physically confined or localized in a certain defined region of space with retention of their catalytic activity and - if possible or even necessary - viability and which can be used repeatedly and continuously."

The advantages of using immobilized cells in fermentations are: (1) a higher amount of cells per unit volume of reactor, (2) a lesser amount of free cells in the effluent, (3)higher dilution rates. The first reason allows for smaller reaction vessels or greater

productivity of existing vessels and the last two reasons directly affect the downstream separations needed for the bio-products.

Recommendations for the case of product expression or over production of cells are shown in Table 1.1 .

TABLE 1.1: Support requirements

1.) High carrier ability
2.) Availability in quantity
3.) Low cost
4.) Easy scale-up
5.) Safety of material

Three methods fitting these requirements are shown in Table 1.2.

TABLE 1.2: Immobilization Methods

Entrapment
Cross-linking
Carrier-binding

Entrapment is the most widely studied method of cell immobilization. Cells are entrapped in the matrices of various polymers. Matrices that have been studied are: collagen, gelatin, agar, alginate, carrageenan, cellulose triacetate, polystyrene and polyacrylamide. Polymer precursors are mixed with cells and the polymer is formed around the cells. Problems faced in the entrapment method are possible death of the cells caused by the polymerizing reagent and low rates of diffusion of substrate to the cell and product out of the matrix.

In the cross-linking method, the cells are linked to one another by a suitable reagent (Chibata et al, 1974). The carrier-binding method

has the cells covalently bound or adsorbed onto insoluble matrices.

Ion exchange resins have been used to separate microorganisms from solution. They have been used in clinical microbiology, waste and water treatment. The separation technique was emphasized in Daniel's review of the use of ion exchange resins with microorganisms (1972). This review covered the separation of algae, diatoms, bacteria, fungi, protozoa, rickettsia and viruses from an aqueous solutions.

Studies of ion exchange resins as cell supports in fermentations has been limited. As of yet no study has shown the qualitative effects that surface area, surface charge and resin morphology have on sorption and adsorption rate.

The focus of this study is to find a ion exchange resin that has good adsorption of E. coli and the adsorption equilibrium and the kinetics of the adsorption based on resin morphology. Other areas of interest are resin geometry, exchange group, resin recyclability and use of the resin in actual growth conditions.

The gram-negative prokaryote Escherichia coli was chosen because the gene locations of the organism are well studied and there are a great many genetically engineered organisms available.

CHAPTER II: LITERATURE REVIEW

Immobilized cells have several advantages. They allow a higher concentration of cells in the fermentor than possible for suspended cell culture, particularly for bacterial cultures. The higher cell mass can result in increased production of cells and products. If the immobilized cells do not release into the media there will be fewer cells in the reactor effluent and less downstream processing of the product stream is required. The increased cell and product concentration in the fermentor as compared to free cell is known as overproduction. Immobilization will also allow a greater dilution rate in a continuous fermentation (Kolot,1981).

If cells express an enzyme needed for a process, then cell immobilization has advantages over enzyme immobilization since enzyme purification is not needed, enzyme loss is held at a minimum, and the enzyme will be inherently more stable; it is in its natural environment (Durand & Navarro,1978).

Due to the large scope of cell immobilization and of cell adsorption, this chapter will cover the use of ion exchange resins for the overproduction of cells and enzymes in both categories.

2.1 ADSORPTION FOR IMMOBILIZATION

Cell adsorption is done at mild conditions as compared to the entrapment and crosslinking techniques. The cells just need to get in

contact with the carrier. Mild conditions and simplicity are the main advantages of adsorption used for cell immobilization (Tampion and Tampion, 1987; Klein & Wagner,1983; Klein & Vorlop,1985). Since adsorption does not put much stress on the system, the cells are usually viable, unlike entrapment where the cells will come in contact with polymerizing agents. There are two types of ion exchange resins: solid gels and macroporous. The solid gels will sorb cells only on their exterior surface while the porosity of macroporous resins will allow cells to sorb on interior surfaces.

Mass transfer of nutrients and products depends on the reactor system. Entrapped cells and cells in macroporous resins rely on mass transfer of nutrients and products into the interior of the particle. In some entrapped gels, the yeast will grow only within 50 μ of the surface (Ollis, 1989). Entrapment gels can be broken by growing cells. Crosslinked resins are less susceptible to disruption (Ollis, 1989). Since adsorption is a surface phenomena, mass transfer of substrates and products has little interference from the carrier, unlike entrapment and encapsulation (Kolot,1981). Since ion exchange resins can be regenerated, this carrier also has the possibility to be reused. The disadvantages of this method are the possibility of large amounts of cell loss from the surface and the adverse effects of pH on adsorption (Tampion & Tampion,1987).

The features sought for carriers used for adsorption are shown in Table 2.1.1 (Tampion & Tampion, 1987).

2.2 ION EXCHANGE

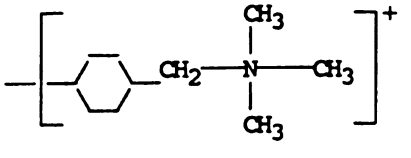
Ion exchange resins are polymeric beads that have a charged matrix with exchangeable ions. The exchangers are divided into two principle categories: structural (the geometry of the polymer matrix) and functional (the ion-active groups).

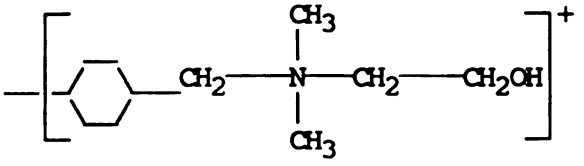
TABLE 2.1.1: Features of supports for adsorption

Non-toxic
High cell retention capacity
High cell loading capacity
Stable to heat sterilization
Stable at appropriate pH values
Resistant to microbial degradation
Availability in appropriate shapes and sizes
Cost ppropriate to application
Density appropriate to reactor type
Reusable

There are a range of functional groups on commercial ion exchange resins. Cation exchangers have a negatively charged matrix that exchanges a cation (positive) charge and anion exchangers have a positive charge that exchange an anionic (negative) charge (Wheaton & Seamster, 1966). These are further divided by charge strength.

The anion exchangers are divided into strong, intermediate and weak base. Strong base exchangers are highly ionized (dissociation into ions) when compared to the intermediate and weak base. Strong base exchangers are of two types:

TYPE I 

TYPE II 

Type I exchangers have a smaller capacity than Type II but are more stable chemically. They also differ in their affinities for chloride and hydroxide ions (Applebaum, 1966). Type II exchangers are more readily regenerated due to their greater affinity for the hydroxide ion. Weak base exchangers generally are a mixture of polyamine functional groups containing primary amine, $-NH_2$, secondary amine,-NHR, and tertiary amine, $-NR_2$ (Wheaton & Seamster,1966). Intermediate base exchangers have a mixture of exchange groups from both strong and weak base exchangers.

Strong base anion exchangers have an operating range of 0-14 pH in both the salt and free-base form. Weak base anion exchanger are highly ionizable over pH of 7 and only in the salt form (Applebaum, 1966). These work best with strong acids such as HCl and H_2SO_4 (Dorfner, 1972).

Cation exchangers are separated into strong and weak acid groups. Strong acid exchangers have sulfonic acid, $-SO_3H$, and weak acid exchangers have carboxylic acid, -COOH, as exchange groups.

The structural portion of exchangers are divided into two categories, gel and macroporous (macroreticular). Macroporous resins have pore sizes ranging up to several thousand angstroms with interconnecting pores. These resins swell very little in aqueous solutions. Gel type exchangers are polystyrene with divinylbenzene (DVB) crosslinks where porosity is inversely proportional to crosslinking. Gel type resins show slight swelling in poor solvents

such as water. Exchangers are normally available as spheres. The specific gravity of exchangers is 1.1 -1.5 (Wheaton & Seamster, 1966).

2.3 EXCHANGE MECHANISM

Ion exchange involves exchanging one ion for another with a simple reversible reaction. As expressed in the following two equations:

Cation Exchange:

$$Z_S \cdot a^+ + b^+ \rightleftharpoons Z_S \cdot b^+ + a^+$$

where the Z_S is the matrix, anion fixed site and a^+ and b^+ are the cations.

Anion Exchange:

$$A_S \cdot c^- + d^- \rightleftharpoons A_S \cdot d^- + c^-$$

where A_S is the matrix and the cation fixed sight and c^- and d^- are the anions.

2.4 MECHANISM OF IMMOBILIZATION

According to a hypothesis made by Daniels and Kempe (1966) bacteria are explained to be "macroscopic zwitterions". At pH's above the isoelectric point of their surfaces, they act as anions and at pH's below the isoelectric point of their surfaces, they act as cations, This hypothetical dipolar ion on the cell surface is shown in Figure 2.4.1 (Daniels,1972). According to this explanation all bacteria should adsorb to all types of exchangers depending on the pH of solution. The adsorption is thought to be an electrostatic attraction between the cell surface and the carrier (Daniels & Kempe,1966). The cell surface

group, carboxyl groups, electrostatically interacted with the quaternary amine groups on the strong base anion exchange resins studied (Zvaginstev and Gusev,1971). This was found to be the common group among cells with differing cell wall structures, gram-negative and gram-positive bacteria and yeast.

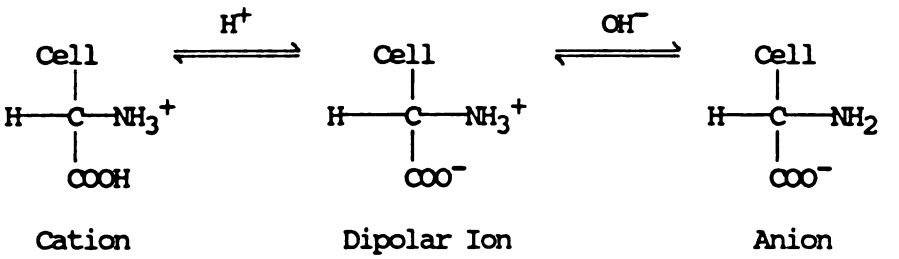


Fig. 2.4.1: Hypothetical dipolar character of cell.

2.5 CELL ADSORPTION

The studies of adsorption of bacteria onto ion exchange resins have shown that the best exchangers are anion exchangers with preference to strong base type (Wood,1980). The capacity of strong base type exchangers have been found to be in excess of 10^{10} cells per gram dry resin (Daniels & Kempe,1966; Wood,1980). Some studies have been shown that cation exchangers gather a few cells, depending on the pH of solution (Gillisen et al,1961).

Gram negative organisms (i.e. E. coli) often have surface structures that have been thought to be ideal for adsorption with appendages such as flagellae and lipopolysaccharide chains. An adsorption study comparing mechanically de-flagellated and normal E. coli showed no difference in the amount of cells adsorbed. A study done with Smooth and Rough strains of Salmonellae typhimurium and Sal. minnesota showed that lipopolysaccharides actually inhibit the

adsorption to anion exchangers (Wood,1980).

Since this research is conducted toward the use of immobilized cells for fermentation purposes the pH range considered for study will be closer to optimum physiological, 7.2, for E. coli(Oriel). The amount of E. coli adsorbed onto an anion exchanger between pH of 5 and 8 showed no distinct maximum level (Niehoff and Echols,1973). This observation agreed
with the adsorption characteristics found for several species of Gram-negative bacteria onto DEAE cellulose over the same pH range (Wood, 1980).

Cation exchangers have been studied for adsorption of bacteria and have been found to adsorb cells at low pH (Daniels & Kempe,1966). For strong acid cation exchangers, negligible adsorption was observed for higher pH, 4-10 (Wood,1980).

Another factor involved with the cell environment is the amount of salts that are in solution. The effect of varying sodium and calcium chlorides concentrations, zero to 1.0 M, on the amount of E. coli adsorbed onto an anion exchanger at pH 6.5 was that the amount of adsorption did not change for polystyrene exchangers (Jarvis, Lach and Wood; 1977).

Physical parameters of the resin will effect the amount of cells adsorbed. For Phase I Haemophilus pertussis, cell adsorption was found to be proportional to the inverse of the number of cross links of Dowex 1 (Kuwajima et al, 1957). The increased water content at low crosslinking was thought to provide a more natural environment for the organism.

According to Zvaginstev and Gusev (1971) only 0.3% of the amount

of actual adsorption sites bind to cell for anion exchangers in the chlorine form, (this is about the small amount of exchange sights found on the resin surface).

2.6 EQUILIBRIUM

The amount of cells adsorbed from solution depends on the amount of adsorbent that is present in solution (Daniels,1972; M. Barr,1957). The adsorption isotherm for bacteria has been determined to be Langmuir in nature (Daniels,1972; Bar et al,1985; Grulke et al,1989). The observation of a Langmuir isotherm suggests that the cells cover the resin in a monolayer. This has been observed by using Scanning Electron Microscopy, S.E.M., where the cells have been observed to layer the surface much like pins in a pin cushion (Daniels,1972).

It is thought that some free cells found in immobilized systems occur from reproduction of the adsorbed cells instead of cell desorption (R. Hattori,1972). The amount of cells that become free is independent of the initial amount of cells adsorbed since the cells divide on the surface until capacity is reached.

2.7 KINETICS

The rate of adsorption has been given the model:

$$A/A_o = k_a t + k'_a \sqrt{t}$$

were A, A_o are adsorbances (at 420 nm) at times t and 0 of the cell suspension. k_a and k'_a are experimentally derived constants that depend on solution conditions, cell and resin type (Daniels,1972).

2.8 CELL AGE

In a study with Zymomomas mobilis (Krug & Dougalis,1983) the age of the cells being immobilized had an effect on the amount of organism adsorbed. As the cells' age increased, the amount adsorbed decreased. This occurred for two strong base macroreticular anion exchangers.

2.9 SURFACE AREA

Since adsorption is a surface effect, the amount of surface area plays a large factor in the amount of cells that can be adsorbed. This was shown by Wood (1980). As the sphere diameter increased, the amount of adsorption decreased. The surface area presented is dependent on the particle size. Daniels (1972) mentioned that better adsorbers were small spheres with large surface area.

2.10 POROSITY

The pores of macroreticular ion exchange resins will have on cell adsorption should be taken into account. The pore size and distribution of pores has been studied for the effect it has on the immobilization of microbes (Messing & Opperman,1979). The research was separated into two categories, dependent upon the type of reproduction that occurred, fission and budding.

For organisms that reproduce by fission, such as E. coli, it was found that the optimum pore size is one that is double the length of the organism. This diameter allows for cell reproduction with very little interference from the pore walls. To get a high accumulation of cells it was found that the carrier should have 70% of the pores

between one to five times the major physical dimension of the cells. This range of pore sizes would allow enough room for two organisms directly across from on another to be able to reproduce, thus doubling their length, and allow passage for another cell through the pore. Pore sizes larger than this would not have as much surface area available for adsorption. The particles used were fritted glass spheres between 18-25 mesh.

2.11 PHYSIOLOGICAL EFFECTS

The effects that adsorption onto Dowex 1, X-4, 100-200 mesh chloride form had on the activity of an organism, E. coli Yamaguchi strain, was

compared to the free cells activity (Hattori and Furuska; 1960). The oxidation activity of the substrates, succinate, lactose, alanine and fumarate were compared. At a pH of 7 the oxidative activities of the adsorbed cells decreased from 7.2 % to 28.0 % of that of the free cells, Table 2.11.1.

Table 2.11.1: Oxidative Activities of Substrates with Free and Adsorbed Cells, pH = 7.0
(Hattori & Furuska, 1960)

Substrate	Activity[1]		Remaining activity after adsorption[2]
	free cell	adsorbed cell	
succinate	108 (μl)	17 (μl)	15.7 (%)
lactose	54	12	20.2
alanine	43	12	28.0
fumarate	97	7	7.2

1) Activity was expressed the amount of O_2 uptake by 10^9 cells for an hour.
2) {(activity of adsorbed cell)/(activity of free cell)} x 100

pH shifts for immobilized enzymes have been observed. These shifts are thought to occur because the support controls the solution environment near the enzymes.

Activity of free cells for the aforementioned substrates show a peak around a pH of 6-7. The peak of activity for the substrates of the adsorbed cells occurs at a pH level one unit greater than that of the free cells. The phenomena of having a peak of activity one pH unit greater for adsorbed cells is referred to as the B effect. The authors hypothesized that a cationic layer of molecules surrounds the anionic layer on the surface. This cationic layer is thought to be hydrogen ions. This would increase the H^+ concentration thus decreasing the pH which the adsorbed cells are exposed.

The rate of growth of adsorbed E. coli was found to be faster than that of the free cells and has an optimum growth rate approximately one pH unit higher than the free cells; this is similar to chemical activities B effect (R. Hattori et al, 1972). This result is surprising since the cells presumably buffer their contents. The mechanism for this effect is not yet explained. Detached cells also show a faster rate of growth than free cells and also show lesser oxygen uptake than that of free cells (R. Hattori et al,1972). The detached cells show a decreased lag time for enzyme induction than there is for free cells (R Hattori et al, 1972).

2.12 IMMOBILIZED FERMENTATIONS

The use of ion exchange resins as an immobilization technique for continuous fermentations is limited. Most of the studies involve ion exchangers as separation devices for microbiology and medicine (Rotman,

B., 1960; Puck, T.T. & Sagik, B. 1953; Zvaginstev & Gusev 1971; Daniels, S. L.,1972, 1966).

A potential advantage of using ion exchange resins in fermentations is that these supports can be regenerated, sterilized and inoculated without leaving the reaction vessel. This is a great advantage when attempting to keep aseptic conditions (Groom et al,1988).

Anion exchangers were chosen as the carrier for all tests in this study. No studies were found that incorporated cation exchange resins. Many types of reactors have been used: packed columns, stirred tank and airlift fermentors.

The fermentation of ethanol using Zymomomas mobilis ATCC 29191 adsorbed onto a weak base anion exchanger, DEAE-cellulose, was studied using a stirred tank reactor (Bar, et al,1987). During the study it was concluded that the use of the stirrer caused shear induced cell detachment. At the impeller speed of 190 rpm the adsorption of cells dropped from 41 mg/g resin to 31.5 mg/g resin after four minutes and eventually leveled off at 20.1 mg/g resin after 90 minutes. When the agitation was decreased to 70 rpm the adsorption increased to 34.5 mg/g resin after ten minutes. For the speeds of 500 and 1000 rpm the final adsorption decreased to 20.1 and 11.0 mg/g resin respectively. Even at the lower speed of 70 rpm the adsorption never returned to the initial value of 41 mg/g resin. The method of calculating sorption was done by extracting the cell protein by boiling in an alkaline solution, followed by a protein assay.

The effects of reactor composition also was investigated (Bar et al,1987). It was found that cell adsorption capacity decreased due to

effects of glucose, ethanol and other unknown components in the fermentation broth. This study concluded that environmental effects in the reactor could change adsorption equilibrium from that observed in a static cultures.

Another investigation using Zymomonas mobilis ATCC 29191 was done using a packed column reactor for the continuous production of ethanol (Krug & Dougalis,1983). The carrier used was the Rohm and Haas macroreticular strong base anion exchanger IRA 938. This reactor showed an increase in ethanol productivity, 89.8 g/L·hr to 377.4 g/L·hr, with an increase in dilution rate, 2.2 hr^{-1} to 11.2 hr^{-1}. Though the productivity increased, the effluent concentration of ethanol decreased from 40.8 g/L to 33.7 g/L and the effluent cell concentration increased from 1.01 g/L to 1.45 g/L, attributed to increased shear due to increased flow. The percentage of glucose used from the feed, 100 g/L, decreased from 97.4% to 79.6% with the increasing dilution rate.

A problem encountered with this system was that it could not run for long periods (greater than 200 hours) due to the reactor fouling because of filamentous growth occurring in the packed bed.

One of the major uses for immobilized cells is to increase production of enzymes and/or specialty chemicals. One study used the organism Bacillus amyloliquefaciens NRC 2147 (National Research Council, Ottawa) with a packed bed reactor and stirred tank reactors, batch and continuous modes, with the Rohm and Haas large pore macroreticular anion exchange resin, Amberlite XE-352, for the production of α-amylase (Groom et al,1988). The results of this experiment showed that the highest concentrations and productivities were achieved with the packed bed reactor system (18700 amylase

activity units per liter (18.7 kU/l) and 9.7 kU/L-hr respectively). Biofouling for this packed bed was avoided by periodically feeding the reactor with medium lacking in soluble starch and yeast extract. This system had a greater productivity than batch and continuous modes of a free cell stirred tank reactor but a smaller concentration of α-amylase than the batch reactor (150 kU/L).

In a study using the thermophile, an organism with optimum growth between 55-75°C, Bacillus stearothermophilus ATCC 29609 was used to produce α-amylase. The support used was the Rohm and Haas macroreticular strong base anion exchanger IRA 938. This resin was chosen because of its large surface area as compared to a solid bead. This structure proved to be too fragile for a stirred tank reactor as the carrier fractured within minutes after agitation started. Due to its high oxygen transfer and low shear, an airlift fermentor was chosen (Grulke et al,1989). The study was done at an operating temperature of 55°C. The continuous production of α-amylase was greater for the immobilized cells than the free cell experiments. This study also shows a particular advantage of ion exchange resins having stability at higher temperatures. Most gels used for entrapment tend to degrade at

TABLE 2.12.1: Summary of Anion Exchangers used in Continuous Fermentations

-overproduction of products occurs -environmental (i.e: impeller, product and substrate concentrations) effects will change the equilibrium adsorption of cells from those found from static cultures -packed bed reactors give a greater productivity than stirred tank reactors -packed bed reactors are susceptible to biofouling -are suitable carrier for thermophiles

temperatures suitable for thermophiles.

A summary of the use of anion exchangers in fermentations is shown in Table 2.12.1.

2.13 RECYCLE

On experimentation with recovery of adsorbed cells, it was found with Staphylococcus aureus that contacting supports with salt solutions can promote the cells to desorb (Wood;1980). The amount of recovery of Staphylococcus aureus is dependent on the pH of the solution and on the salt concentration, reaching a peak at 0.6 M NaCl. Concentrations of sodium and calcium chloride were varied and showed that conditions that may inhibit adsorption are not necessarily going to promote recovery. The same experiments were replicated for E. coli and little promotion of recovery was found to occur (Wood, 1980).

2.14 SUMMARY

The quality sought after is a large amount of cells adsorbed. The ion exchanger best suited for this are anion exchangers with a large surface area. The environmental effects of pH and salt concentration should have a negligible effect on the amount of E. coli adsorbed for optimum growth conditions.

A summary of ion exchange for E. coli is given in Table 2.14.1.

TABLE 2.14.1: Summary of Ion Exchange for *E. coli*

-is an electrostatic interaction with cell surface carboxyl groups -depends on surface area -has Langmuir type adsorption isotherm -anion exchangers show best adsorption -negligible change in capacity noticed for pH of 6-10 and for varying salt concentrations -will change the optimum activity by one pH unit

CHAPTER III: MATERIALS & METHODS

Methods needed for this study are growth plates of the organism, overnight cultures of organism, preparation of resins for adsorption and analysis of the adsorption.

3.1 ORGANISM AND CULTURES

Organism

The genetically engineered organism Escherichia coli 246 (EC246), an organism created in Patrick J. Oriels laboratory (Michigan State University) was used for this study. It has a resistance to 10 x 10^{-6} g/ml solution of the antibiotic chloramphenicol and produces thermostable α-amylase than a wild type Bacillus stearothermophilus (Oriel).

Other data used concerning E. coli are in Tables 3.1.1 and 3.1.2.

TABLE 3.1.1: Physical Parameters E. coli

Mass:	9.5 x 10^{-13} g/cell
Dimensions:	
length:	2 μm
width:	.5 μm

TABLE 3.1.2: Optimum Growth Conditions E. coli

pH:	7.2
Temp. (°C):	37

Growth Plate

A growth plate of the frozen E. coli culture are made by the following technique. The growth support composition is shown in Table 3.1.3 (Oriel).

The LB (Table 3.1.4), agar and H_2O are mixed, taken to a pH 7.2 and autoclaved for 30-45 minutes. The solution is cooled to 50°C

TABLE 3.1.3: Growth plate composition

20 g LB broth
20 g Bacto Agar
1000 g dH_2O
10 mg. chloramphenicol

TABLE 3.1.4: LB broth composition

Ingredient	wt. %
Select Peptone 140 (Pancreatic digest of Casein)	50
Select Yeast Extract	25
Sodium chloride	25

before adding of the chloramphenicol. Since chloramphenicol does not dissolve in water, a solution of 10 mg/ml chloramphenicol/ethanol solution is used to transfer the antibiotic into the plate solution. When the antibiotic is added, the solution should be gently agitated. The solution is poured into petri dishes that are resting on a level surface
until a thickness 0.5-1.0 cm. is obtained. The dish is covered with its lid and allowed to cool to room temperature.

At this time the solution is in a gel form and some condensation may occur on the inside of the cover. The plate is placed in a 37°C

oven until condensation dissipates. The plate is ready for inoculation.

A thawed sample of the organism is required to inoculate the plate. A sterile inoculating loop (heated over a flame until red) is used to pick up a sample of the organism and spread a thin layer of it onto the growth plate. The plate is covered and placed in a 37°C oven so the organism can grow (24-48 hrs.).

When the plate is grown it is stored at 4°C to help prevent further growth. This plate is sealed with Para-film and is used to obtain cells for overnight cultures.

Overnight Cultures

Overnight cultures were started with 100 ml. of sterile LB broth containing 10 $x10^{-6}$ g/ml of chloramphenicol in a 250 ml. shaker flask. A heat sterilized inoculating loop was used to take a sample of the organism off the growth plate and place it into the LB broth. When a few samples of organism had been placed in the broth, the shaker flask was covered and placed in a 37°C shaker at 180 rpm and left overnight. The growth plate was sealed and placed back into 4°C storage.

3.2 MEASURING CELL CONCENTRATION

Optical Density

Optical density is used to measure the concentration of Escherichia coli in solution. A Klett-Summerson colorimeter with a green filter (400-450 $x10^{-8}$ m wave length) was used.
The concentration at low Klett readings are 1 x 10^{6} cells/ml.
solution per 1 Klett. (Oriel)

Klett vs. cell concentration calibration

To get a Klett vs. concentration function, an overnight

culture was grown and the solution centrifuged at 8,000 rpm for ten minutes to separate cells from solution. The cells were then resuspended with .05 M Tris buffer at pH 7.5 to a Klett of 500. This solution was then diluted with a known amount of Tris buffer solution and a Klett reading was taken after each dilution. This step was repeated until a Klett of 50 was obtained. This procedure was done twice on each sample.

Knowing the amount of cells at the lowest Klett reading the amount of cells for the entire solution can be found by using:

$$\text{total \# cells} = (\text{Klett})(1 \times 10^{6})(\text{Vol. solution})$$

Assuming a negligible amount of cells lost during the procedure the concentration to corresponding Klett reading can be found by:

$$\text{Klett} = \text{total \# cells}/(\text{volume})$$

To obtain the cell mass multiply the above by 9.5×10^{-13} g/cell. This calibration curve is shown in Figure 3.2.1 and the equations used for calculating concentrations, found by least square linear regression, are found in Table 3.2.1.

TABLE 3.2.1: Linear equations for the calculation of concentration

Klett (x)	Slope (m)	Y-intercept (b)
0 - 125	9.500×10^{-7}	0.0
126 - 300	1.242×10^{-6}	-3.75×10^{-5}
300 - 500	1.711×10^{-6}	-1.80×10^{-4}

The Klett meter should warm up for 10-15 minutes after being turned on. It should be zeroed with distilled water in the Klett test tube. After the zero is initially set take the test tube out and set the dial to a Klett reading of sixty. Reinsert the test tube and take

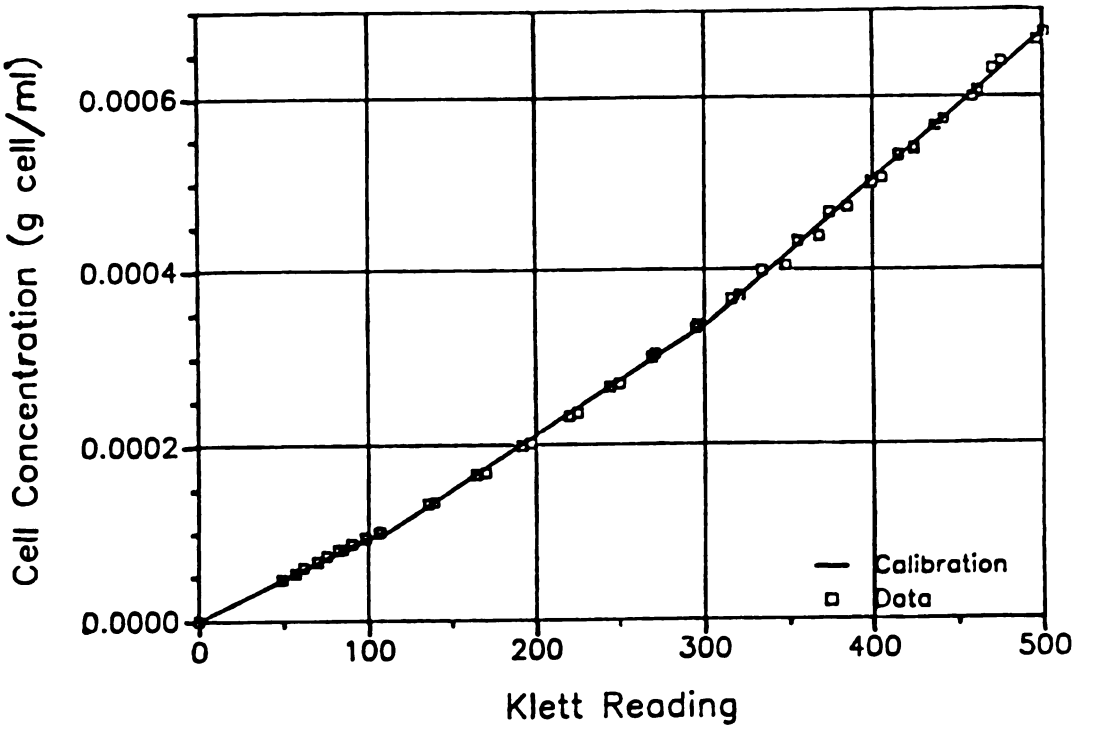


Fig. 3.2.1: Calibration of Klett optical density for Escherichia coli

the dial back to the zero reading. When the arrow matches with the center line the machine has been zeroed.

Any undue vibrations on the meter's foundation can lead to false readings.

3.3 ION EXCHANGE RESIN

Resin Preparation

Ion exchange resins need to be conditioned prior to use. The ion exchange resin was prepared in its chlorine form by the following procedure as suggested to Patrick J. Oriel by Rohm and Haas. This procedure is specific for anion exchangers. The hydroxide form is avoided as it can cause changes in pH.

1.) Contact the resin with a 10% NaCl solution for one hour at 37°C.

2.) Filter resin from NaCl solution and contact with a 4% HCl solution for one hour.
3.) Filter resin from the HCl solution and thoroughly rinse resin with distilled H_2O.
4.) Let resin dry overnight at 55°C.

3.4 ADSORPTION

Equilibrium Isotherms

Equilibrium isotherms were determined by the following procedures.

1.) Grow an overnight culture of EC246 in sterile LB broth 37°C.
2.) Centrifuge cells of the overnight culture at 8,000 rpm for 10 minutes.
3.) Pour off supernate and resuspend cells in a Tris buffer solution of known molarity and pH until solution has a Klett reading of 450.
4.) Contact two 0.4 g samples of dry ion exchange resin with 9 ml of resuspended cell solution and put into a 37°C shaker at 180 rpm.
5.) Dilute resuspended cell solution to approximately Kletts of 400, 350, 300, 250, 200, 150, 100, 50 and 0 and repeat step 4 for each different concentration.
6.) After 24 hr. take optical reading of each solution.
7.) Perform material balance on system to obtain adsorption of cells onto ion exchange resin.

Kinetics

To acquire kinetic data prepare ion exchange resin as before and perform the following procedure.

1.) Repeat steps 1-3 of previous procedure.
2.) Contact three 0.5 g samples of dry resin with 10 ml of known concentration resuspended cell solution.
3.) Take optical density readings of cell solutions at .25, .50, .75, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0 and 24 hours.
4.) Perform material balance on system to obtain adsorption of cells onto ion exchange resin.

A 5 ml. syringe with a fine mesh screen was used to separate the cell solution from the ion exchange resin. This prevented the large resin particles from interfering with the optical density.

3.5 RESIN POROSITY & AREA

Porosimetry

The Micrometrics Poresizer 9310 mercury poresemeter was used with 3 cc powder penetrometer to obtain surface area information. The data for this study were pore sizes larger than 1 micron in diameter. No adsorption should occur in smaller diameters because the minimum dimension of E. coli is .5 μm .

3.6 SCANNING ELECTRON MICROSCOPY

The Jeol T-330 scanning electron microscope was used with Polaroid type 52 black and white film with a f-stop of 16 was used to examine the resin and the cells sorbed to the resin particles. (Appendix 1)

The pictures with the immobilized cells on the resin were produced with the help of the Electronic Imaging Center at Michigan State University. The steps used to prepare the immobilized cells are a series of ethanol drying steps and a critical drying step. (Appendix 2)

3.7 PRODUCTION

Batch Growth

The batch growth of the cells and α-amylase production were compared by the following procedure.

1.) Grow an overnight culture of EC246 at 37°C in sterile LB broth.
2.) Prepare ion exchange resin in chloride form.
3.) After 24 hrs. put 5.0 g of ion exchange resin into 100 ml. of sterilized LB media with corresponding amount of chloramphenicol and inoculate with 10 ml. of overnight culture.
4.) At periods of 24, 48 72, and 96 hours after inoculation take a Klett reading and take a small (approx. 1ml.) sample of fluid, for all cultures.
5.) Spin cells out of fluid sample and keep refrigerated.

6.) Do an α-amylase assay on the fluid samples, Campbell and Manning as modified by Oriel and Schwacha (Appendix 3).

3.8 RESIN RECYCLE

To check on the recyclability (the reuse of a resin already used for adsorption) of the ion exchange resins, the following procedure was followed:

1.) Prepare chloride form of resin as previously mentioned.
2.) Perform an equilibrium experiment on resin, as already described.
3.) When equilibrium experiment is through, separate the resin from the cell solution and rinse resin with 500 ml. distilled water.
4.) Prepare chloride form of resin as done in step one.
5.) Repeat step two.
6.) Compare cell loading for each recycle run.

CHAPTER IV: RESULTS & DISCUSSION

This study used anion exchangers and cation exchangers from Dow Chemical Company. This series contains a variety of particles sizes, morphologies and charges. Due to its use by other researchers for immobilization (Krug & Dougalis, 1983; Grulke et al, 1989) the Rohm and Haas anion exchange resin IRA-938 was studied also. The purpose was to determine how various resin properties affected sorption of cells. All resins investigated are shown in Table 4.0.1.

4.1 BATCH SORPTION EVALUATION OF RESINS

All resins were evaluated in batch sorption tests to determine the sorption of cells per gram of resin, at typical pH for growing E. coli of 7.5. To obtain the amount of cell adsorption onto the resin a cell balance was performed on the slurry. The final concentration of cells was subtracted from the initial concentration of cells, multiplied by the volume of solution and divided by the dry mass of resin in solution. (Cell mass on a wet basis).

$$Ads = \frac{(C_0 - C)\cdot V}{M}$$

Ads ≡ amount of cells adsorbed (g cells/g dry resin)
C_0, C ≡ concentration cells in solution (g cells/ml), initial and time t
V ≡ volume of solution (ml)
M ≡ mass dry resin (g)

The assumptions used for this adsorption study are listed in Table 4.1.1.

TABLE 4.0.1: Information on Resins Studied*

Name (Codes)	C/A	S/I/W	Type	Structure, Mesh
XU-40123.00	A	W	——	Epoxy (14-40)
XU-40091.00	A	I	——	Epoxy (14-40)
XU-40196.01	A	S	I	Gel 550 μm
XU-434-200.00	A	S	I	Gel (200-400)
XU-434-200.01	A	S	I	Gel (100-200)
XU-434-200.02	A	S	I	Gel (50-100)
XY-40032.00	C	S	——	Macro. (16-45)
XF-43356.00	A	W	——	Macro. (16-50)
XUS-43419.00	A	W	——	Macro. (16-50)
IRA-938**	A	S	I	Macro. (20-50)
XUS-40187.00	A	S	I	Macro. (16-45)
XU-40170.00	A	S	II	Macro. (16-50)
XUR-0525-187-239.00	A	S	I	Macro. (20-50)
XUR-0525-187-239.01	A	S	II	Macro. (20-50)
XUR-0525-187-239.02	A	S	——	Macro. (20-50)
XUR-0525-187-239.03	A	W	——	Macro. (20-50)
XUR-0525-187-239.04	A	W	——	Macro. (20-50)
XUR-0525-187-239.05	A	W	——	Gel (20-50)
XUR-0525-187-239.06	N/A	N/A	N/A	Acrylic (20-50)
XUR-0525-187-239.07	N/A	N/A	N/A	Acrylic (20-50)
XUR-0525-187-239.08	N/A	N/A	N/A	Acrylic (20-50)
XUR-0525-187-239.09	N/A	N/A	N/A	Acrylic (20-50)
XUR-0525-187-239.10	N/A	N/A	N/A	Acrylic (20-50)

C= cation, A= anion, S= strong, I= intermediate, W= weak
*All Dow resin except were noted, ** Rohm & Haas, N/A= not avail.

The first set of resins investigated were the XUR-0525-187-239 series. This set was chosen because they were the first

TABLE 4.1.1: Assumptions made for study

- all cells viable - cells do not reproduce - negligible amount of cells adsorbed onto glass vial - negligible amount of cells and/or solution lost during separation for optical density readings - negligible amount of resin in solution while taking optical density - negligible amount of swelling occurred

resins obtained from Dow to study. As can be seen (Fig. 4.1.1) of the ten XUR-0525-187-239 resins samples .02, .03 and .04 show the best adsorption. Resin XUR-0525-187-239.00 poor adsorption may have been caused by the exchange groups being produced with a OH^- group instead of the usual Cl^- ion (Greenberg, 1989).

Mechanical Strength

A more thorough kinetic study was done on XU-43419.00, XF-43356.00, XU-40123.00, XU-40091.00, XUS-40187.00 and XU-40170.00. A "negative" sorption is due to the presence of small pieces of resin sorbing in the light path. As shown in Figure 4.1.2 the resins XU-43419.00 and XF-43356.00 have a "negative" adsorption. XU-43419.00 started to fracture and continue to fracture throughout the trial and XF-43356.00 had good adsorption and then fractured. XU-40123.00 initially gave a "negative" adsorption then the cells sorbed onto the support with time, suggesting that it initially fractured a small amount and then stopped.

XF-43356.00 suggests that resins should be agitated for some time to

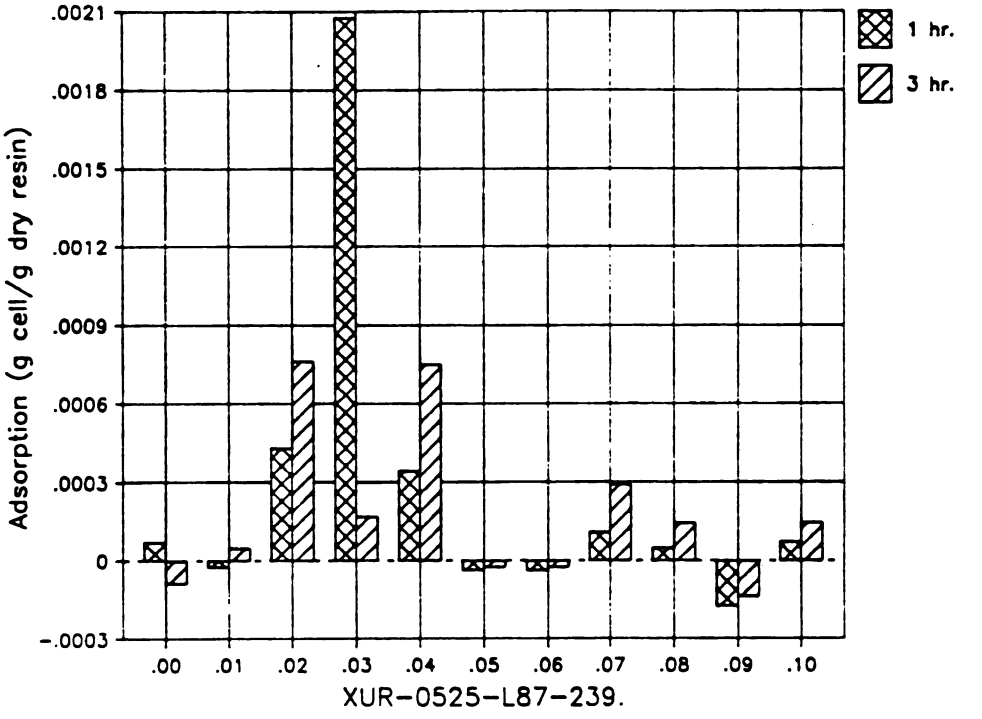


Fig 4.1.1: Batch Adsorption of XUR-0525-187-239. series of resins
Init. cell conc. = 3.24 x 10^{-4} g cell/ml
pH = 7.5, .05 Tris buffer

determine wether they have good mechanical strength. When these solutions were observed under a light microscope small portions of resin were found floating. This verified that resin fragments result in an increase in light adsorption.

The resins XU-43419.00 and XF-43356.00 are both macroporous suggesting that some macroporous resins are not strong enough for the agitation that may be encountered.

XU-40091.00 also gave an initial "negative" adsorption (Fig.4.1.3). It was concluded that the resins were too fragile at the conditions used. The epoxy resins XU-40123.00 and XU-40091.00 have a tendency to degrade in basic conditions (Greenberg, 1989).

Further Evaluation

Figure 4.1.4 show the resins, XUS-40187.00 and XU-40170.00, compared during a kinetic study, show a positive adsorption thus good mechanical strength.

The resins XU-434-200.00, XU-40196.01, XY-40032.00 and IRA-938 were screened by comparing the samples after contact for twenty-four hours with cell solution at a typical cell concentration of E. coli under normal conditions (Table 4.1.2).

TABLE 4.1.2: Adsorption of cells by ion exchangers
Initial Concentration: .00043 g cell/ml

Resin	Adsorption (g cell/g dry resin)
IRA-938	0.00855
XU-434-200.00	0.00590
XU-40196.00	0.00195
XY-40032.00	0.00197

To further study a resin it was decided that a positive adsorption greater than .001 g cell/g dry resin was required and in the case of the XUR-0525-187-239. series the largest sorptions were required. From this initial screening period the resins suitable for further study are shown in Table 4.1.3. There is a possibility of missing good resins by this evaluation technique since the equilibrium isotherm is not known.

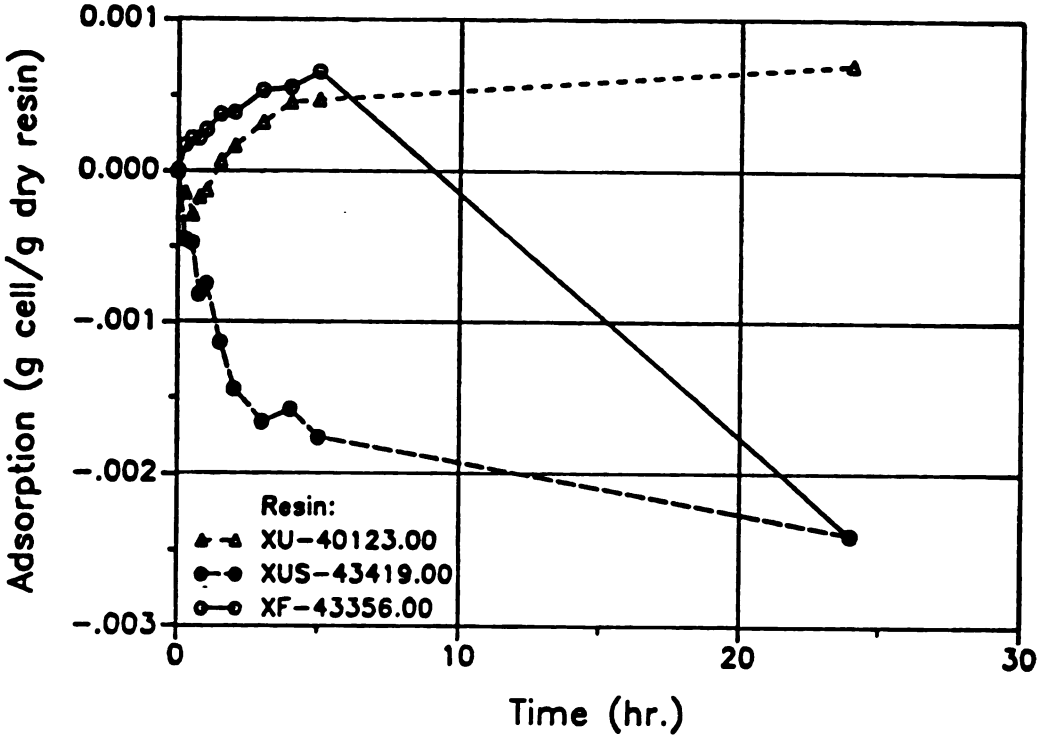


Fig. 4.1.2: Comparison of kinetics of three resins
pH = 7.5, .05 M Tris buffer

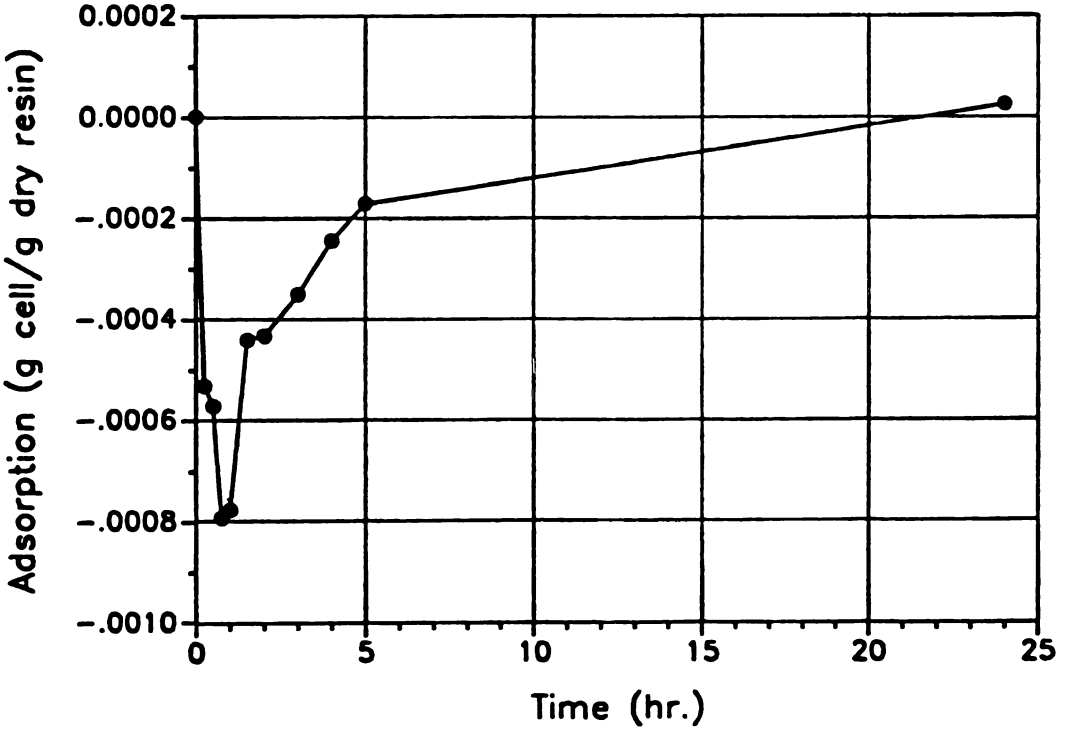


Fig. 4.1.3: Kinetics of XU-40091.00
pH = 7.5, .05 M Tris buffer

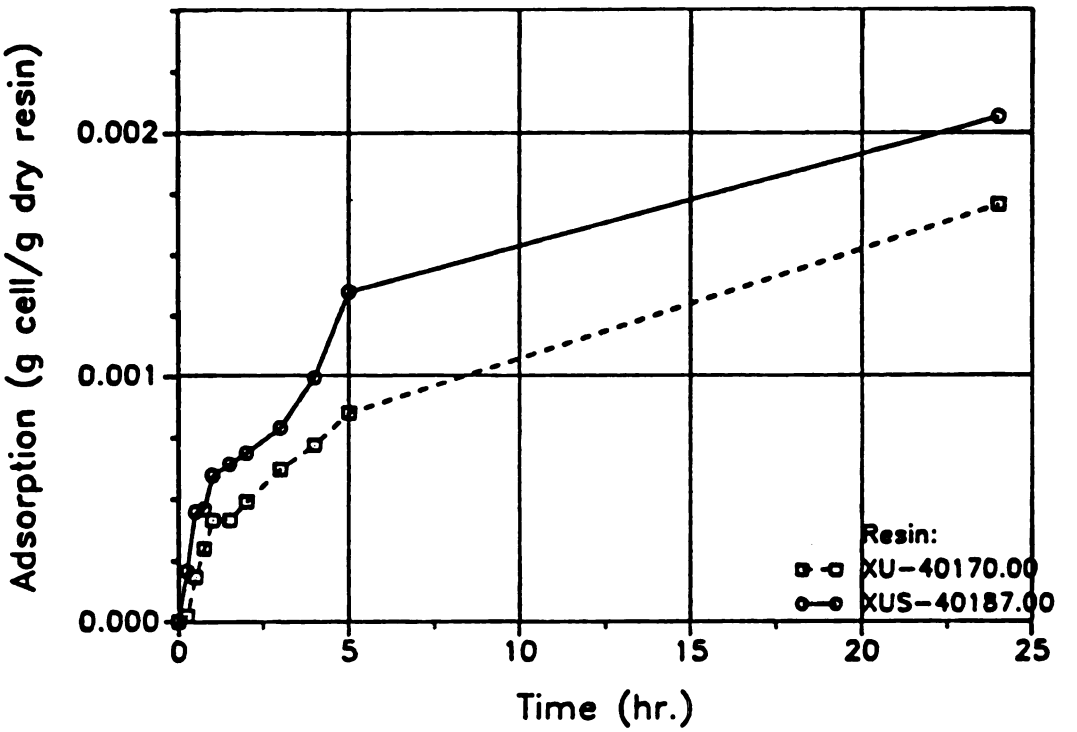


Fig. 4.1.4: Comparison of kinetics of two resins
pH = 7.5, .05 M Tris buffer

TABLE 4.1.3: Positive Resins after initial Evaluation

Resin	C/A	S/I/W	Type	Structure
XUS-40187.00	A	S	I	Macro.
XU-40170.00	A	S	II	Macro.
XU-40196.00	A	S	I	Gel
XY-40032.00	C	S	———	Macro.
XUR-05250187-239.02	A	S	I	Macro.
XUR-05250187-239.03	A	W	———	Macro.
XUR-05250187-239.04	A	W	———	Macro.
XU-434-200.00	A	S	I	Gel
IRA-938	A	S	I	Macro.

Table 4.1.3 shows that gel and macroporous resins have good sorption while the acrylic resins had poor adsorption of E. coli. All but one resin giving good adsorption were anion exchangers.

For further evaluation, samples of the resins XY-40032.00, XUR-

0525-187-239.02, .03 and .04 were contacted with a cell solution for twenty-four hours and compared with XU-434-200.00 and IRA-938 (Table 4.1.4 & Table 4.1.5). From Tables 4.1.4 and 4.1.5 it is shown that the resin XU-434-200.00 has a much better sorption than the resins XY-40032.00, XUR-0525-187-239.02, .03 and .04, from 1.76 - ∞ times better sorption. The resin XUR-0525-187-239.03 looks like it might not be mechanically strong after noticing the decrease in adsorption during the initial batch adsorption (Fig. 4.1.1). When all the resins that had positive cell adsorption during these batch adsorption tests are compared IRA-938 had the best adsorption. The resins XUS-40187.00 and XU-40170.00 were kept after initial screening to observe the differences between Type I and Type II strong base anion exchangers.

TABLE 4.1.4: Adsorption of cells by anion exchangers
Initial Concentration: .00017 g cell/ml

Resin	Adsorption (g cell/g dry resin)
IRA-938	0.00300
XU-434-200.00	0.00214
XUR-0525-187-239.02	0.00121
XUR-0525-187-239.04	0.00000

TABLE 4.1.5: Adsorption of cells by anion exchangers
Initial Concentration: .00035 g cell/ml

Resin	Adsorption (g cell/g dry resin)
IRA-938	0.00799
XU-434-200.00	0.00526
XUR-0525-187-239.03	0.00299
XY-40032.00	0.00110

4.2 CELL SORPTION EQUILIBRIA

Resins with high cell sorption were tested over a range of conditions to determine the effects of pH, surface charges, buffer concentration, surface area, cross-linking and porosity on cell sorption. Comparison of pairs of resins will allow us to determine the effects of each variable on sorption.

Isotherms

As discussed in Chapter 2, the Langmuir Isotherm fits the cell sorption data for this system (Eqn. 1).

$$Ads = \frac{K1 \cdot C}{K2 + C} \qquad (1)$$

Ads ≡ amount of cells adsorbed (g cells/g dry resin)
C ≡ concentration cells in solution (g cells/ml)
K1 ≡ resin capacity (g cells/g dry resin)
K2 ≡ inverse of equilibrium constant (g cells/ml)

K1 and K2 can be determined by several methods. The cell concentration times the inverse of equation 1 is linear (Eqn 2). A least squares linear regression was done to calculate the slope and Y-intercept.

$$\frac{C}{Ads} = \frac{C}{K1} + \frac{K2}{K1} \qquad (2)$$

As shown in Figure 4.2.1 the data does come out to be linear and the calculated K1 and K2 of the Langmuir equilibrium provided good fit to the data (Fig. 4.2.2). K1 gives the capacity of the resin when the cell concentrations in the medium are high. K2 is the inverse of the equilibrium constant this parameter is better when it is small because the resin will reach its cell capacity at a lower cell concentrations and at lower K2 the equilibrium constant will increase thus favoring

conditions for the immobilized cells. A low K2 suggests a stronger interaction between the cells and resin surface.

The reason for an equilibrium between the cells and the binding sites of the ion exchange resin to exist are not known. One would suspect that the cells would bind with all the available sites until few sites are left. Possible causes of the equilibria phenomena are thought to be the resin binding with lipopolysaccharides that may have come off the cells and put in solution or the cells hindering adjacent binding sites.

The purpose of our study is to find resins having the largest amount of cells sorbing onto the resin. This parameter, K1, will be the center of this section since it provides a good comparison of resin capacity.

The results of the equilibrium tests for the various resins are shown in Table 4.2.1 for pH of 7.5 and .05 M Tris buffer solution. From this test it is shown that the best resins for adsorption, have the largest K1, are XU-434-200.00 and IRA 938. Both of the best adsorbers are Type I strong base anion exchangers.

TABLE 4.2.1: Equilibrium Constants K1 & K2 at pH = 7.5, Tris = [.05]

RESIN	K1 (g cell/g dry resin)	K2 (g cell/ml)	r
XUS-40187.00	.004664	.0004187	.920
XU-40170.00	.000961	.0000089	.946
IRA-938	.008819	.0000039	.995
XU-434-200.00	.005050	.0000008	.998

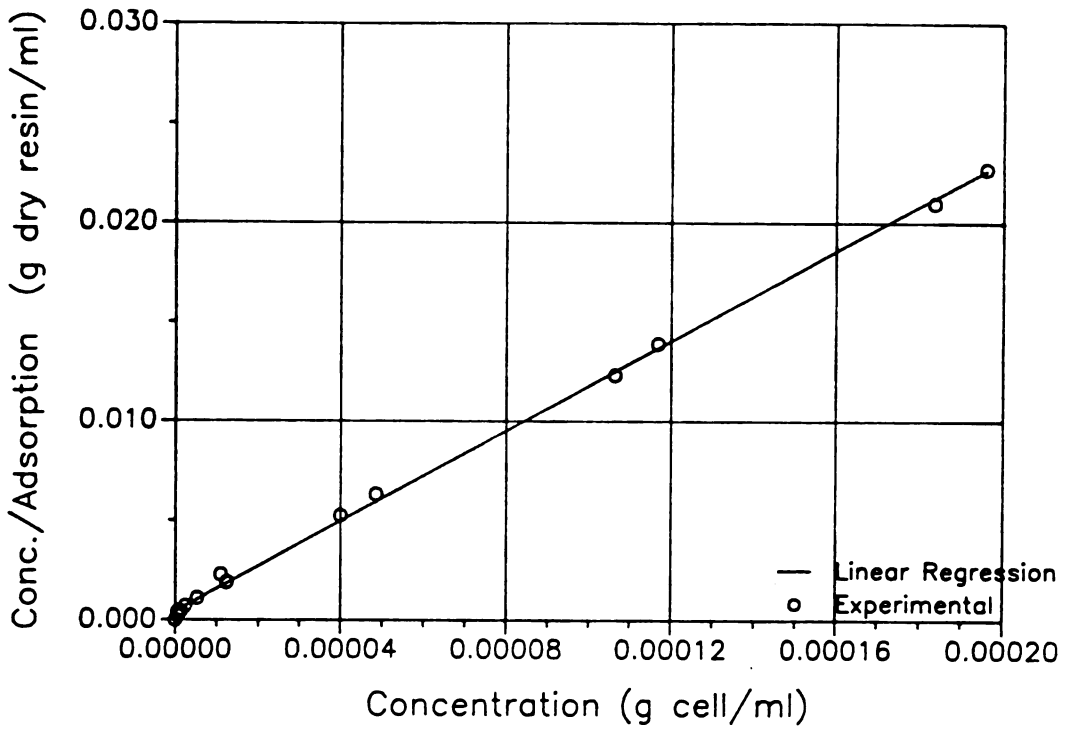


Fig. 4.2.1: Linearization of Langmuir Isotherm
Resin: XU-434-200.00
pH = 7.5, .05 M Tris buffer

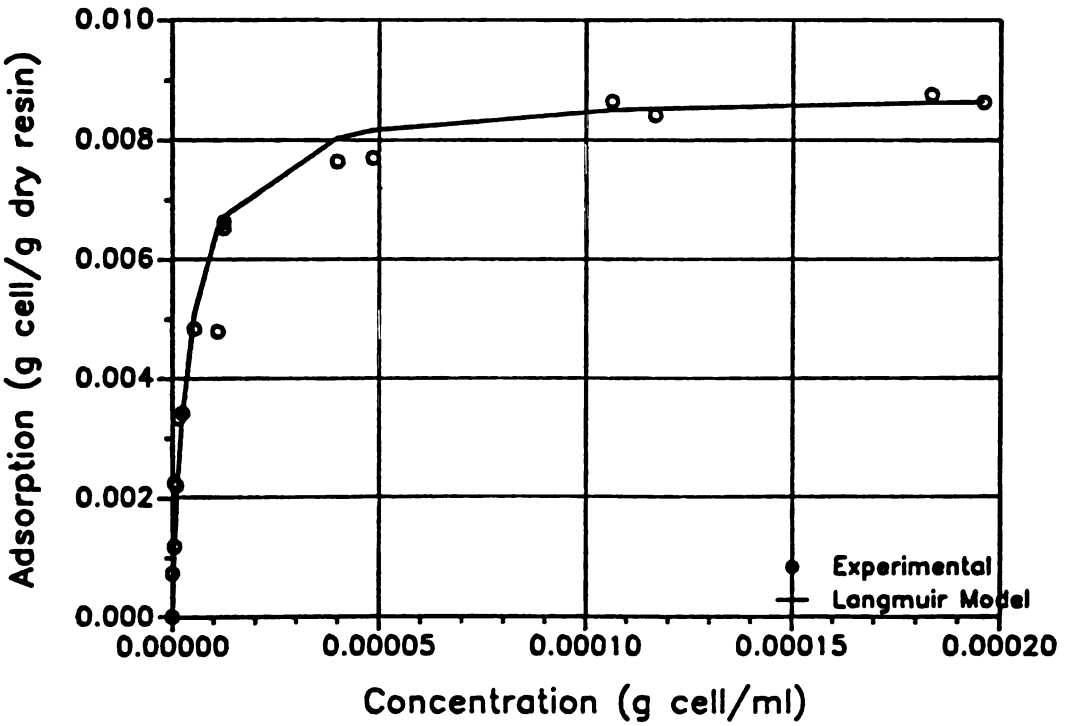


Fig. 4.2.2: Langmuir Model of Adsorption
Resin: XU-434-200.00
pH = 7.5, .05 M Tris buffer

Ion Type

The resins XUS-40187.00 and XU-40170.00 differ only in their exchange groups; Type I and Type II. They both have similar charge densities. Table 4.2.2 and Fig. 4.2.3 show that the Type I strong base anion exchangers are better than the Type II strong base anion exchangers. The Type II exchanger is "saturated" at coverage of .001 g cells/g dry resin at solution concentrations below a typical level of .0003 g cell/ml. Therefore, a Type II support would not add significant amount of cells to a reactor at normal cell loadings. However Type II support might be good at low cell concentrations due to it having a smaller K2. These two resins are a good comparison since they are made with the same polymer matrix and are of the same mesh sizes, hence similar surface areas.

TABLE 4.2.2: Equilibrium Constants K1 & K2 for Type I & II strong base anion exchangers at pH = 7.5, Tris = [.05]

RESIN	Type	K1 (g cell/g dry resin)	K2 (g cell/ml)
XUS-40187.00	I	.004664	.0004187
XU-40170.00	II	.000961	.0000089

Surface Area

Since Langmuir type equilibrium suggests monolayer adsorption, the amount of surface area available is of great importance to the amount of adsorption that may occur. Surface areas of the resin were measured using mercury porosimetry. The surface areas (m^2/g) are reported for pore sizes greater than 1 μm diameter. The cells cannot fit into of smaller pores. The XU-434-200.00 studied for surface area has a different adsorption capacity than reported in section 4.2.1 because

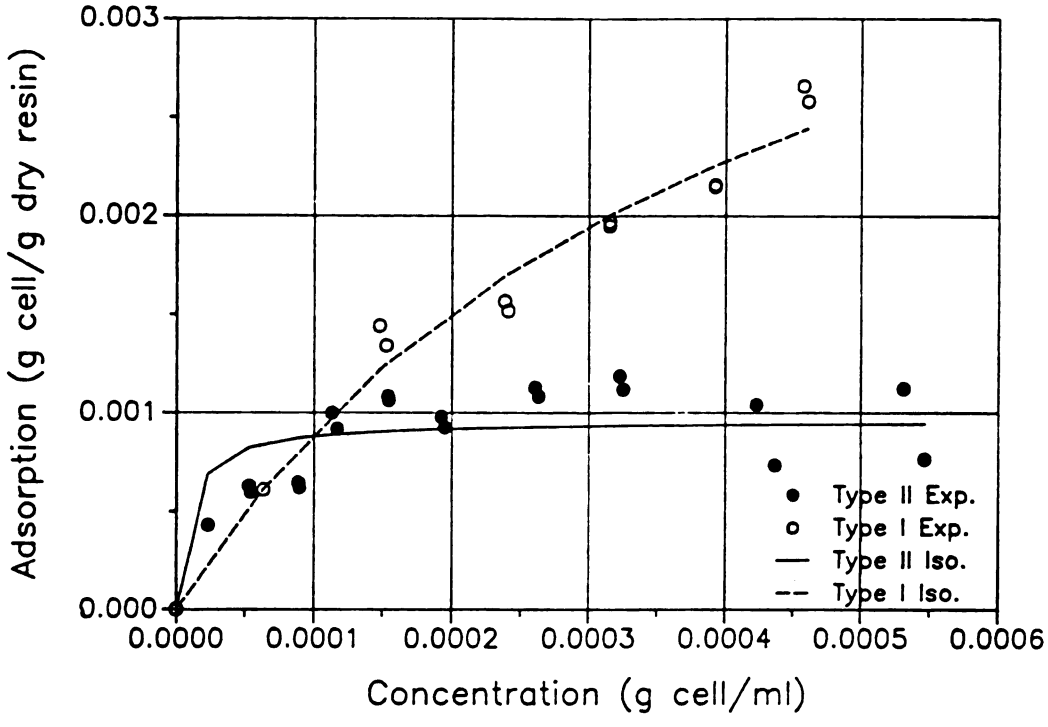


Fig. 4.2.3: Comparison of Type I & Type II Strong Base Anion Exchangers
Resin: Type I: XUS-40187.00
Type II: XU-40170.00
pH = 7.5, .05 M Tris buffer

the resin studied forsurface area came from a different stock bottle of material and may of had a different surface area. Spheres this small, mesh size 220-400, are currently state of the art and could have some variances due to production (Greenberg, 1988). Differing stocks of sphere (mesh) sizes of the anion exchanger XUS-434-200 were available in 50-100 mesh, 100-200 mesh and 200-400 mesh. As the mesh sizes increase the diameter of the spheres decrease thus, assuming the density is constant, the surface area per mass of resin should increase.

TABLE 4.2.3: Adsorption Capacity and Surface Area

RESIN	K1 (g cell/g)	K2 (g cell/ml)	AREA (m^2/g)	POROSITY (cc/g)
IRA-938[1]	.008819	.0000039	.4279	1.1435
XU-434-200.00	.008867	.0000081	.0922	0.6787
XU-434-200.01	.006051	.0000082	.0549	0.6406
XU-434-200.02	.002660	.0000025	.0269	0.5917

Table 4.2.3 shows that IRA-938 has the greatest amount of surface area of the resins checked and that it is also has the largest porosity. The adsorption capacity, K1, shows to depend on the amount of surface area present and the inverse equilibrium constant, K2, does not show any trend with either the porosity or the surface area available. As shown in Figures 4.2.4 and 4.2.5 that the surface of the XU-434-200.00 is smooth with no visible pores and that the IRA-938 is very convoluted and has many pores. By looking at the cells on the surface of a resin (Fig. 4.2.6) the cell dimensions were found to be 3 μm x .5 μm. By taking the cell area, cell width times length, and multiplying it by the number of cells adsorbed onto the resin the amount of area can be obtained (Eqn. 3).

$$\text{Area used by cells} = \frac{(\text{area } (m^2/\text{cell})) \cdot K1 (\text{g cell/g dry resin})}{9.5 \times 10^{-13} \text{ g/cell}} \qquad (3)$$

The dimensions used for this study, cell length and width, are for the minimum cell packing on the resin. The cell diameter, 0.5 μm, would be for the maximum cell packing.

---

[1] The reported surface area and porosity for IRA-938 are for pores greater than 1 μm in diameter. The absolute values are higher but not accessible to cells.

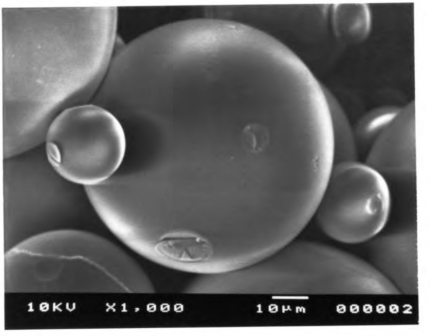


Fig. 4.2.4: Surface of resin XU-434-200.00

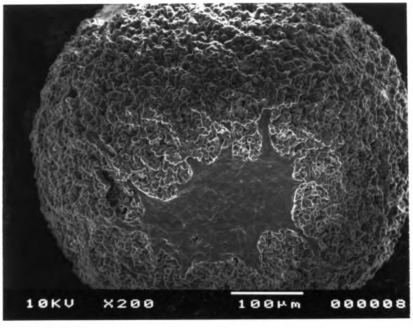


Fig. 4.2.5: Surface of resin IRA-938

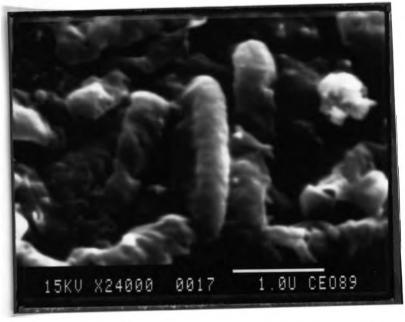


Fig. 4.2.6: Cells immobilized on the surface of the resin XU-434-200.00

The percentage of surface area used by the cells is found by multiplying the surface area used by cells by 100 and dividing by the surface area of the resin (Eqn. 4).

$$\% \text{ surface area used} = \frac{\text{Area used } (m^2/g \text{ dry resin}) \cdot 100}{\text{area of resin } (m^2/g \text{ dry resin})} \quad (4)$$

Table 4.2.4 shows that the macroporous resin shows a greater percentage of unused area than the gel type resin. This is probably due to the cells needing to diffuse into the pores to use the available surface area, and causing possible fouling of the pores, preventing further use of the pore. This shows that the macroporous resin uses the

least amount of available surface area of the resins studied. This is hypothesized to be due to the cells having to diffuse into the pores

TABLE 4.2.4: Percentage of unused surface area*
cell area = 1.50×10^{-12} m^2

RESIN	% unused surface area
IRA-938	96.75
XU-434-200.00	84.82
XU-434-200.01	82.60
XU-434-200.02	84.39

* Area taken for pores greater than 1μm diameter.

and consequently interfering with a free path into the pores due to the immobilized cells accumulating at the surface of the resin and the entrance of the pores or the cells are not able to diffuse deeply into the pores.

Figures 4.2.7 show the macroporous IRA-938 Type I strong base anion exchanger cut through the middle and that the majority of the cells are on the outer surfaces of the resin. Figures 4.2.8 - 4.2.11 show a closer view of the cells on the resin surface at various depths into the resin. As can be seen in Figure 4.2.8 the majority of the cells are in the first 30 μm depth of the resin. A cell distribution according to resin depth is shown in Figure 4.2.12 were, using the photographs, the cells were counted on the resin surface and the depth measured. This distribution seems to agree with the calculated amount of area used (Table 4.2.4).

The predicted amount of surface area (Table 4.2.4) used by the gel resins, ≈ 15%, does not agree with the Figures 4.2.13 and 4.2.14 which show complete coverage of the surface. A possible cause of this may be that the minimum pore diameter used, 1.0 μm, from the porosity data may

have been too small thus giving a larger surface area than what is available to the cells.

Fig. 4.2.7: Immobilized cells on strong base Type I anion exchanger IRA-938 sliced through middle.

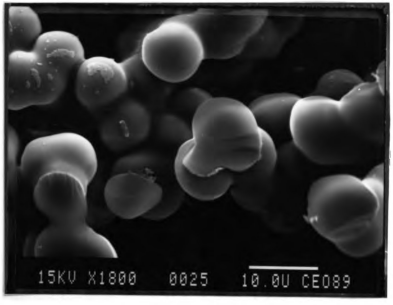


Fig. 4.2.8: Immobilized cells on strong base Type I anion exchanger IRA-938 sliced through middle.
Depth 0 - 26 μm

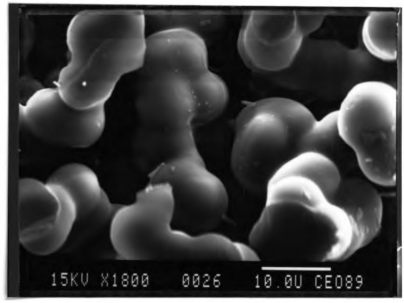


Fig. 4.2.9: Immobilized cells on strong base Type I anion exchanger IRA-938 sliced through middle.
Depth 24.5 - 76 µm

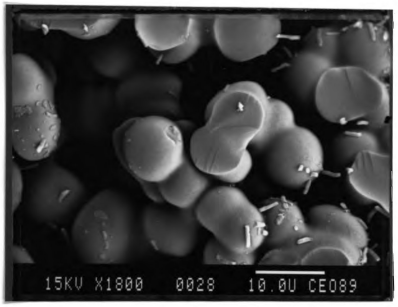


Fig. 4.2.10: Immobilized cells on strong base Type I anion exchanger IRA-938 sliced through middle.
Depth 74.5 - 126 μm

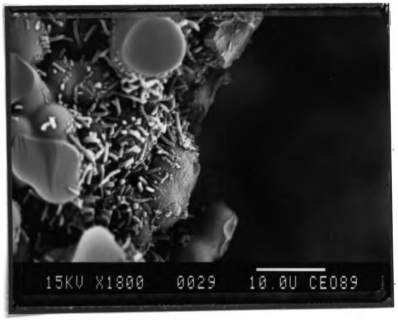


Fig. 4.2.11: Immobilized cells on strong base Type I anion exchanger IRA-938 sliced through middle.
Depth 121 μm - center

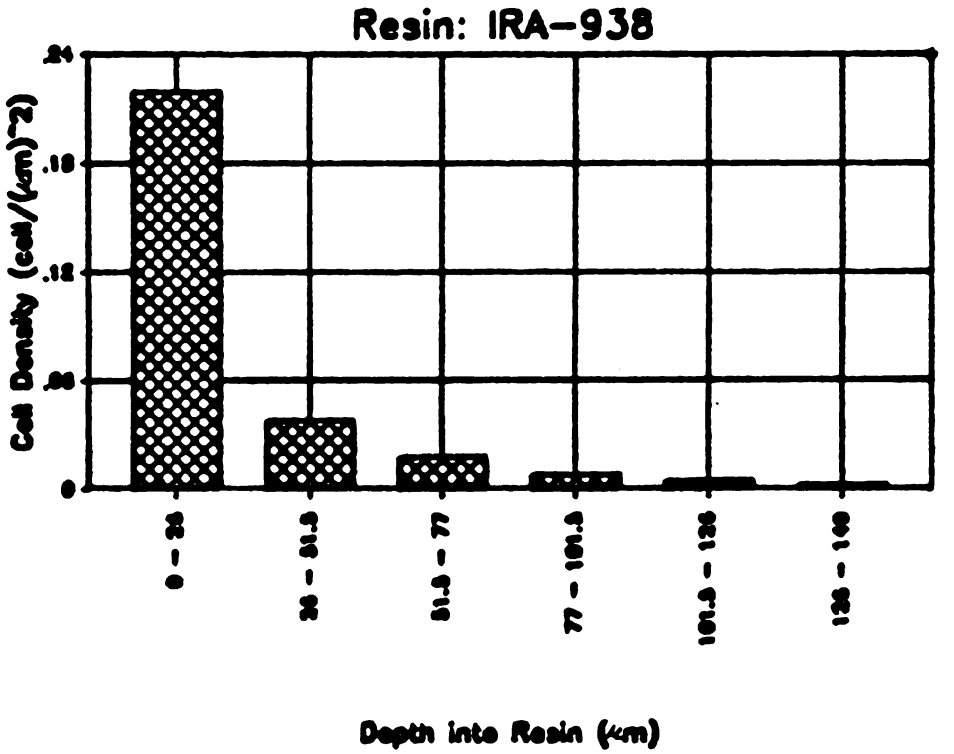


Fig. 4.2.12: Cell distribution for depth into Type I strong base macroporous anion exchanger IRA-938.

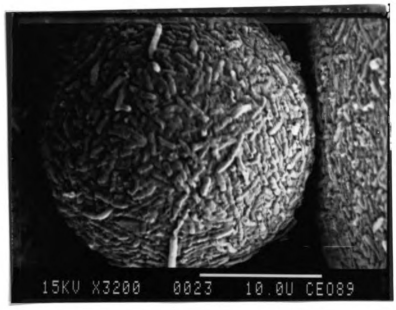


Fig. 4.2.13: Type I strong base gel anion exchanger XU-434-200.00 with immobilized cells, entire exchange bead.

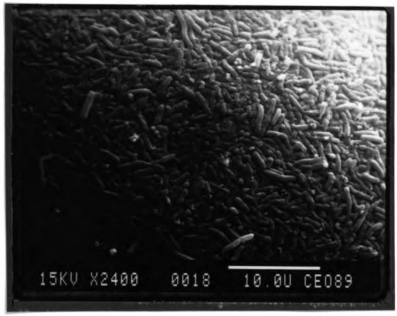


Fig. 4.2.14: Type I strong base gel anion exchanger XU-434-200.00 with immobilized cells, surface of exchange bead.

Crosslinking

The effect that cross-linking of the polymer matrix was investigated with 200-400 mesh Type I strong base anion exchangers. As shown in Table 4.2.5 the adsorption capacity, K1, decreases with increasing cross-linking. This observation agrees with Kuwajima et al (1957) observation for Haemophilus pertussis. The cross-linking of the polymer is the cause of this effect since the surface areas are similar.

No trends were found for the effect that cross-linking has upon the inverse equilibrium constant.

As the cross-linking of a resin increases the water retention of the polymer decreases thus the permeability into the resin decreases (Greenberg, 1989). This decreased permeability

TABLE 4.2.5: Adsorption Capacity and Surface Area of differing crosslinking of 200-400 mesh gel spheres, Type I strong base anion exchanger

RESIN	Percent Crosslink	K1 (g cell) (g dry resin)	K2 (g cell) (ml)	Area* (m^2/g)	Porosity (cc/g)
XU-434-200.00	2	.008867	.0000081	.0912	.6707
XM-1062-1824	4	.004545	.0000112	.0804	.6574
XY-40013.00	8	.001918	.0000057	.1257	.6263

* Area taken for pores greater than 1μm diameter.

suggest that the cell has to actually reach into the resin in order to anchor itself to the resin. This decrease in the permeability is shown in Table 4.2.5 by the decrease in the porosity as the percent cross-linking increases.

Macroporous resins usually have fourteen percent cross-linking (Greenberg, 1989). This suggests that the amount of volume inside a resin could play a large factor in the amount cells adsorbed.

pH

The solutions pH was studied on its effect on the adsorption capacity of XU-434-200.00 and IRA-938. The pH of solution was kept $\pm$1 pH unit from optimal pH, 7.2 for *E. coli*, so as to replicate variances that could be found in an actual fermentation system. Figures 4.2.15 and 4.2.16 show that around a neutral pH adsorption capacity is at its

lowest and increases as the pH gets away from neutrality. Theadsorption capacity increases faster when the solutions get more basic. The change in adsorption capacity from lowest to highest observed was ≈20%, and the highest capacity occurred at pH of 8.2. This increase of adsorption away from neutrality might be caused by the cells becoming a stronger ion. This would depend on the isoelectric point for E. coli.

This observation is in conflict with that found by Niehoff and Echols (1973) for E. coli were it was shown that adsorption was not effected within the pH of 6-10.

K1 can be fit with a quadratic formula with a function of pH (Eqn. 5).

$$K1 = a \cdot (pH)^2 + b \cdot pH + c \qquad (5)$$

TABLE 4.2.6: Constants for the function of K1 on pH (Eqn. 5)

Resin	a x 10^4	b x 10^3	c x 10^2
	(g cell/g dry resin)		
XU-434-200.00	2.80098	-3.78542	1.76204
IRA-938	13.10045	-18.60613	7.43702

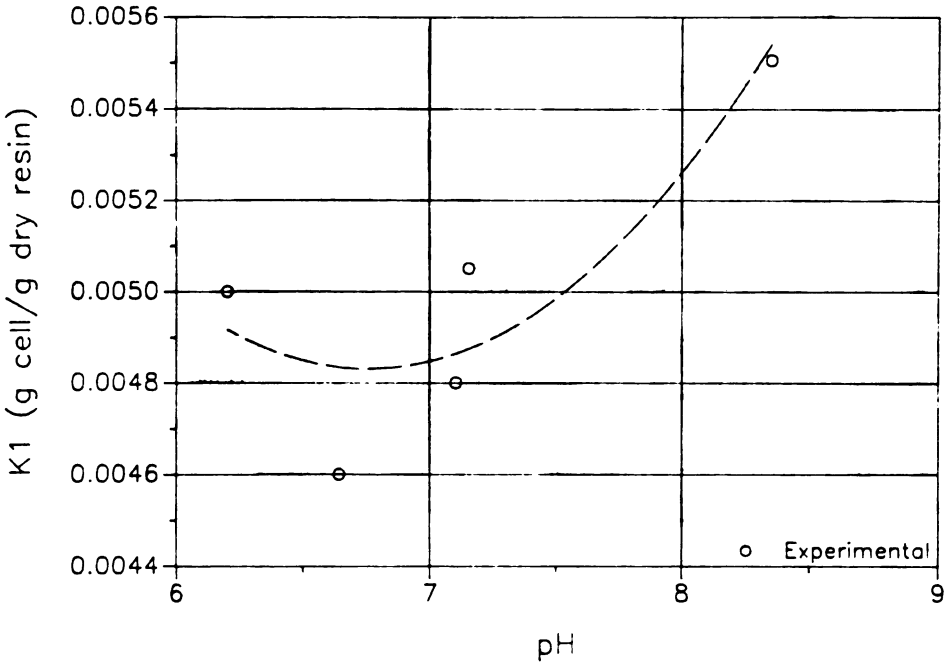


Fig. 4.2.15: K1 as a function of pH, Resin: XU-434-200.00
.05 M Tris buffer

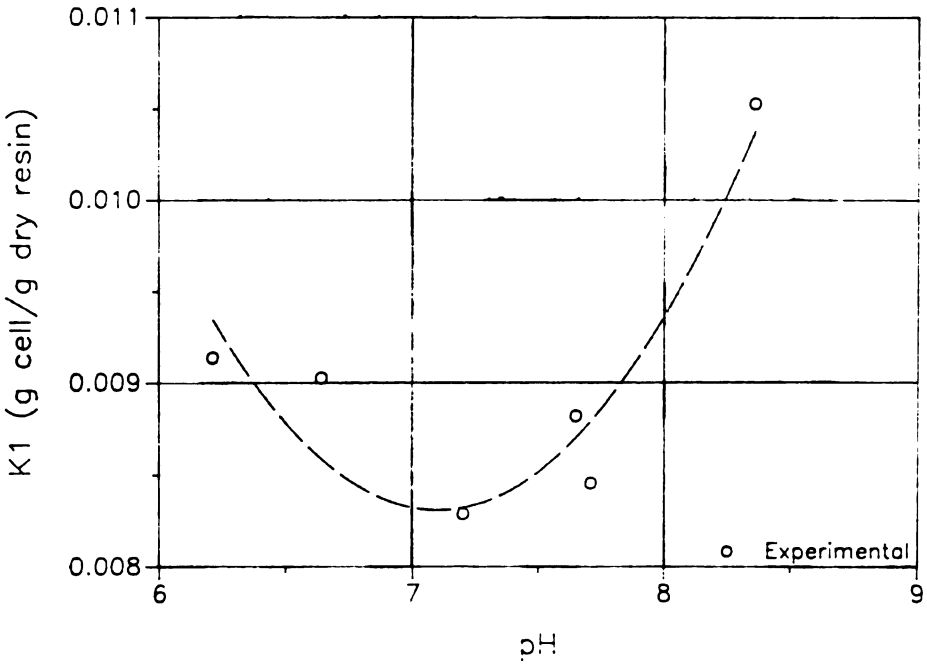


Fig. 4.2.16: K1 as a function of pH, Resin: IRA-938
.05 M Tris buffer

pH does not show any trend for the effect it might have upon the inverse equilibrium constant for IRA-938 and XU-434-200.00 (TABLES 4.2.7 & 4.2.8).

TABLE 4.2.7: Effect of pH upon K2
Resin: IRA-938
.05 M Tris buffer

pH	K2 (g cell/ml)
6.21	.0000157
6.64	.0000147
7.20	.0000116
7.65	.0000039
7.71	.0000164
8.36	.0000029

TABLE 4.2.8: Effect of pH upon K2
Resin: XU-434-200.00
.05 M Tris buffer

pH	K2 (g cell/ml)
6.20	.0000085
6.64	.0000018
7.10	.0000057
7.15	.0000004
8.35	.0000008

Ion Concentration

The amount of the Tris buffer dissolved in solution was varied for the resins IRA-938 and XU-434-200.00. Figures 4.2.17 and 4.2.18 show that the adsorption capacity tends to decrease as the buffer solution increases in molarity. The effect of varying the Tris buffer gives a variance from upper to lower adsorption capacities of ≈10%. The decrease in adsorption capacity as Tris buffer concentration increases might be caused by the Tris competing with the cells for available exchange sites or the Tris buffer concentration changing the dielectric

constant of cell surface; this would change the attraction of the cell for the resin.

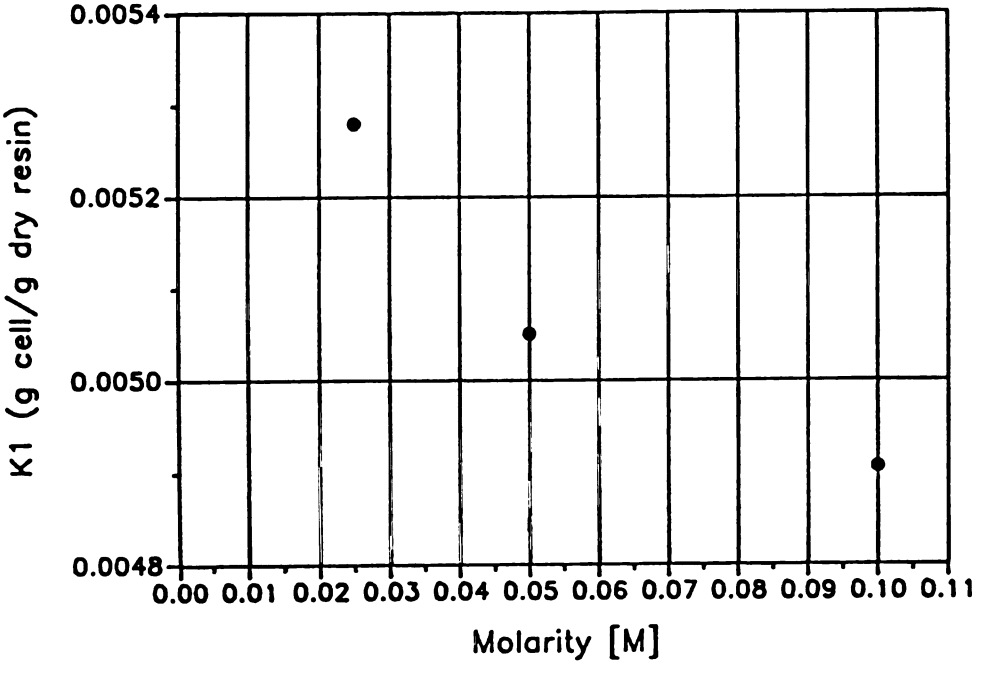


Fig. 4.2.17: Resin XU-434-200.00: K1 as function of buffer concentration. pH = 7.5

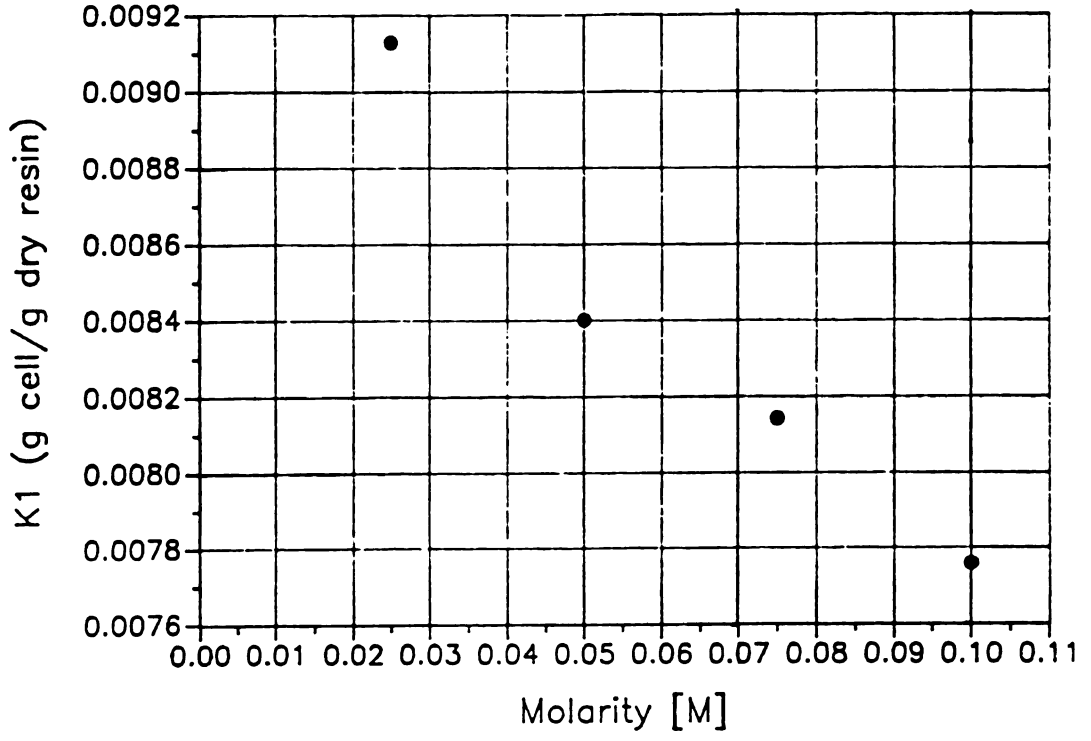


Fig. 4.2.18: Resin IRA-938: K1 as a function of buffer concentration. pH = 7.7

The ion concentration does not show any trend for effecting the inverse equilibrium constant, K2, for IRA-938 and XU-434-200.00 (TABLES 4.2.9 & 4.2.10).

TABLE 4.2.9: Effect of buffer concentration upon K2
Resin: XU-434-200.00
pH = 7.5

Tris [M]	K2 (g cell/ml)
.025	.0000044
.050	.0000057
.075	.0000078
.100	.0000004

TABLE 4.2.10: Effect of buffer concentration upon K2
Resin: IRA-938
pH = 7.7

Tris [M]	K2 (g cell/ml)
.025	.0000104
.050	.0000164
.075	.0000026
.100	.0000083

4.3 KINETICS

Using the simple reaction used for ion exchange and modifying the reactants used to our system a simple second order reaction, as shown below, was assumed to take place.

Reaction:

$$\text{Cells} + \text{Resin} \underset{k_d}{\overset{k_a}{\rightleftharpoons}} \text{Cells}\cdot\text{Resin}$$

NOMENCLATURE:

$[C]$, $[C]_0$ ≡ # cells in solution (# cells/ml soln.)
$[R]$, $[R]_0$ ≡ # sites available for cells in solution (# sites/ml soln.)
$[R\cdot C]$ ≡ # sites occupied on resin (# sites occupied/ml soln.)
k_a ≡ adsorption constant (ml/# sites(or cells)·hr)
k_d ≡ desorption constant (1/hr.)

$$[R]_0 \equiv \frac{\text{K1 (g cell/g dry resin)}\cdot\text{mass dry resin (g)}}{\text{(vol. solution (ml.))}\cdot 9.5 \times 10^{-13}\ \text{g/cell}}$$

$$[C]_0 \equiv \frac{\text{(Init. conc. cell(g cell/ml))}}{\text{(mass cell } (9.5 \times 10^{-13}\ \text{g/cell}))}$$

Assume all cells lost from solution are gained by resin then:

Cell balance: $[C] = [C]_0 - [R\cdot C]$

Open site balance: $[R] = [R]_0 - [R\cdot C]$

Occupied site balance: $[R\cdot C] = [C]_0 - [C]$

Reaction Equation:

Irreversible: $\frac{d[R\cdot C]}{dt} = k_a\,[C]\,[R]$

Substituting and separating the irreversible reaction the equation becomes:

$$k_a \cdot t = \int_0^t \frac{d[R\cdot C]}{([R]_0 - [R\cdot C])([C]_0 - [R\cdot C])}$$

Integrating:

$$k_a \cdot t = \left[\frac{1}{[R]_0 - [C]_0}\right] \ln\left[\frac{[R]_0\,([C]_0 - [R\cdot C])}{([R]_0 - [R\cdot C])\,[C]_0}\right]$$

Reversible: $\frac{d[R\cdot C]}{dt} = k_a\,[C]\,[R] - k_d\,[R\cdot C]$

At equilibrium:

$$K_{eq} = k_a/k_d$$

then:

$$k_d = k_a \cdot K2/(9.5 \times 10^{-13}\ g/cell)$$

Substituting and separating the reversible reaction equation becomes:

$$k_a \cdot t = \int_0^t \frac{d[R\cdot C]}{[R]_0[C]_0 - [R\cdot C]([C]_0 + [R]_0 + 9.5 \times 10^{-13}/K2) + [R\cdot C]^2}$$

let:

$a = [R]_0\,[C]_0$

$b = -([R]_0 + [C]_0 + 1/K2)$

$c = 1$

$q = 4ac - b^2$

If $q > 0$ then:

$$k_a \cdot t = \frac{2}{\sqrt{q}}\ \tan^{-1}\left[\frac{2c[R\cdot C] + b}{\sqrt{q}}\right] - \frac{2}{\sqrt{q}}\ \tan^{-1}\left[\frac{b}{\sqrt{q}}\right]$$

If $q < 0$ then:

$$k_a \cdot t = \frac{1}{\sqrt{-q}} \ln \left[\frac{2c[R \cdot C] + b - \sqrt{-q}}{2c[R \cdot C] + b + \sqrt{-q}} \right]$$

$$- \frac{1}{\sqrt{-q}} \ln \left[\frac{b - \sqrt{-q}}{b + \sqrt{-q}} \right]$$

Table 4.3.1 shows the adsorption capacity, K1, and the equilibrium constant, K2, used for the kinetic study as found from the equilibrium isotherm.

TABLE 4.3.1: Constants used for kinetic study
pH = 7.5, Conc. = .05 M Tris

Resin	K1 (g cell/g)	K2 (g cell/ml)	r
IRA-938	.008819	.0000039	.999
XU-434-200.00	.0088671	.0000081	.989
XU-434-200.01	.0060509	.0000082	.998
XU-434-200.02	.00266	.0000025	.994

Reaction Order

Figure 4.3.1 shows that the irreversible kinetic model gave a straight line for the XU-434-200.00 and that irreversible and reversible reactions have differing calculated adsorption constants, k_a (Table 4.3.2). This also shows that second order overall kinetics is occurring for this system. The desorption constant, k_d, is seven orders of magnitude greater than the adsorption constant. Figure 4.3.2 shows that the irreversible kinetics is a better model than the reversible when compared to the experimental data. This suggests that little desorption of cells is occurring and that the irreversible kinetics is

the better model for this system.

For the following example :

Init. cell conc. = .0000988 g cell/ml
Solution Vol. = 10 ml
Tris buffer = .05 M
pH = 7.5
Resin: XU-434-200.00
Mass Resin = .500

From Table 4.3.1 : K1 = .0088671 g cell/g dry resin

From the equations already given for $[R]_0$ and $[C]_0$ it is found:

$$[R]_0 = \frac{.0088671 \text{ g cell/g dry resin} \cdot .500 \text{ g dry resin}}{10 \text{ ml} \cdot 9.5 \times 10^{-13} \text{ g/cell}}$$

$$= 4.667 \times 10^8 \text{ sites/ml}$$

$$[C]_0 = \frac{.0000988 \text{ g cell/ml}}{9.5 \times 10^{-13} \text{ g/cell}}$$

$$= 1.04 \times 10^8 \text{ cells/ml}$$

TABLE 4.3.2: Kinetic Constants for XU-434-200.00
pH = 7.5, .05 M Tris buffer
$[C]_0$ =1.04 x 10^8 cells/ml, $[R]_0$ =4.667 x 10^8 site/ml
10 ml cell soln., .5 g dry resin

Reaction Type	$k_a \times 10^8$	k_d	r
Irreversible	1.068	———	.998
Reversible	1.396	.11905	.998

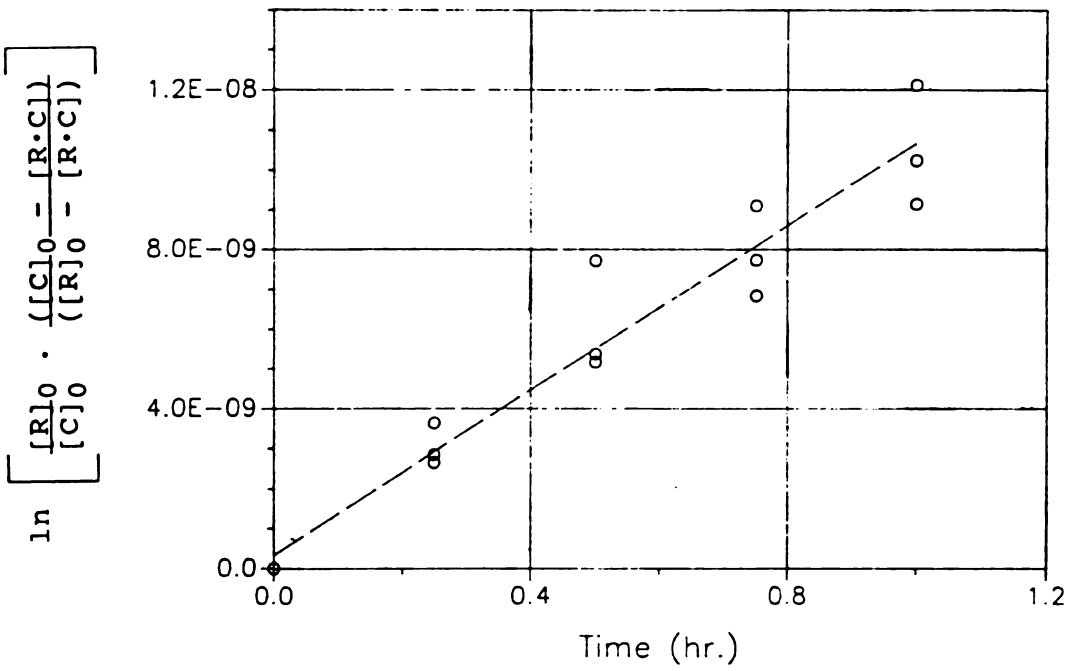


Fig. 4.3.1: Linearization to calculate adsorption constant
Resin XU-434-200.00, pH = 7.5, .05 M Tris buffer
$[C]_0$ =1.04 x 10^8 cells/ml, $[R]_0$ =4.667 x 10^8 site/ml

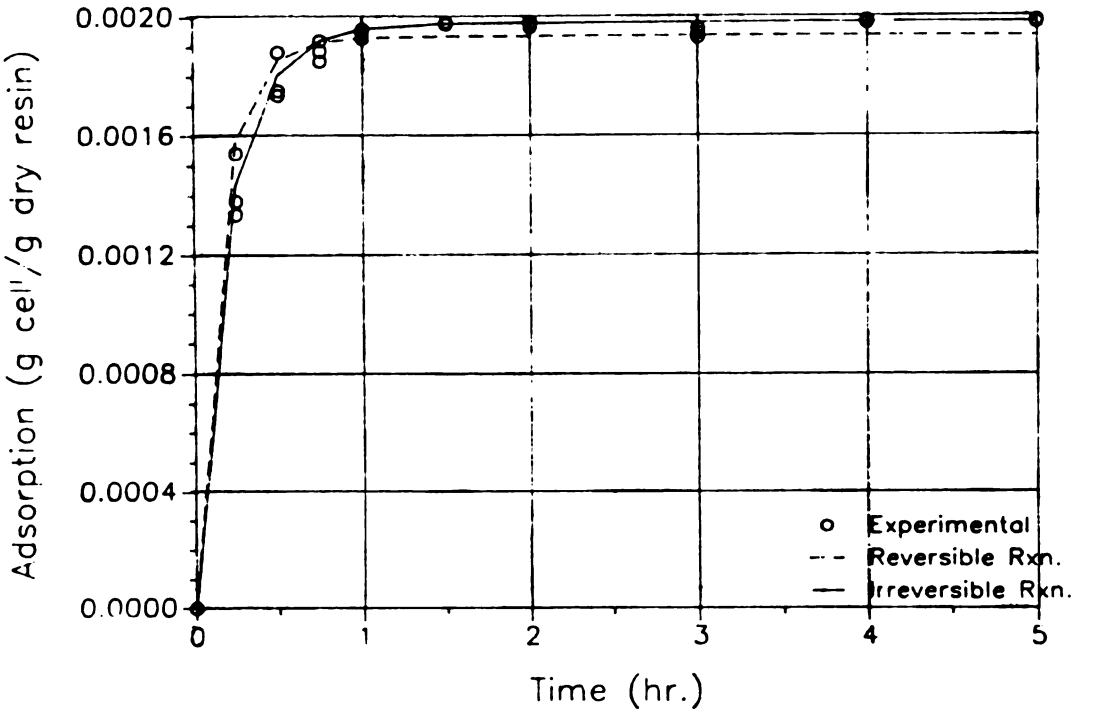


Fig. 4.3.2: Reaction models of cell adsorption
Resin XU-434-200.00, pH = 7.5, .05 M Tris buffer
$[C]_0$ =1.04 x 10^8 cells/ml, $[R]_0$ =4.667 x 10^8 site/ml

To find the reaction order for the individual reactants for a binary reaction the amount of one reactant should be much greater than the other. The initial adsorption sites available on the ion exchange resin were approximately four times greater than the cell concentration. Using irreversible kinetics:

$$-\frac{d[C]}{dt} = k_a \cdot [C]^A \cdot [R]^B$$

Taking the natural log of the above equation the following is used to find the reaction order of the reactants.

$$\ln(-(d[C]/dt)) = A \cdot \ln([C]) + \ln(k_a) + B \cdot \ln([R])$$

Knowing the reaction is second order overall then: A + B = 2.

$\ln(k_a) + B \cdot \ln([R])$ is considered a constant because k_a is a constant and [R] is considered a constant, since [R] >> [C]. Then the above equation becomes a linear equation where A is the slope. Figure 4.3.3 and Table 4.3.3 show a straight line for $\ln(-(d[C]/dt))$ vs. $\ln([C])$ using least square linear regression a slope of ≈1.0 was found. This indicates that the kinetics are first order for both reactants.

TABLE 4.3.3: Reaction order of Cells
Resin: XU-434-200.00
pH = 7.5, .05 M Tris buffer
$[C]_0 = 1.04 \times 10^8$ cells/ml, $[R]_0 = 4.667 \times 10^8$ site/ml
10 ml. cell soln., .5 g dry resin

Slope (A)	Y-int. ($\ln(k_a) + b \cdot \ln([R])$)	r
1.0526	0.0269	.9996
1.0956	-0.79068	.9960
1.1187	-1.01935	.9900

Macroporous Resins

The macroporous resin, IRA-938, shows slower kinetics than the gel type resin, XU-434-200.00, for the same initial concentration of cells (Fig. 4.3.4). This suggests that the cells have to diffuse into the pores of the resin to reach unoccupied surface. The kinetic model was based on the gel type resin, which has pores to small for the cells, and did not take into account changes due to pore fouling. The model for the gel, which takes into account the initial mass transfer, is compared to data for the macroporous resin IRA-938 in Figure 4.3.5. This suggests that the apparent adsorption constant changes over time. This is a typical result for systems in which the rate-controlling step can change from extra to intra particle mass transfer.

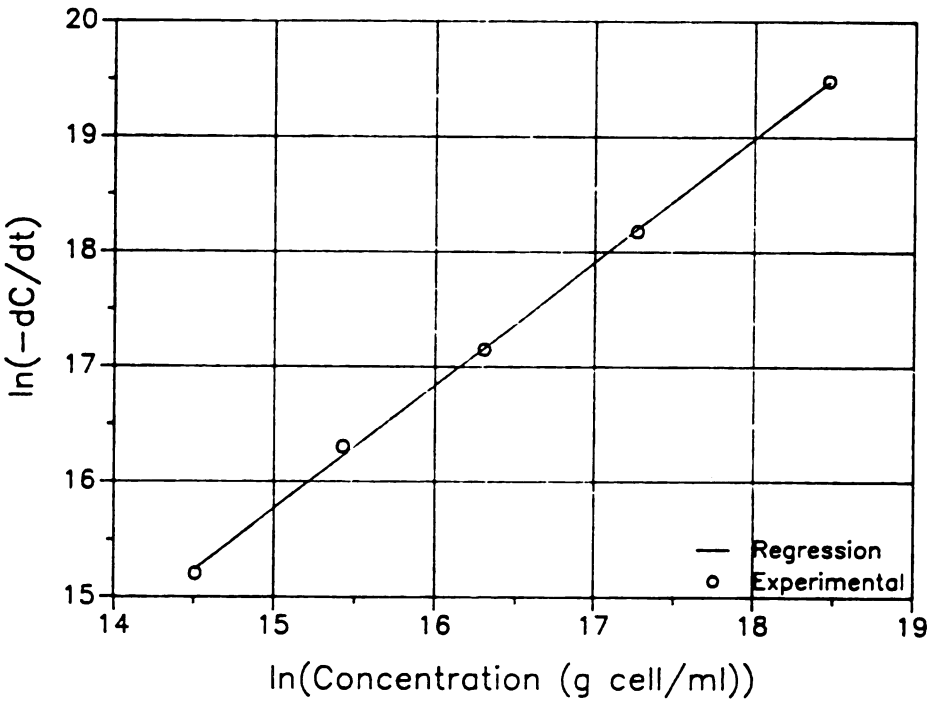


Fig. 4.3.3: Reaction order of cells
Resin XU-434-200.00, pH = 7.5, .05 M Tris buffer
$[C]_0$ =1.04 x 10^8 cells/ml, $[R]_0$ =4.667 x 10^8 site/ml

This is further shown by the change in the adsorption constant as time progressed. Table 4.3.4 shows that as time increased the k_a decreased. This indicates that the cells must diffuse into the pores and as time increases the adsorption constant decreases due to the cells

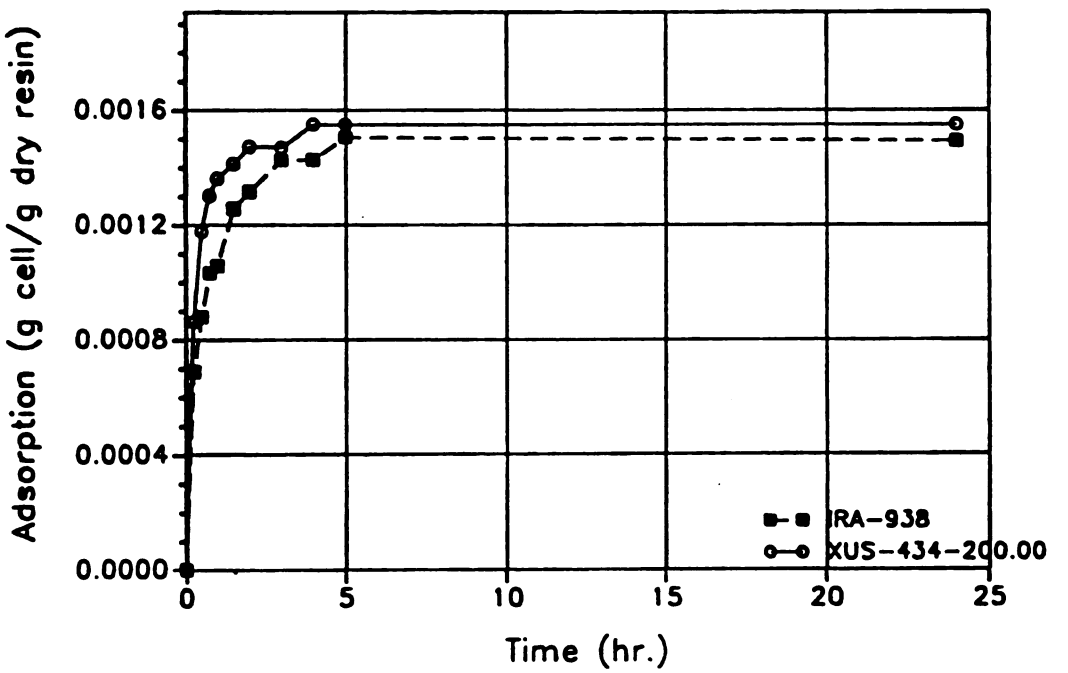


Fig. 4.3.4: Kinetics of macroporous (IRA-938) and gel (XU-434-200.00) anion exchanger.
pH = 7.5, .05 M tris buffer, $[C]_0$ =8.21 x 10^7 cell/ml
0.500 g dry resin

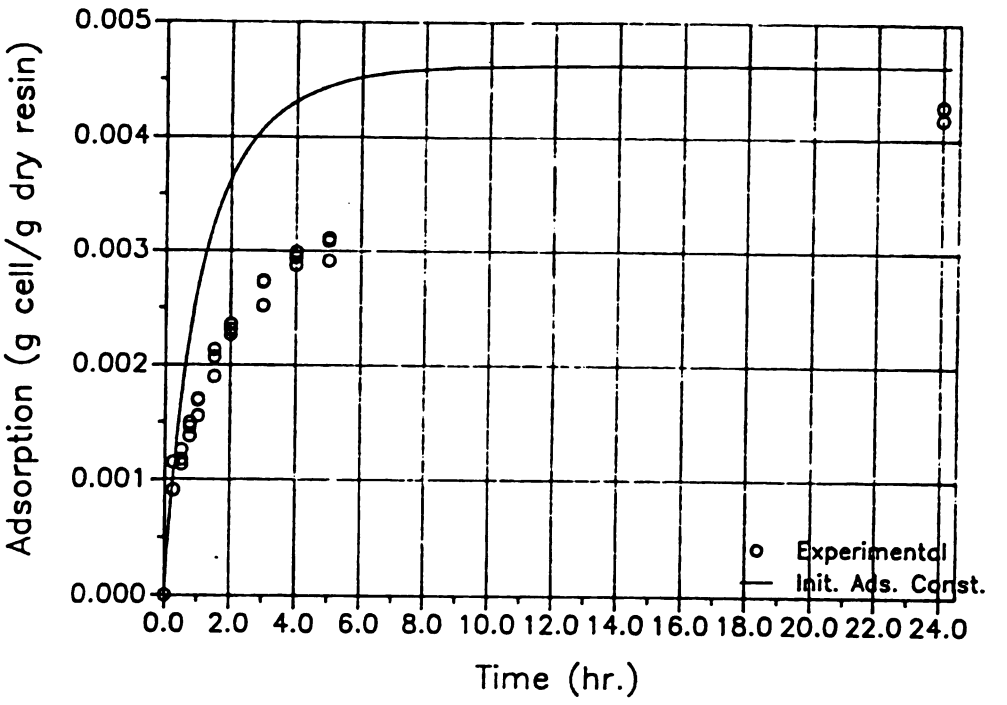


Fig. 4.3.5: Macroporous resin (IRA-938) using initial adsorption constant. 10 ml cell soln., .5 g dry resin
$[C]_0$ = 2.45 x 10^8 cell/ml, $[R]_0$ =4.64 x 10^8 sites/ml
pH = 7.5, .05 M Tris buffer

TABLE 4.3.4: Adsorption constant and time for macroporous resin IRA-938.
$[C]_0$ = 2.45 x 10^8 cell/ml, $[R]_0$ =4.64 x 10^8 sites/ml
10 ml cell soln., .5 g dry resin
pH = 7.5, .05 M Tris buffer

Time (hr.)	k_a (ml./# cells·hr) x 10^9
0 - 0.25	1.965
0.25 - 1.5	0.7031
1.5 - 3.0	0.5600
3.0 - ∞	0.3400

having to get past cells that are fouling the pores to contact unoccupied surface.

These constants were used to make a kinetic curve and is compared to he experimental data and show a close fit (Fig.4.3.6).

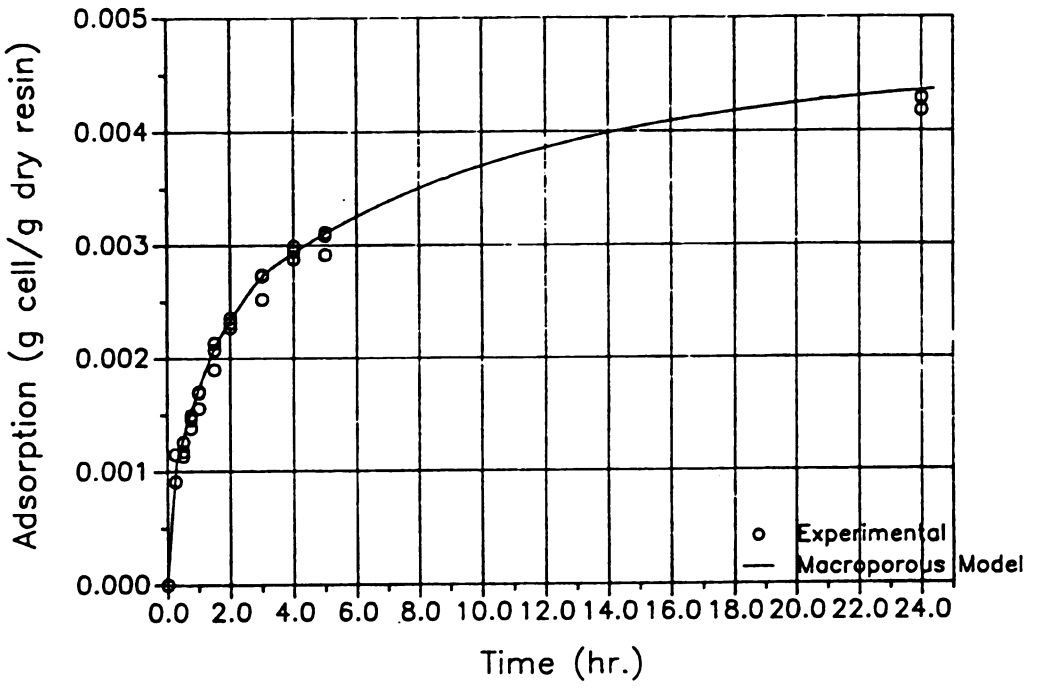


Fig. 4.3.6: Macroporous resin (IRA-938) adsorption using constants from Table 4.3.4. 10 ml cell soln., .5 g dry resin, pH = 7.5, .05 M Tris buffer $[C]_0 = 2.45 \times 10^8$ cell/ml, $[R]_0 = 4.64 \times 10^8$ sites/ml

Mass Transfer

The effects that resin size and initial concentration have upon the adsorption constant were studied. Various sphere sizes of a gel Type I anion exchange resins XU-434-200.00, .01 and .02 were used.

The equation below, modified from Eqn. 21.2-25 from Bird, Stewart and Lightfoot, was used for constant surface composition and small mass transfer rates.

$$\frac{k_a \cdot D}{c_f \cdot D_{ABf}} = 2.0 + 0.60 \left[\frac{D \cdot v_\infty \cdot \rho_f}{\mu_f}\right]^{1/2} \left[\frac{\mu}{\rho \cdot D_{AB}}\right]_f^{1/3}$$

By multiplying the above equation by $c_f \cdot D_{ABf}$ the equation

becomes:

$$k_a \cdot D = 2.0 \cdot c_f \cdot D_{ABf} + 0.60 \cdot D^{\frac{1}{3}} \left[\frac{v_\infty \cdot \rho_f}{\mu_f}\right]^{1/2} \left[\frac{\mu}{\rho \cdot D_{AB}}\right]_f^{1/3} \cdot c_f \cdot D_{ABf}$$

Assuming c_f, D_{ABf} and everything in the brackets of the above equation to be constant the equation becomes linear in form, as shown in the equation below.

$$k_a \cdot D = b + m \cdot D^{\frac{1}{3}}$$

Were k_a is the adsorption constant and D is the particle diameter.

Figure 4.3.7 shows $k_a \cdot D$ vs. $D^{\frac{1}{3}}$ is linear and that $k_a \cdot D$ decreases with increasing cell concentration. This shows that known mass transfer

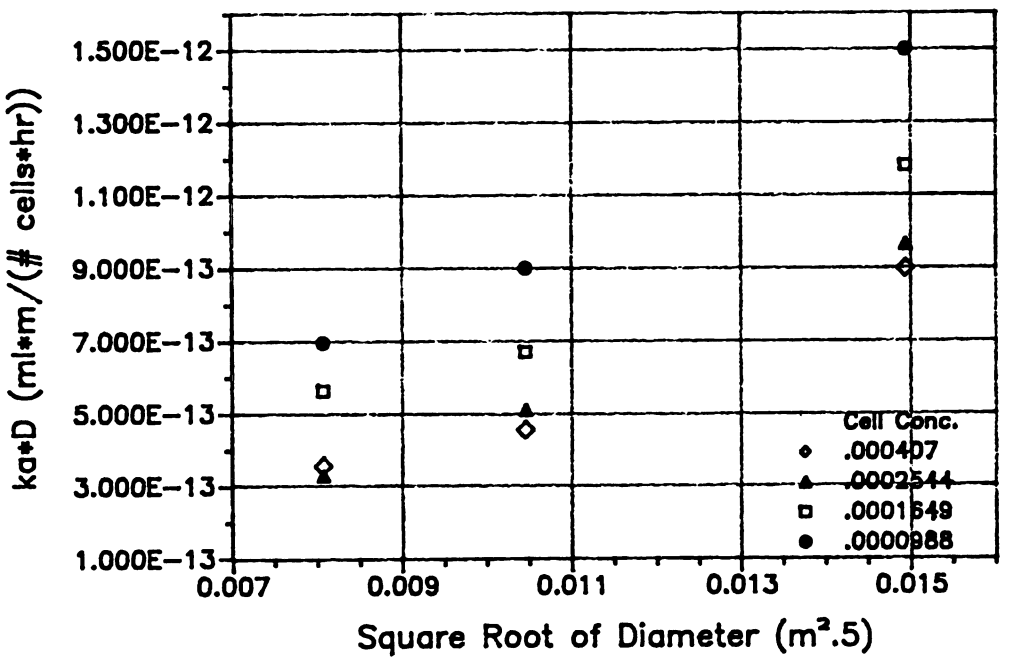


Fig. 4.3.7: $k_a \cdot D$ vs. $D^{\frac{1}{3}}$ for Type I strong base gel type anion exchanger
Resins: XU-434-200.00, .01 &.02
pH = 7.5, .05 M Tris buffer

phenomena can explain this system. Table 4.3.5 shows the constants found for a least squares linear regression done on the equation for each cell concentration studied.

At cell concentrations greater than .00025 g cells/ml the $k_a \cdot D$ decreases little. This point of cell concentration may be a

TABLE 4.3.5: Linear Regression of $k_a \cdot D$ vs. $D^{\frac{1}{3}}$
Resins: XU-434-200.00, .01 & .02
pH = 7.5, .05 M Tris buffer

Cell Conc. g cell/ml	m x 10^{11} ml·$D^{\frac{1}{3}}$/(# cell·hr)	b x 10^{13} ml·D/(# cell·hr)	r
.0000988	11.90	-3.00	.995
.0001649	9.30	-2.30	.982
.0002544	9.35	-4.40	.998
.0004028	8.13	-3.40	.984

critical concentration were the free path and the number of collisions do not change.

Observing that concentration has an effect on the adsorption a least squares linear regression was performed on the effect that initial cell concentration has on the $k_a \cdot D$ for cell concentrations up to .0002544 g cells/ml for the various $D^{\frac{1}{3}}$ (Table 4.3.6).

TABLE 4.3.6: Linear Regression of $k_a \cdot D$ vs. Cell Conc.
Resins: XU-434-200.00, .01 & .02
pH = 7.5, .05 M Tris buffer

$D^{\frac{1}{3}}$ $m^{\frac{1}{3}}$	m x 10^{9} ml·$D^{\frac{1}{3}}$/(# cell·hr)	b x 10^{13} ml·D/(# cell·hr)	r
.01493	-3.39	18.00	-.980
.01045	-2.46	11.20	-.982
.00806	-2.36	9.37	-.997

Mass Transfer Model

Since both the initial cell concentration and the square root of the resin diameter seem to have a linear effect on the $k_a \cdot D$ a linear model was proposed for these combined effects. By doing a least squares linear regression on the slopes and Y-intercepts of the lines of cell concentrations effect on the $k_a \cdot D$ for each of the $D^{\frac{1}{2}}$ the following was found:

For the slopes of the concentrations:

$m = -1.567 \times 10^{-7}$ $(ml^2 \cdot m^{\frac{1}{2}}/(g\ cells \cdot \#\ cell \cdot hr))$

$b = -9.875 \times 10^{-10}$ $(ml^2 \cdot m/(g\ cells \cdot \#\ cell \cdot hr))$

$r = -.996$

and for the Y-intercepts of the concentrations:

$m = 1.290 \times 10^{-10}$ $(ml \cdot m^{\frac{1}{2}}/(\#\ cells \cdot hr))$

$b = -1.541 \times 10^{-13}$ $(ml \cdot m/(\#\ cells \cdot hr))$

$r = .989$

This gives the equation for $k_a \cdot D$ as a function of the resin diameter and cell concentration as:

$$k_a \cdot D(dia, conc) = (-1.567 \times 10^{-7} \cdot (dia.)^{\frac{1}{2}} - 9.875 \times 10^{-10}) \cdot (conc.) + (1.29 \times 10^{-10} \cdot (dia.)^{\frac{1}{2}} - 1.541 \times 10^{-13})$$

The use of the above equation with concentrations greater than .000255 g cell/ml is done by keeping the concentration at .000255 g cell/ml. The fit of the above equation to the experimental data is shown in Fig. 4.3.8.

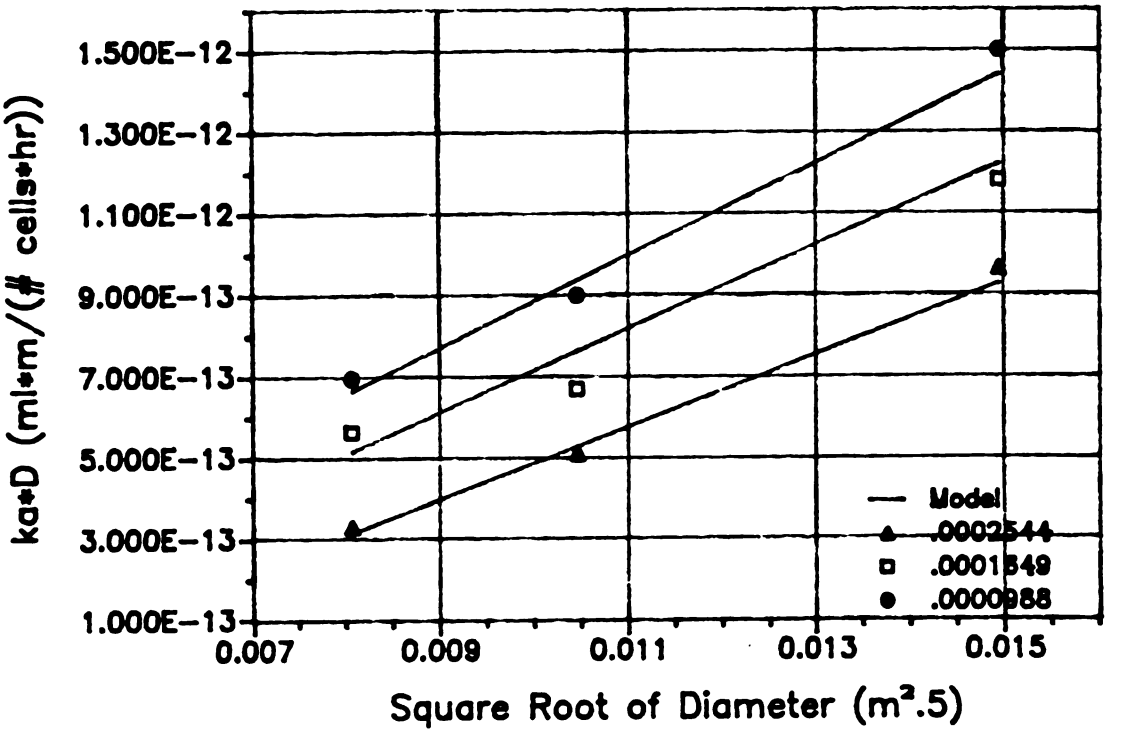


Fig. 4.3.8: Linear model for $k_a \cdot D$ vs. $D^{\frac{1}{2}}$ for Type I strong base gel type anion exchanger.
Resins: XU-434-200.00, .01 & .02
pH = 7.5, .05 M Tris buffer

Effects of Diameter & Surface Area

Figure 4.3.9 shows that the particle diameter has an effect on the adsorption constant. As the diameter of the particle increases the adsorption constant decreases. This effect looks like the amount of surface area exposed to the solution is the cause for the decrease because, as the sphere diameter increases, the surface area should decrease for the same mass of resin, assuming constant density. Figure 4.3.10 shows that the k_a increases with increasing available surface area. This increase is linear with the surface area.

The effect that the initial cell concentration has upon the adsorption constant is shown also in Fig. 4.3.9 and 4.3.10. As the cell concentrations increase the k_a decreases until a concentration of

.000255 g cell/ml is obtained, at this concentration the adsorption constant steadies at around 5 x 10^{-9} ml/# cell·hr. This increase of k_a with smaller cell concentrations can be attributed to the cells having a longer free path and less collisions with one another at lower cell concentrations. This occurred for all three resin areas studied.

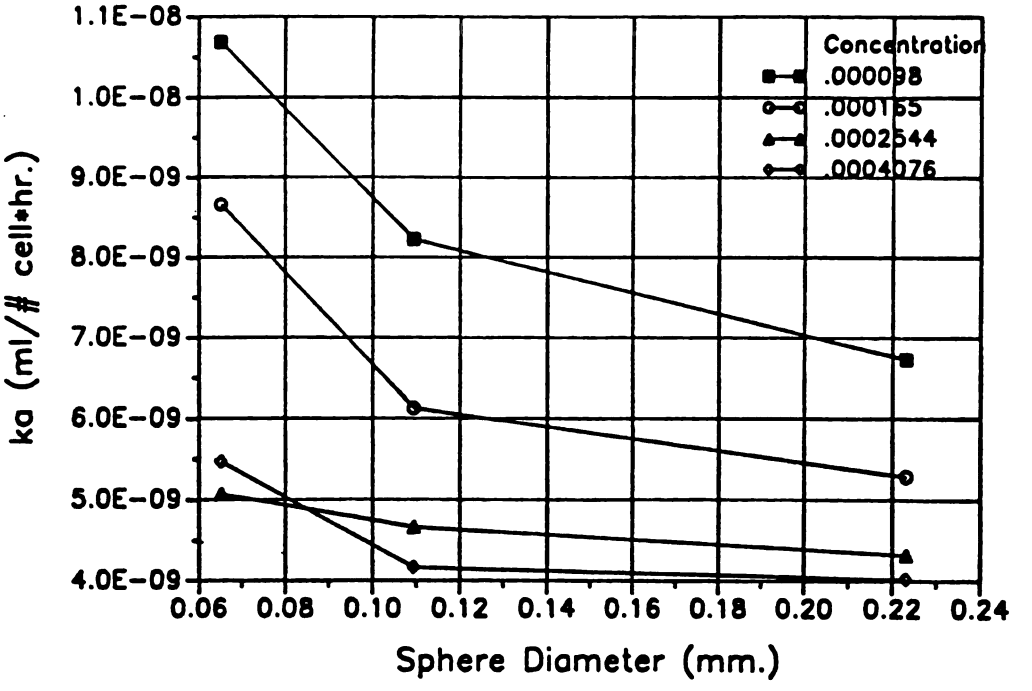


Fig. 4.3.9: Resin diameters effect on k_a for Type I strong base gel type anion exchanger.
Resins: XU-434-200.00, .01 & .02
pH = 7.5, .05 M Tris buffer

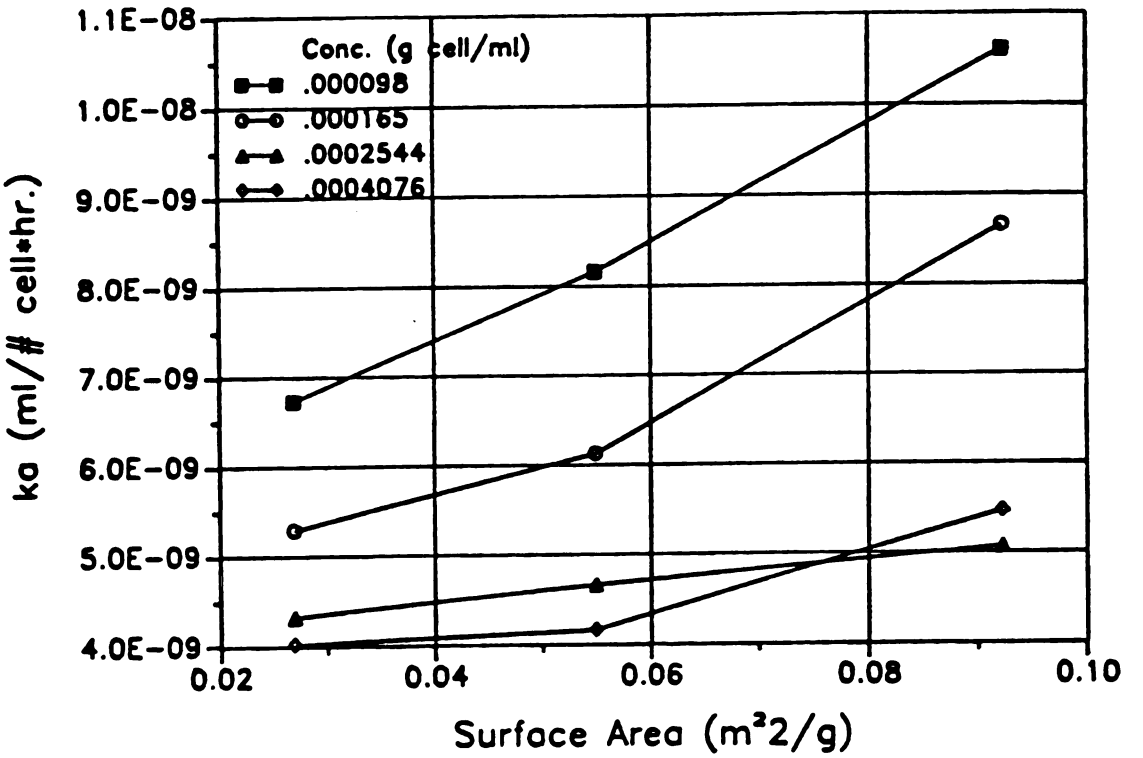


Fig. 4.3.10: Surface area effect on k_a for Type I strong base gel type anion exchanger.
Resins: XU-434-200.00, .01 & .02
pH = 7.5, .05 M Tris buffer

The linear model for k_a was used on the macroporous resin IRA-938 for the initial k_a and can only take into account its external surface area. As shown in Table 4.3.6 the fit is good with low concentrations and poor with the large using the resin diameter of .00042 m.

TABLE 4.3.7: Predicted and experimental initial adsorption rate, k_a, for macroporous IRA-938

Initial Conc. (g cell/ml)	k_a (ml/# cell·hr) x 10^9	
	Predicted	Experimental
.00023	3.918	1.965
.00008	4.818	5.372

Table 4.3.7 indicates that the porosity of the resin must effect the initial k_a of the macroporous resin since at large cell concentrations the k_a is much slower than that predicted. This could mean that the external surface area taken into account for that diameter could be larger for the macroporous Type I strong base anion exchanger IRA-938.

It is known that the adsorption constant changes with time for macroporous resins and that the predictive equation of k_a for gel type resins does not work well for macroporous resins. To create a model for k_a for macroporous resins is beyond the scope of this study.

4.4 GROWTH

Free Cells

A batch growth of cells was performed to compare the amount of free cells produced, total cells produced and the amount of extracellular α-amylase produced in a shaker flask. Three runs of this experiment were performed with a control batch, no ion exchange resin in solution, and a 4.35 weight percent solution of the anion exchange resins XU-434-200.00 and IRA-938 with the initial solutions at the same pH. Figures 4.4.1, 4.4.2 and 4.4.3 show that the control batch has a greater amount of free cells than both flasks with ion exchange resins. For all three experiments the IRA-938 had the smallest amount of free cells. The smaller amount of free cells in solution will allow less downstream processing to occur for the separation of cells.

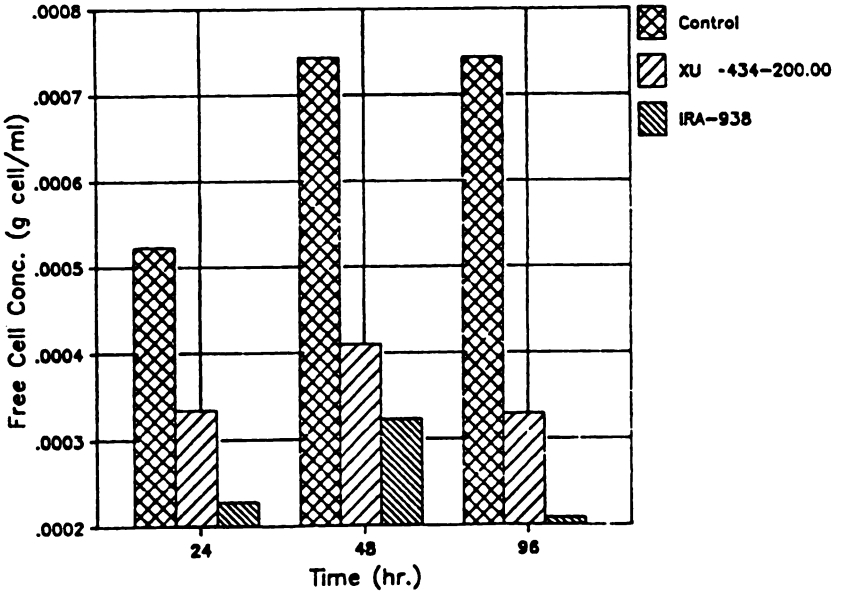


Fig. 4.4.1: Free cell concentration of production experiment 1 4.35 wt. % Type I strong base anion exchange resin, macroporous and gel type structures.

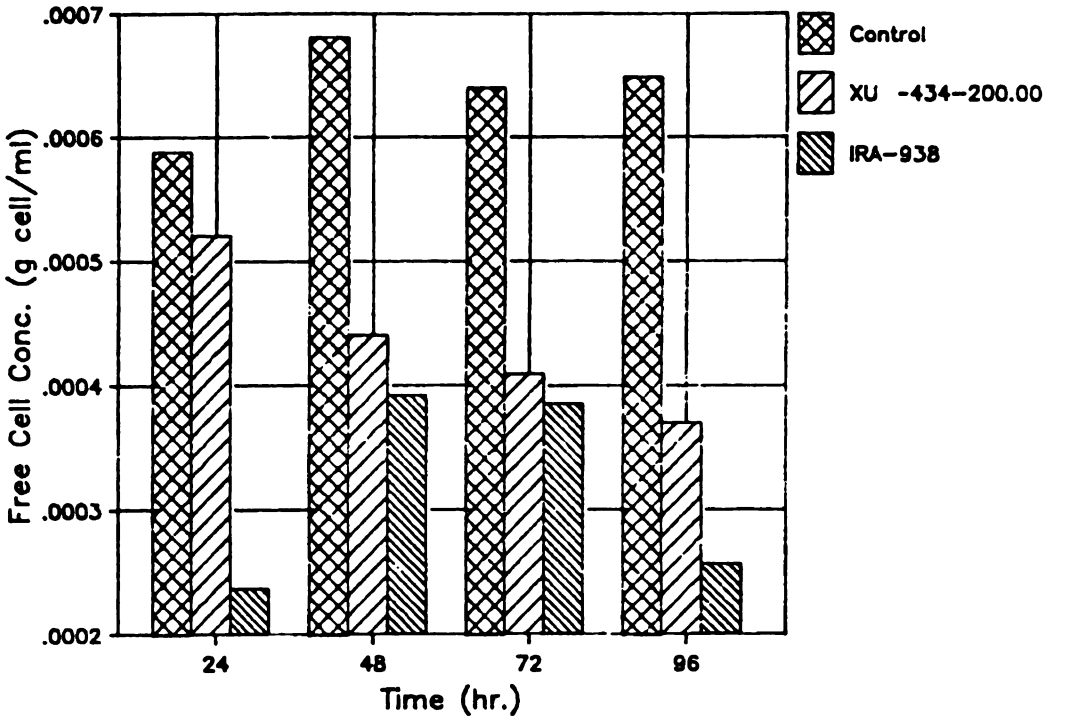


Fig. 4.4.2: Free cell concentration of production experiment 2 4.35 wt. % Type I strong base anion exchange resin, macroporous and gel type structures.

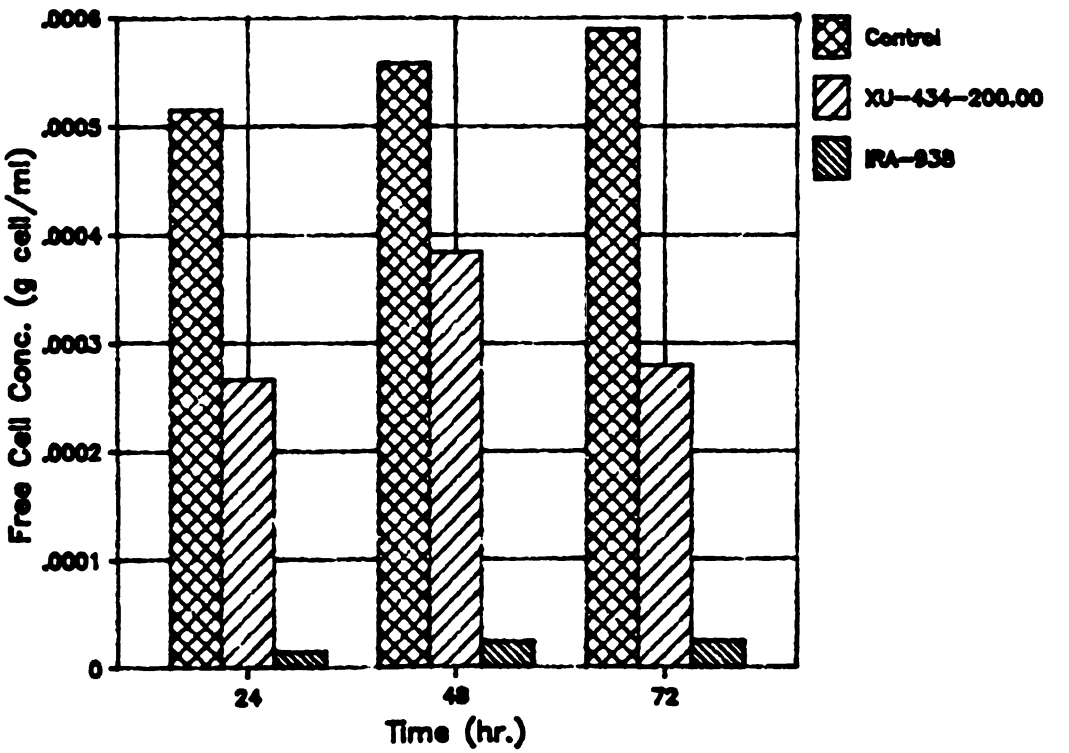


Fig. 4.4.3: Free cell concentration of production experiment 3 9.1 wt. % Type I strong base anion exchange resin, macroporous and gel type structures.

Total Cells

Using the adsorption constants for XU-434-200.00 and IRA-938 (Table 4.2.3) the total amount of cells adsorbed onto the resin was found and by adding this to the amount of free cells the total amount of cells in the flasks were estimated for the three experiments (Fig. 4.4.4 - 4.4.6). The total cell concentration was greater for the resins than for the sample for the last two growth experiments (Fig. 4.4.5 & 4.4.6). This shows that a greater amount of cells can be accumulated in a reactor vessel than that without ion exchange resin. The first growth experiment (Fig. 4.4.4) shows a greater amount of cells for the immobilized systems at twenty-four hours but a larger total cell concentration was noticed for the control, no ion exchange resin, after forty-eight hours. No explanation is known of why this would occur.

The total cell concentrations were calculated to be higher for the immobilized cells even though no new nutrients were added. The immobilized cell systems may have depleted the nutrients sooner than the suspended cell system since the cell concentrations seemed to decrease or level off quicker than the suspended cells.

These calculations of total cell concentration were assumed to follow the adsorption that was studied for a .05 M Tris buffer concentration at ≈ pH 7.2.

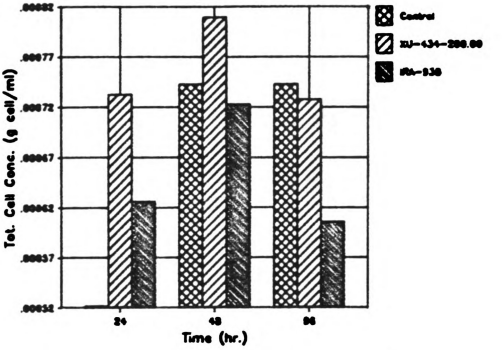


Fig. 4.4.4: Total cell concentration of production experiment 1 4.35 wt. % Type I strong base anion exchange resin, macroporous and gel type structures.

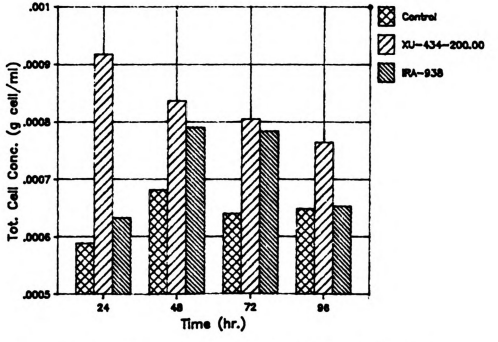


Fig. 4.4.5: Total cell concentration of production experiment 2 4.35 wt. % Type I strong base anion exchange resin, macroporous and gel type structures.

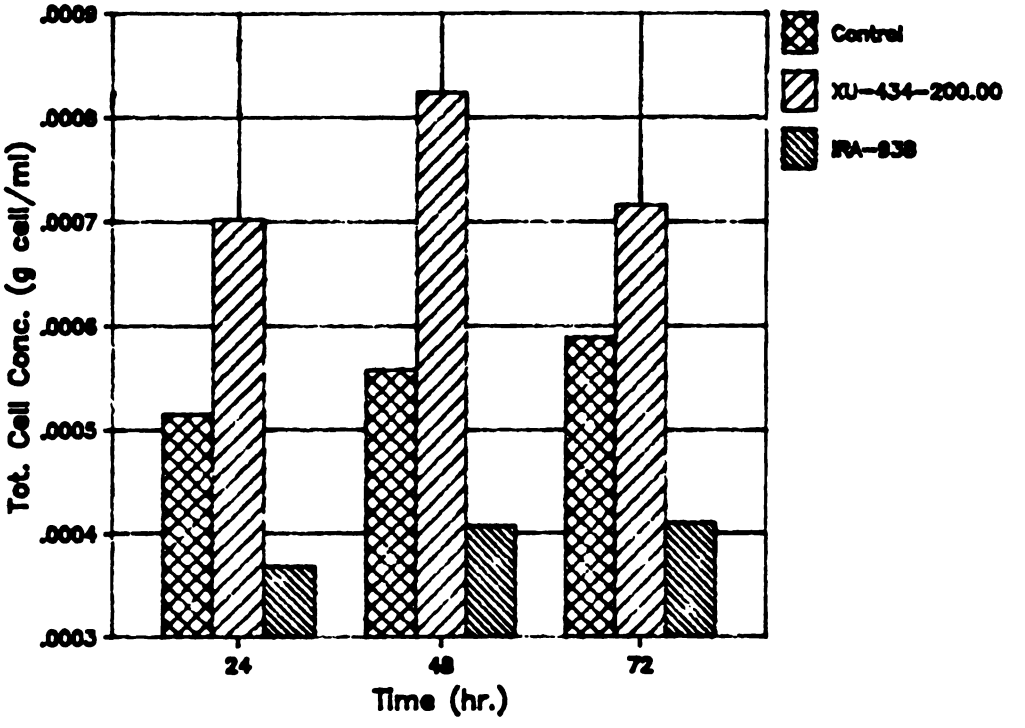


Fig. 4.4.6: Total cell concentration of production experiment 3 9.1 wt. % Type I strong base anion exchange resin, macroporous and gel type structures.

Production

The production of the enzyme α-amylase from E. coli was studied (Fig. 4.4.7 - 4.4.9). In Fig. 4.4.7 and Fig. 4.4.8 after 24 hours the XU-434-200.00 gave a slightly larger amount of extracellular α-amylase. This was not the case for times greater than 24 hours. At 48 hours the XU-434-200.00 gave the lowest concentration of enzyme for Fig. 4.4.7 and 4.4.8. These differences in the enzyme concentration could be an effect of the immobilized cells growth rates and differences in the structures of the two resins used. During the first growth experiment the concentration of enzyme dropped drastically for the IRA-938 between 48 and 96 hours. It was hypothesized that due to large porosity of this resin that the enzyme could have diffused into the pores and been adsorbed due to the greater surface area. This was not the case for the

second growth experiment (Fig. 4.4.8) The amount of α-amylase for the second growth experiment was higher for the immobilized cultures for the free cells. When the amount of resin was increased to ≈9% by weight (Fig. 4.4.9) the amount of enzyme for the XU-434-200.00 was the lowest of the three cultures and the IRA-938 was as large as the free cells for the twenty-four and seventy-two hour periods. Due to the scatter of the data for the α-amylase between all experiments, it is concluded that the analytical methods used for the α-amylase concentration should be reviewed. Therefore, no conclusions for what is better for the production of this enzyme can be made.

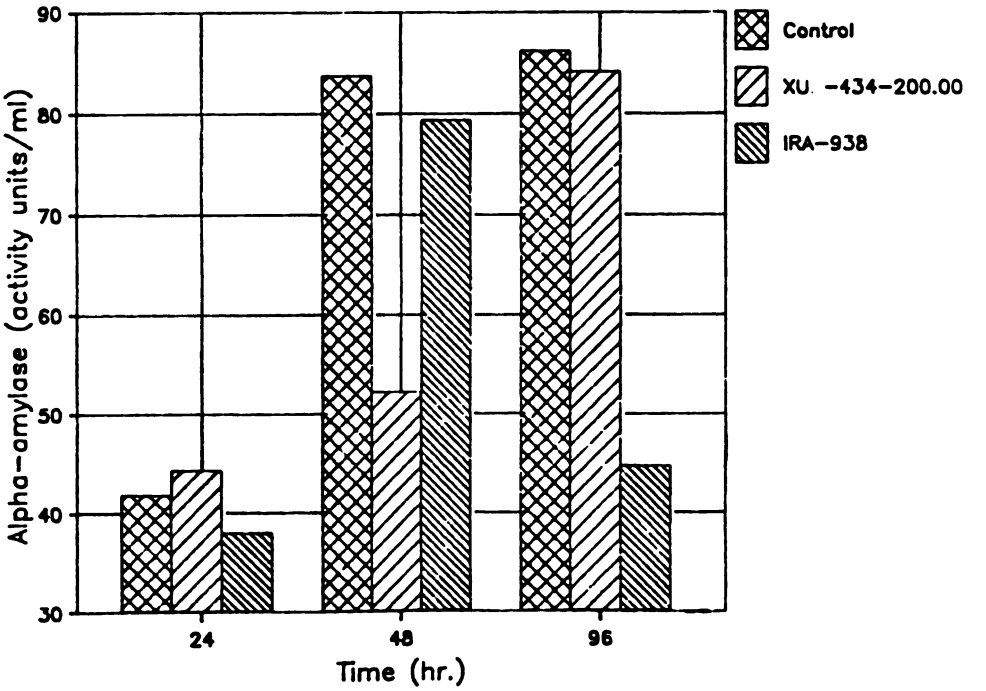


Fig. 4.4.7: Production of α-amylase, production experiment 1
4.35 wt. % Type I strong base anion exchange resin, macroporous and gel type structures.

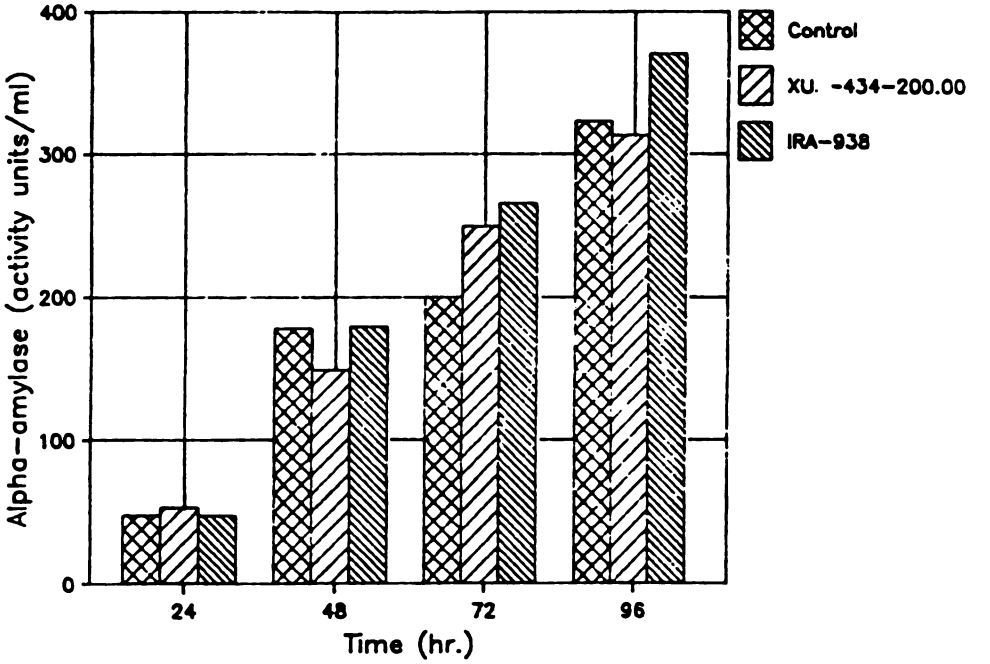


Fig. 4.4.8: Production of α-amylase, production experiment 2
4.35 wt. % Type I strong base anion exchange resin,
macroporous and gel type structures.

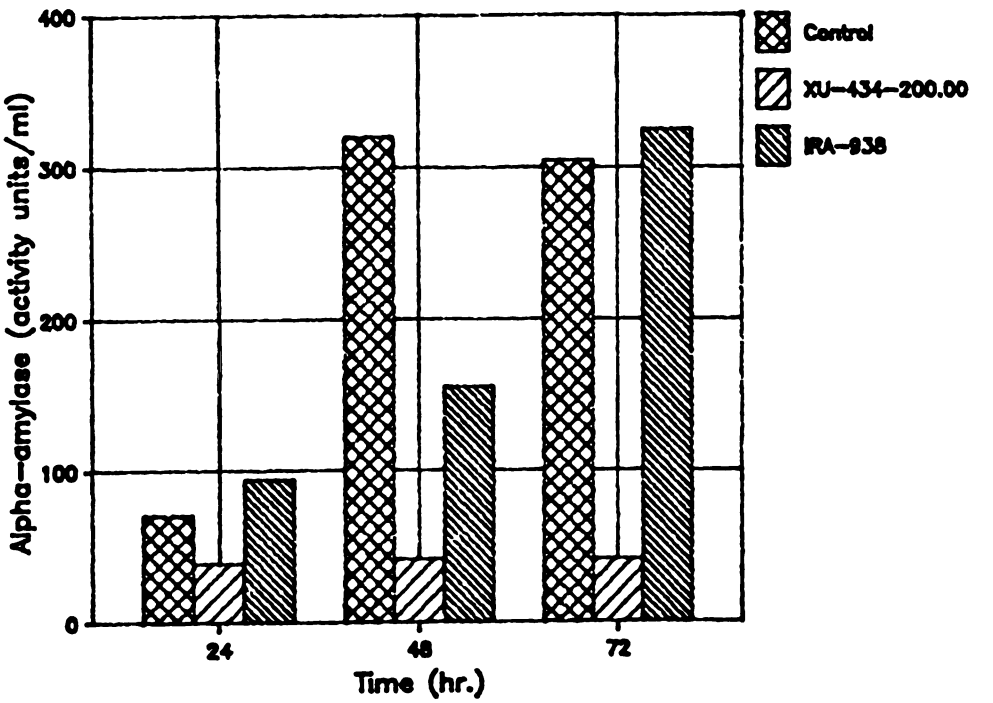


Fig. 4.4.9: Production of α-amylase, production experiment 3
9.1 wt. % Type I strong base anion exchange resin,
macroporous and gel type structures.

4.5 RECYCLE

One potential advantage for the use ion exchange resins for the use of whole cell immobilization is the possibility of re-use. Three separate experiments were performed for multiple adsorption of whole cells. The initial concentration of cells was kept constant for all trials. A normal resin preparation was performed between sorptions by contacting them with acid and salt solutions. Figures 4.5.1 - 4.5.3 show the amount of adsorption obtained for the samples of resin recycled. For all three experiments, the second cycle of the resins gave lesser amount of adsorption than the original use of the sample. The macroporous IRA-938 gave a better adsorption than the gel XU-434-200.00. For the two cases were a third cycle was done (Fig. 4.5.1; 4.5.3) both showed better adsorption than the second cycle. The poorer performances of the recycled resins could be caused by the cells leaving proteins or polysaccharides on the resin surface that could not be removed by the regular acid and salt contact, thus causing some fouling. The better recyclability of the macroporous resin could be from the resin having a smaller percentage of its surface being used and more surface area left unaffected by the cell attachment. The cases when the third cycle had a better adsorption than the initial adsorption could be due to the effects the age of the cells being used. Younger cells might adsorb better than older cells. The age of the cells was not taken into account during these runs.

This set experiments show that normal resin preparation steps may not be sufficient to clean resin surfaces. Further study should be done to find what further steps are needed to clean the resin for re-use.

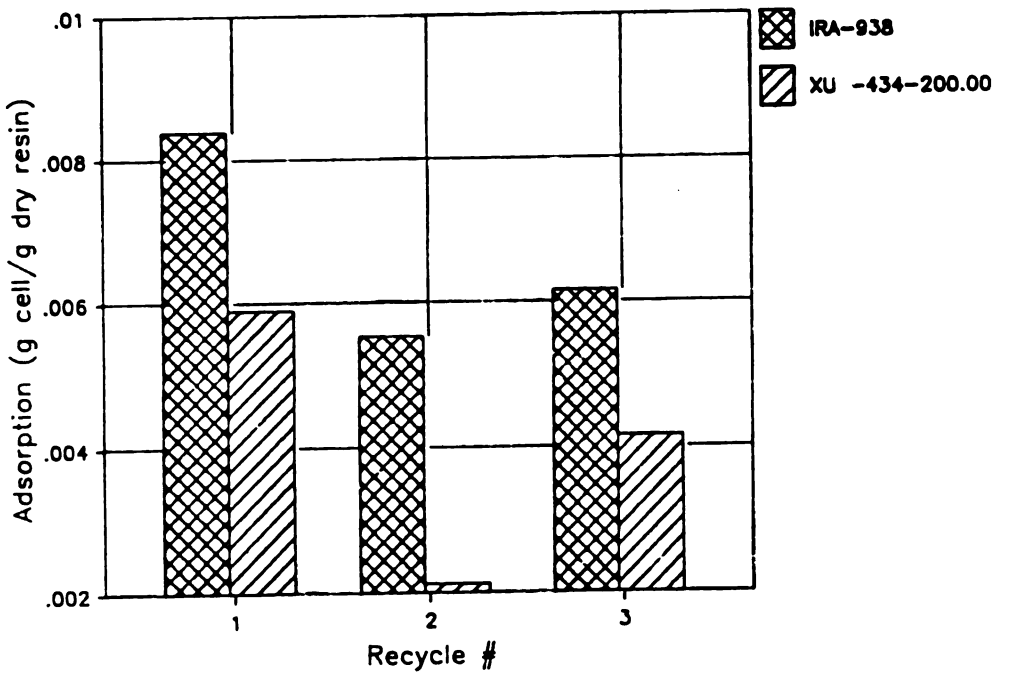


Fig. 4.5.1: Recycle experiment 1:5 wt. % Type I strong base anion exchange resin, macroporous and gel type structures.
Init. cell conc. = .00049 g cell/ml
Solution vol. = 10 ml., pH = 7.5, .05 M Tris buffer

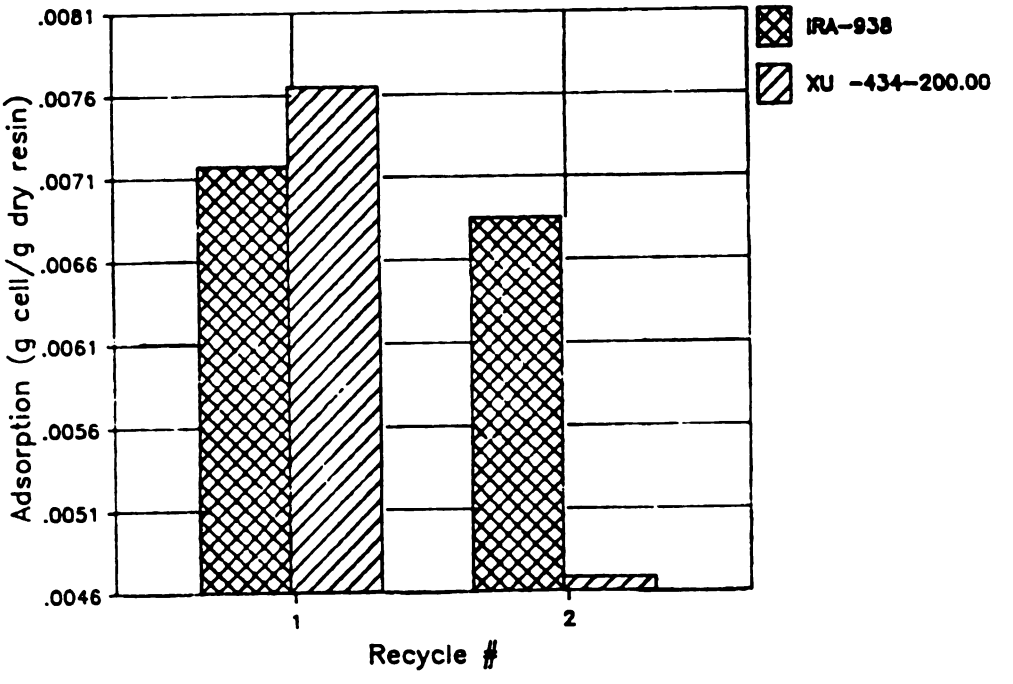


Fig. 4.5.2: Recycle experiment 2:5 wt. % Type I strong base anion exchange resin, macroporous and gel type structures.
Init. cell conc. = .00049 g cell/ml
Solution vol. = 10 ml., pH = 7.5, .05 M Tris buffer

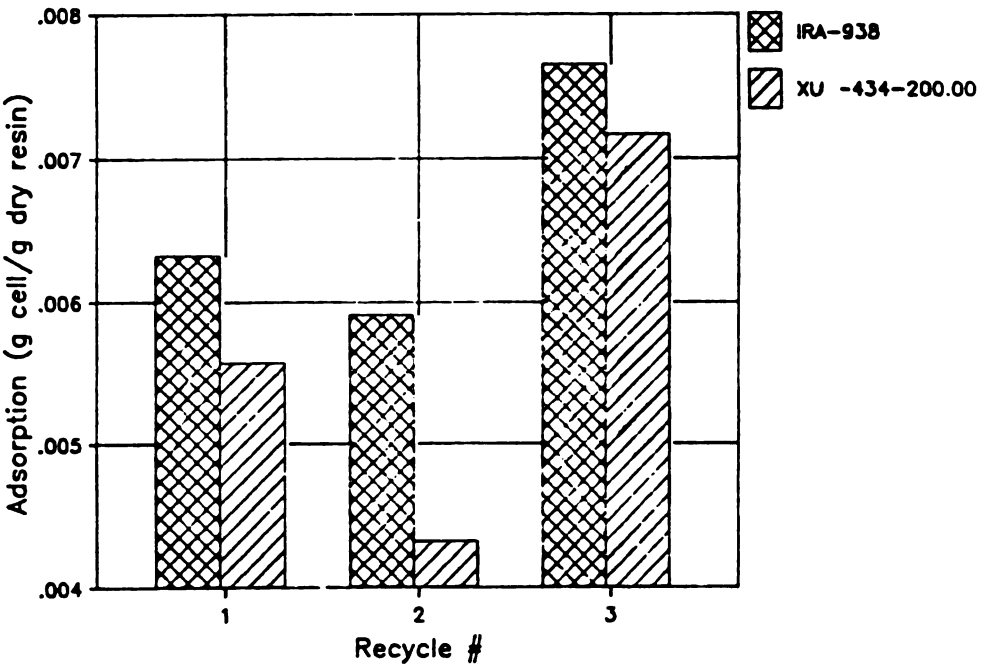


Fig. 4.5.3: Recycle experiment 3:5 wt. % Type I strong base anion exchange resin, macroporous and gel type structures.
Init. cell conc. = .00037 g cell/ml
Solution vol. = 10 ml., pH = 7.5, .05 M Tris buffer

CHAPTER V: CONCLUSIONS

1.) Type I strong base anion exchangers are preferred by E. coli for adsorption.

2.) A greater percentage of surface area is used by gel type resins were there is a large external surface area.

3.) Adsorption of cells decrease with increasing crosslinking of the ion exchange resin.

4.) Little diffusion of cells into macroporous resin occurs.

5.) Equilibrium of cells and resin follows a Langmuir isotherm.

6.) The adsorption reaction is second order overall reaction which follows irreversible kinetics with the reaction order of the cells and resin being first order.

7.) Mass transport of the cells onto the resin can be explained by normal mass transport phenomena for surfaces with constant composition.

8.) The rate of adsorption decreases with increasing cell concentration and reaches a minimum around .000255 g cells/ml.

9.) The rate of adsorption increases with increasing external surface area.

10.) The pH of solution has a parabolic effect on the amount of cells adsorbed, with minimum adsorption occurring around pH 7.

11.) As the amount of Tris buffer in solution increases the amount of cells adsorbed decreases.

12.) The use of ion exchange resins in batch fermentations will give lesser amounts of free cells and greater amount total cells in solution.

13.) Ion exchange can be reused for adsorbing cells, though their capacity is slightly diminished.

CHAPTER VI: RECOMMENDATIONS

1.) Investigate what compounds are left on the resin surface after and during the adsorption of cells.

2.) Further modelling of the rate of adsorption onto macroporous resins should be investigated with the effects of extra and intra particle diffusion.

3.) Investigate the effects of various porosities and pore size distribution have on the adsorption capacities of macroporous resins.

4.) Review the analytical methods for the estimation of α-amylase concentration.

5.) Further investigate the use of gel type and macroporous resins in actual fermentations, batch and continuous.

6.) Investigate cleaning methods of the used ion exchange resin for possible resin reuse.

7.) Investigate ion exchange resins for the use of mammalian whole cell immobilization.

8.) Further investigate how resin and solution properties effect the equilibrium constant of cell adsorption.

APPENDICES

APPENDIX 1: S.E.M.

The intent of this instruction manual is to provide the novice with a set of abreviated instructions that can be usedto begin observation of a specimen in the JEOL T-330 SEM. It should be emphasized that this document does not attempt to make the prospective SEM user an expert in the techniques of electrom microscopy. Rather, this document presents a set of instructions on how to obtain an image using the Secondary Electron Imaging mode on the T-330. It shold be noted that this particlar SEM can perform backscattered electron imaging also. In addition prospective users should consult with me to determine more on the following accessories which are not discussed herein:

GAMMA: Used for enhancing the image when extremes in contrast and brightness occur.

Objective Lens Operatures: Used for selecting higher resulution or greater depth of field.

DFU (Dynamic Focusing Unit): Used to bring into focus the entire field when specimen is tilted.

Working Distance: Used for selecting higher resolution or greater depth of filed.

The Composite Materials and Structures Center has several texts and manuscripts that discuss in detail electrom microscopy theory and techniques. These contain information that should be reviewed by prospective SEM users so that they can operate the unit at its greatest potential for the specific sample that is to be observed.

1. Start Up.

This sequence of steps should be followed to power-up the JOEL T-330 if the unit has been turned off.

1. Turn on the Neslab recirculating water chiller located behind the SEM.

2. Insert key into SEM. This key is spring loaded and operates the same as an ignition key of an automobile. To turn on the power, turn key to the START position momentarily and then allow the key to return to the ON position. Power is now supplied to the SEM.

3. Place CHECKER in the PWR position. The ammeter needle of the CHECKER should be at 0.5, perfectly vertical. If not, the incoming voltage must be regulated using the VARIAC transformer located on the wall adjacent to the outlet supplying voltage to the SEM. Adjust the transformer so that the CHECKER ammeter reads 0.5.

The SEM should now be fully powered. It will take 15-20 minutes for the diffusion pump oil to reach operating temperature. During this period full vacuum in the column can not be achieved and thus it is not possible to view a sample. The operator should observe the SEQUENCE panel to determine when the column is fully evacuated. The SEQUENCE panel provides the following information when the adjacent red light is illuminated:

POWER	The SEM is on.
DP	Diffusion Pump oil is heated; DP is now ready.
PRE EVAC	The column has been roughed pumped; the DP will now engage.
EVAC	The column has been fully evacuated.
HT READY	Column has been fully evacuated and High Tension (high voltage) can now be applied to the filament.
HT	The High Tension is on.
FILAMENT	Voltage is being applied to the filament and the filament is engaged.

When the SEQUENCE light adjacent to HT READY is illuminated it is now possible to begin observation of the sample. If there is no sample on the SEM stage, proceed to section 2, SPECIMEN INTRODUCTION AND EXCHANGE. If a sample has been introduced into the column and the column has been evacuated, proceed to the next section entitled SPECIMEN OBSERVATION.

2. SPECIMEN INTRODUCTION AND EXCHANGE.

1. Turn FILAMENT knob to zero.

2. Turn off the high tension by depressing the red HT button.

3. WAIT 5 MINUTES to allow filament to cool down.

4. Depress VENT. The sample chamber and column will be returned to ambient pressure. Failure to wait for the filament to cool as directed in step 3 will cause the filament to burn upon exposure to oxygen.

5. When the PRE EVAC light is extinguished the stage may be pulled open. Always wear lint-free nylon gloves when opening chamber.

6. Remove sample mounting pedestal.

7. Insert new specimen. Use squeeze bulb to blow away dust on specimen or in chamber. Close the stage.

8. Depress PUMP DOWN. Observe the lights on the SEQUENCE panel which will indicate when chamber has been fully evacuated so that imaging may proceed.

9. Proceed to section 3 SPECIMEN OBSERVATION.

3. SPECIMEN OBSERVATION.

This section describes specimen observation using the SEI mode (Secondary Electron Imaging). The DET (Detector) selector light should indicate that the sem is in the SEI mode. In addition the SEI button should be depressed on the IMS (Image Selection) panel.

A series of adjustments must be executed to obtain high resolution images of the specimen. These adjustments are grouped into 5 categories:

1. Adjustment of filament current and filament alignment.

2. Adjustment of contrast and brightness.

3. Focusing

4. Astigmatism correction.

5. Objective lens alignment.

3.1. Adjustment of filament current and filament alignment.

1. Select the desired amount of ACCELERATING VOLTAGE. Do not exceed 20 KV. Consult Mike Rich for determining an appropiate initial level of accelerating voltage.

2. Set SPOT SIZE to the 2 O'Clock position.

3. Adjust BRIGHTNESS to the 12 O'Clock position.

4. Depress the red HT button and observe that the red light next to HT on the SEQUENCE panel is illuminated.

5. Put CHECKER in the LD CUR. (Load Current) position.

6. Slowly turn the FILAMENT knob clockwise to the 2:30 position while observing the CHECKER ammeter. Do not allow the needle to deflect beyond 0.6 mamps. If amperage exceeds 0.6 mamps prior to attaining the 2:30 position on the FILAMENT knob, the GUN BIAS must be decreased. The GUN BIAS control knob is located in the tabletop to the immediate righthand side of the column. To decrease the GUN BIAS amperage turn the knob clockwise.

7. With FILAMENT knob at the 2:30 position, adjust GUN BIAS so that the ammeter reads 0.5 mamps, perfectly vertical.

8. Select LSP (Line Scan Profile) on the MODE panel and EXP (Exposure) on the SPEED panel.

9. Use BRIGHTNESS knob to place waveform at center of CRT. Use CONTRAST to set the peak to valley amplitude of waveform so that it is approximately 1 inch on CRT.

10. While observing the waveform slowly turn the FILAMENT knob counterclockwise. As soon as the waveform begins to travel down the CRT stop turning the filament knob. Now slowly turn the FILAMENT knob clockwise the minimum amount necessary to set the waveform so that it is loacated at the highest point on the CRT. Do not turn the FILAMENT knob past the 3 O'Clock position. See Mike Rich should you encounter difficulty in adjusting the waveform to its peak height.

11. After the waveform has been adjusted the filament alignment must be checked. Filament alignment is controlled by the 2 pair of black knobs located atop the electron beam column. To align the filament, simultaneously turn in opposite directions (one clockwise, the other counterclockwise) a pair of knobs located across from each other while observing the waveform. Adjust the waveform so that a peak is achieved. Repeat this process with the remaining pair of knobs. Normally little adjustment if any will be needed. With new filaments, alignment should be checked every hour for the first 5 hours of filament time.

3.2. Adjustment of contrast and brightness.

Before contrast and brightness can be adjusted the specimen must be located and placed into the path of the electron beam. The following sequence is helpful in locating your sample within the chamber. Remember, never remove the cap of the viewing port while the FILAMENT is on.

1. Select PIC on MODE and TV on SPEED panels.

2. Select minimum magnification level.

3. At this point you may have to decrease the CONTRAST to obtain a clear image on the CRT.

4. Use the X-Y stage controls to locate the sample.

5. Use the UP/DOWN ARROWS of FOCUS panel to bring the image into focus

7. Select SLOW 1 on SPEED panel and proceed to the next sections on contrast and brightness adjustment

Contrast and brightness may be adjusted manually or automatically by the unit itself. However the automatic mode may not be used during picture taking. Because of the necessity of performing manual contrast and brightness adjustment when photodocumenting the specimen, automatic contrast and brightness adjustment will not be presented here. See Mike Rich should you desire more information on this operation.

Manual Adjustment of Contrast and Brightness.

1. Select EXP on SPEED panel and PIC on MODE panel.

2. Use the CONTRAST knob to achieve 3-bars on the tophat as shown in Fig 2.

3. Use the BRIGHTNESS knob to center the 3 bars of the tophat as shown in Fig 1.

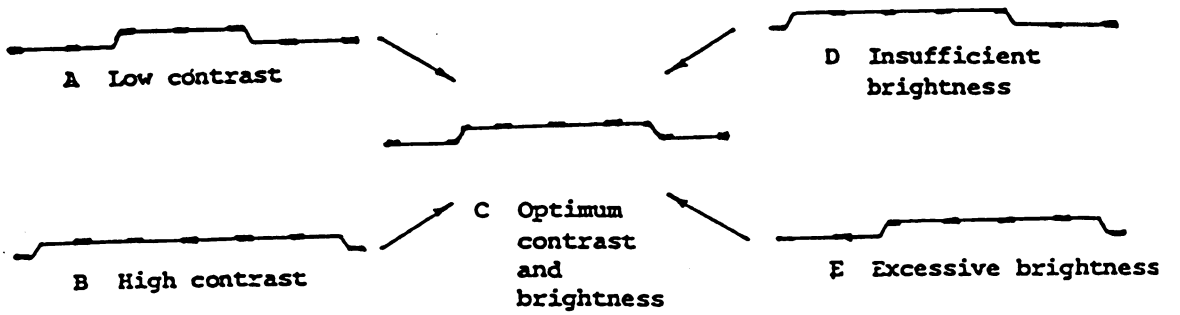


Figure 1. Contrast and Brightness Adjustment.

The contrast and brightness will require adjustment whenever the magnification is changed and immediately prior to taking a photograph of the image.

3.3. Focusing.

The JOEL T-330 has both automatic as well as manual focusing. The AFD (Automatic Focusing Device) does not work well if insufficient contrast exists. At high magnifications manual focusing is superior to automatic focusing. Thus automatic focusing is not discussed here. See Mike Rich should you desire more information on this operation.

Manual Focusing.

1. Depress the RESET button on the FUNCTION panel.

2. To adjust the coarse focus depress either the up-arrow or down-arrow located on the FOCUS panel while observing the image on the CRT.

3. To adjust the fine focus turn the FINE focusing control knob on the FOCUS panel.

It may be necessary to adjust the contrast and brightness during the focusing sequence.

3.4. Astigmatism Correction.

Astigmatism correction can be performed automatically using ASD (Automatic astigmatism correction device) or manually. Generally the manual adjustment is superior as well as quicker than ASD. Due to these limitations ASD is not discussed here. See Mike Rich should you desire more information on the ASD.

Manual Astigmatism Correction.

1. Select SLOW 1 on the SPEED panel.

2. Adjust focus at a sufficiently high magnification. Astigmatism corrections are best made at magnifications of 5000 X or greater. Adjust the focus so that it is centered, which means that the focus is set to the middle of the blurring direction.

3. Turn X control of STIGMATOR while observing image. Adjust the X control to achieve the best focus.

4. Repeat step 3 using the Y control of STIGMATOR.

5. Refocus the specimen using the fine focusing controls.

6. If difficulty is encountered in adjusting the astigmatism, set both the X and Y STIGMATOR controls to their mid-positions and strart again with step 1.

It may be necessary to repeat the astigmatism correction operation several times to obtain the highest quality image.

3.5. Objective Lens Alignement.

The objective lens should be aligned at the beginning of every session. It should also be adjusted if the accelerating voltage or if the aperature size is changed during the sem session. Aperature #2 should be used for most work.

1. Switch to TV MODE and manually adjust the CONTRAST and BRIGHTNESS to achieve a good image on the CRT.

2. Depress ALIGN on the FUNCTION panel. Notice that the image on the CRT is wobbling.

3. To align the objective lens turn the 2 knobs located on the Objective Lens Selector which is situated on the lefthand side of the column. The intent is to adjust the image so that it is not moving horizontally or vertically as

it wobbles. Use the end knob to adjust the horizontal movement, and the side knob to adjust the vertical movement. When perfectly alinged the image will seem to be moving in and out of the CRT and have the appearance of a fast beating heart.

4. When aligned depress the ALIGN button to stop the image from wobbling. At this time it may be necessary to again correct for astigmatism as described in section 3.4.

4. Photography.

1. Using SLOW1 on SPEED panel, obtain a well focused image at the desired magnification. Generally it is best to focus at a higher magnification and then lower the magnification to the desired level without further focusing.

2. Adjust CONTRAST and BRIGHTNESS as described in section 2.2.

3. Select exposure marker by depressing ON/OFF-DATA. The exposure marker will label the photograph with the accelerating voltage, magnification, magnification bar, and 6 digit identification number.There are 2 choices on the way this information is displayed on the micrograph: 1)on the photo itself when IMAGE BASE is selected, or 2) on a darkened background at the base of the micrograph when BLANK is selected.

4. Use thumbwheels of COUNTER FILM NUMBER to select the desired 6 digit identification number. To engage automatic incrementing of identification number, depress ON/OFF of the COUNTER FILM NUMBER and note that the last 2 digits of number become illuminated on panel.

5. Select exposure duration. Use QUICK for faster processing time. Use NOR (Normal) for longer exposure and higher resolution micrographs.

6. Load film.

7. Depress SHUTTER to initiate exposure. Specimen image will reappear on CRT upon completion of exposure.

8. Process the film.

9. Recommended f stop setting:

Film Type	ASA	f-stop
55	50	5.6
52	400	16
53	800	22

5. WHAT TO DO WHEN FINISHED.

1. Turn FILAMENT knob fully counterclockwise and turn off the red HT button.

2. Remove the sample from the SEM. See Section 2 for instructions.

3. Fully evacuate the column by depressing PUMP DOWN

4. Increase magnification to its highest level: 200 000X.

5. Select SLOW 2 on the SPEED PANEL.

POLARON SPUTTER COATER

1. Lift top to insert specimen to be coated. Place specimen in center of chamber.

2. Turn the collar lock atop chamber to release the target locking mechanism. Adjust the target so that it is approximately 50 mm (2 in) from specimen surface. Note: the target is the gold ring located inside the top of the chamber. Do not ever touch the target.

3. Close top of chamber and open valve on argon cylinder. Adjust flow to 5 psi on the regulator.

4. Turn OPERATION SWITCH to PUMP. The rotary pump will begin to evacuate the chamber.

5. When 0.1 mbar vacuum achieved, open LEAK valve about 3 rotations and allow pressure to rise to about 0.5 mbar. Flush chamber with argon for 30 seconds, then close LEAK valve.

6. Repeat step 5.

7. Allow system to pump down to between .04 and .03 mbar. This may take up to 10 minutes.

8. Set OPERATION SWITCH to SET HT.

9. While observing the ammeter, slowly increase the VOLTAGE knob to about 2.5 KV. If amperage exceeds 30 mamps decrease the voltage. A purple plasma discharge should now be evident coming from the target. Keep increasing VOLTAGE till 25 mamps or 2.5 kV is attained.

10. The vacuum will begin to degrade and amperage decline as the plasma is generated. Thus to maintain 25 mamps at 2.5 kV a small amount of argon is required to be added to the chamber. Slowly open LEAK valve while observing the ammeter. Adjust the LEAK valve so that 25 mamps is maintained at 2.5 kV.

11. The thickness of the deposited gold film can be estimated by using the empirically derived relationship:

$$T = 7.5It$$

where T is thickness in Angstrom units, I is current in mamps, and t is time in minutes. This relationship is valid only for a specimen to

target distance of 50 mm and at 2.5 kV. Thus a 1 minute exposure to the plasma at 25 mamps, 2.5 kV, with a target to specimen distance of 50 mm, a thickness of approximately 200 Angstroms will be deposited on the sample surface.

12. After subjecting the sample to the plasma for the desired amount of time, return VOLTAGE to the zero position.

13. Turn OPERATION SWITCH to OFF.

14. Open VENT valve to return chamber to ambient pressure.

15. Remove specimen from chamber.

16. Close VENT and LEAK valve on sputter coater and close argon cylinder valve.

17. Clean the inside of the glass chamber using a Kim-wipe wetted with ethanol.

APPENDIX 2: Preparation of Biological Samples for S.E.M.

APPENDIX

Biological Specimen Preparation for S.E.M.

The tissue and/or cells are placed in a solution with a fixative, usually 5% glutaraldehyde. The fixative is used in a buffer solution which maintains constant pH, usually 7.2. This is allowed to sit for one to two hours at room temperature or in an ice bucket.

The samples are then washed with the buffer once or twice to remove the fixative.

The sample is next dehydrated with ethyl alcohol. The sample is contacted with gradually increasing graduations of ethanol, 25%, 50%, 75%, 95% and 100%. About fifteen minutes of contact is required at each graduation. The 100% ethanol step is repeated to make sure that the cells are fully dehydrated.

The last step is the critical point drying step, ideally this should occur directly after the ethanol drying step. Critical drying prevents the cells from shriveling or distorting because of the cells are not allowed react to the surface tension forces caused by the tissue water. Critical step drying is done with carbon dioxide at 700-900 psig. The contact with the CO_2 allows the ethanol in the sample to be replaced with CO_2. This soaking with liquid CO_2 is repeated a few times to be sure the ethanol is removed. The last contacting of liquid CO_2 is then heated and the vapor removed from the sample container until the critical point, 1120 psi and 32°C, is reached. After this the sample should be completely dry and ready to be sputter coated for the S.E.M..

APPENDIX 3: Assay for α-amylase

AMYLASE ASSAY-STARCH IODINE METHOD

REAGENTS:

0.2M acetate buffer (6.56 g NaAc + 1.2 g\HAc, adjust to 500 ml with H2O, adjust pH to 5.25 with NaOH)
0.5M CaCl2
1% starch (Difco soluable starch) solution in H2O
1N HCL
3% KI/0.3% I2

REACTION COCKTAIL:

5 ml 0.2M NaAc
1 ml CaCl2
1 ml starch solution
3 ml dd H2O

REACTION:

1) To 0.8 ml of reaction cocktail, add enzyme solution (about 25 microliters of a 1.5 unit/ml solution gives a reasonable change in optical density in 5 minutes)

2) Stop reaction at desired time by immersing tube in ice, add 1 ml of 1N HCl

3) Add 200 microliters of 3% KI/0.3% I2 solution for color rxn

4) After tubes have warmed to room temp. (about 15 min.) read on spectrophotometer at 620 nm (iodine binding is temperature dependent)

Zero time points should be made by adding enzyme directly before iodine solution with no exposure to assay temperature. Blanks are made by substituting dd H2O for enzyme solutions.

Standard assay temperature for B. stearothermophilus amylase is 70 deg C. One unit of activity is defined as the amount of enzyme required to release 1 micromole of maltose/minute. We assume that all of the starch can be quantitatively converted into maltose. Thus, the subsequent loss of optical density is directly proportional to the amount of maltose released, which in turn is directly proportional to the enzyme activity. The assay is significantly non-linear with time and enzyme concentration. One should set up the assay such that replicates of several time points are used, and that changes in optical density between times points are small. Beware of the source of the starch; there are great differences in iodine binding capacity with different suppliers. Blanks containing water instead of enzyme should have an optical density of between 1.0 to 1.3

ref. Manning and Campbell 1961. J. Biol. Chem. 236:2952-2957.

α-amylase Assay

$$\left[\frac{\text{\# units } \alpha\text{-amylase}}{\text{ml}}\right] = \left[\frac{S_0 - S_t}{S_0}\right] \cdot \left[\frac{25}{V}\right] \cdot \left[\frac{1}{t}\right] \cdot \left[\frac{1}{d}\right] \cdot 93.567$$

NOMENCLATURE

S_0, S_t = spectrometer reading at time 0, at time t
V = sample of enzyme solution used in assay (μl)
t = time enzyme allowed to react (min.)
d = dilution of original enzyme solution

APPENDIX 4: Diameters of Mesh Size

U.S Sieve and Tyler Equivalents
(ASTM-E-11-61)

Tyler equivalent Mesh	Diameter (mm.)
2.5	8.000
3.0	6.730
3.5	5.660
4	4.760
5	4.000
6	3.360
7	2.830
8	2.380
9	2.000
10	1.680
12	1.410
14	1.190
16	1.000
20	0.841
24	0.707
28	0.595
32	0.500
35	0.420
42	0.354
48	0.297
60	0.250
65	0.210
80	0.177
100	0.149
115	0.125
150	0.105
170	0.088
200	0.074
250	0.063
270	0.053
325	0.044
400	0.037

APPENDIX 5: Equilibrium Spreadsheet

```
Equilibrium Spreadsheet
A1          = "Resin: XU-434-200.00
F1          P= "Int./Fin.
A2          = "Date:
B2          = "7/13/88
D2          P= "Solution    pH
F2          = "7.9/7.7
G2          P= "      K1=
H2          P= AI7
D3          P= "Tris buffer
F3          = .05
G3          P= "M     K2=
H3          P= AI8
G4          P= "       r=
H4          P= AH26
AA4         P= "Conc./Ads=(1/k1)*Conc. + k2/k1
AI5         P= .1
C6          P= "Volume of Sample (ml)
D6          P= "Sample Volume (ml)
F6          = 9
AA6         P= "Conc./Ads. vs. Conc.
AI6         P= 0
AH7         P= "Coeff. 1
AI7         P= 1/AH23
C8          P= "     Resin
E8          P= "Initial
F8          P= "    Final Klett
G8          P= "Initial
G8          P= "    Adsorbed
AA8         P= "Exp
AH8         P= "Coeff. 2
AI8         = AI7*AH24
C9          P= "     Mass (g)
E9          P= "Klett
F9          P= "     (24 hrs.)
J9          P= "Init.
L9          P= "Conc.
N9          P= "Conc.
O9          P= "Conc.
Q9          P= " (g cell/g resin)
S9          P= "Exp.
T9          P= "L.R.
U9          P= "  Conc. (g cells/ml)
Z9          P= "Exp.
AA9         P= "Langmuir 2
AB9         P= "    X
AH9         P= "  r^2=
AI9         P= AH26^2
A10         P= "    Sample #
C10         P= "    #1
D10         P= "    #2
F10         P= "    #1
G10         P= "    #2
```

```
Equilibrium Spreadsheet
J10          P= "Conc.
L10          P= "  #1
N10          P= "  #2
O10          P= "(g cell/ml)
Q10          P= "    #1
R10          P= "    #2
S10          P= "Average
T10          P= "(L.R.)
U10          P= "    #1
V10          P= "    #2
W10          P= " Ave.
Y10          P= "Conc.
Z10          P= "Adsorb
AA10         P= "Conc./Ads.
AB10         P= "Conc.
AC10         P= 20
AD10         P= "x^2
AE10         P= "y^2
AF10         P= "  x*y
AI10         P= "Mdl
A11          P= 1
C11           = .407
D11           = .395
E11           = 435
F11           = 269
G11           = 243
I11          P= IF(E11<300,1.242E-6*E11-3.75E-5,1.711E-6*E11-1.81E-4)
J11          P= IF(E11<125,9.5E-7*E11,I11)
K11          P= IF(F11<300,1.242E-6*F11-3.75E-5,1.711E-6*F11-1.81E-4)
L11          P= IF(F11<125,9.5E-7*F11,K11)
M11          P= IF(G11<300,1.242E-6*G11-3.75E-5,1.711E-6*G11-1.81E-4)
N11          P= IF(G11<125,9.5E-7*G11,M11)
O11          P= J11
Q11          P= (J11-L11)*F6/C11
R11          P= (J11-N11)*F6/D11
S11          P= (Q11+R11)/2
T11          P= U26*O11+U27
U11          P= L11
V11          P= N11
W11          P= (U11+V11)/2
Y11          P= IF(U11<=0,0,U11)
Z11          P= IF(F11<=0,0,Q11)
AA11         P= IF(F11<=0,0,Y11/Z11)
AB11         P= IF(AA11=0,0,Y11)
AC11         P= IF(Y11<=0,AC10-1,AC10)
AD11         P= AB11^2
AE11         P= AA11^2
AF11         P= AB11*AA11
AI11         P= AI7*Y11/(AI8+Y11)
AJ11         P= AH23*AB11+AH24
A12          P= A11+1
C12           = .411
```

```
Equilibrium Spreadsheet
D12          = .408
E12          = 382
F12          = 151
G12          = 155
I12         P= IF(E12<300,1.242E-6*E12-3.75E-5,1.711E-6*E12-1.81E-4)
J12         P= IF(E12<125,9.5E-7*E12,I12)
K12         P= IF(F12<300,1.242E-6*F12-3.75E-5,1.711E-6*F12-1.81E-4)
L12         P= IF(F12<125,9.5E-7*F12,K12)
M12         P= IF(G12<300,1.242E-6*G12-3.75E-5,1.711E-6*G12-1.81E-4)
N12         P= IF(G12<125,9.5E-7*G12,M12)
O12         P= J12
Q12         P= (J12-L12)*F6/C12
R12         P= (J12-N12)*F6/D12
S12         P= (Q12+R12)/2
T12         P= U26*O12+U27
U12         P= L12
V12         P= N12
W12         P= (U12+V12)/2
Y12         P= IF(U12<=0,0,U12)
Z12         P= IF(F12<=0,0,Q12)
AA12        P= IF(F12<=0,0,Y12/Z12)
AB12        P= IF(AA12=0,0,Y12)
AC12        P= IF(Y12<=0,AC11-1,AC11)
AD12        P= AB12^2
AE12        P= AA12^2
AF12        P= AB12*AA12
AI12        P= AI7*Y12/(AI8+Y12)
AJ12        P= AH23*AB12+AH24
A13         P= A12+1
C13          = .41
D13          = .413
E13          = 325
F13          = 125
G13          = 111
I13         P= IF(E13<300,1.242E-6*E13-3.75E-5,1.711E-6*E13-1.81E-4)
J13         P= IF(E13<125,9.5E-7*E13,I13)
K13         P= IF(F13<300,1.242E-6*F13-3.75E-5,1.711E-6*F13-1.81E-4)
L13         P= IF(F13<125,9.5E-7*F13,K13)
M13         P= IF(G13<300,1.242E-6*G13-3.75E-5,1.711E-6*G13-1.81E-4)
N13         P= IF(G13<125,9.5E-7*G13,M13)
O13         P= J13
Q13         P= (J13-L13)*F6/C13
R13         P= (J13-N13)*F6/D13
S13         P= (Q13+R13)/2
T13         P= U26*O13+U27
U13         P= L13
V13         P= N13
W13         P= (U13+V13)/2
Y13         P= IF(U13<=0,0,U13)
Z13         P= IF(F13<=0,0,Q13)
AA13        P= IF(F13<=0,0,Y13/Z13)
AB13        P= IF(AA13=0,0,Y13)
```

```
Equilibrium Spreadsheet
AC13        P= IF(Y13<=0,AC12-1,AC12)
AD13        P= AB13^2
AE13        P= AA13^2
AF13        P= AB13*AA13
AI13        P= AI7*Y13/(AI8+Y13)
AJ13        P= AH23*AB13+AH24
A14         P= A13+1
C14          = .4
D14          = .391
E14          = 266
F14          = 54
G14          = 69
I14         P= IF(E14<300,1.242E-6*E14-3.75E-5,1.711E-6*E14-1.81E-4)
J14         P= IF(E14<125,9.5E-7*E14,I14)
K14         P= IF(F14<300,1.242E-6*F14-3.75E-5,1.711E-6*F14-1.81E-4)
L14         P= IF(F14<125,9.5E-7*F14,K14)
M14         P= IF(G14<300,1.242E-6*G14-3.75E-5,1.711E-6*G14-1.81E-4)
N14         P= IF(G14<125,9.5E-7*G14,M14)
O14         P= J14
Q14         P= (J14-L14)*F6/C14
R14         P= (J14-N14)*F6/D14
S14         P= (Q14+R14)/2
T14         P= U26*O14+U27
U14         P= L14
V14         P= N14
W14         P= (U14+V14)/2
Y14         P= IF(U14<=0,0,U14)
Z14         P= IF(F14<=0,0,Q14)
AA14        P= IF(F14<=0,0,Y14/Z14)
AB14        P= IF(AA14=0,0,Y14)
AC14        P= IF(Y14<=0,AC13-1,AC13)
AD14        P= AB14^2
AE14        P= AA14^2
AF14        P= AB14*AA14
AI14        P= AI7*Y14/(AI8+Y14)
AJ14        P= AH23*AB14+AH24
A15         P= A14+1
C15          = .401
D15          = .403
E15          = 210
F15          = 4
G15          = 8
I15         P= IF(E15<300,1.242E-6*E15-3.75E-5,1.711E-6*E15-1.81E-4)
J15         P= IF(E15<125,9.5E-7*E15,I15)
K15         P= IF(F15<300,1.242E-6*F15-3.75E-5,1.711E-6*F15-1.81E-4)
L15         P= IF(F15<125,9.5E-7*F15,K15)
M15         P= IF(G15<300,1.242E-6*G15-3.75E-5,1.711E-6*G15-1.81E-4)
N15         P= IF(G15<125,9.5E-7*G15,M15)
O15         P= J15
Q15         P= (J15-L15)*F6/C15
R15         P= (J15-N15)*F6/D15
S15         P= (Q15+R15)/2
```

```
Equilibrium Spreadsheet
T15          P= U26*O15+U27
U15          P= L15
V15          P= N15
W15          P= (U15+V15)/2
Y15          P= IF(U15<=0,0,U15)
Z15          P= IF(F15<=0,0,Q15)
AA15         P= IF(F15<=0,0,Y15/Z15)
AB15         P= IF(AA15=0,0,Y15)
AC15         P= IF(Y15<=0,AC14-1,AC14)
AD15         P= AB15^2
AE15         P= AA15^2
AF15         P= AB15*AA15
AI15         P= AI7*Y15/(AI8+Y15)
AJ15         P= AH23*AB15+AH24
A16          P= A15+1
C16           = .402
D16           = .396
E16           = 165
F16           = 4
G16           = 2
I16          P= IF(E16<300,1.242E-6*E16-3.75E-5,1.711E-6*E16-1.81E-4)
J16          P= IF(E16<125,9.5E-7*E16,I16)
K16          P= IF(F16<300,1.242E-6*F16-3.75E-5,1.711E-6*F16-1.81E-4)
L16          P= IF(F16<125,9.5E-7*F16,K16)
M16          P= IF(G16<300,1.242E-6*G16-3.75E-5,1.711E-6*G16-1.81E-4)
N16          P= IF(G16<125,9.5E-7*G16,M16)
O16          P= J16
Q16          P= (J16-L16)*F6/C16
R16          P= (J16-N16)*F6/D16
S16          P= (Q16+R16)/2
T16          P= U26*O16+U27
U16          P= L16
V16          P= N16
W16          P= (U16+V16)/2
Y16          P= IF(U16<=0,0,U16)
Z16          P= IF(F16<=0,0,Q16)
AA16         P= IF(F16<=0,0,Y16/Z16)
AB16         P= IF(AA16=0,0,Y16)
AC16         P= IF(Y16<=0,AC15-1,AC15)
AD16         P= AB16^2
AE16         P= AA16^2
AF16         P= AB16*AA16
AI16         P= AI7*Y16/(AI8+Y16)
AJ16         P= AH23*AB16+AH24
A17          P= A16+1
C17           = .395
D17           = .395
E17           = 114.5
F17           = .5
G17           = .5
I17          P= IF(E17<300,1.242E-6*E17-3.75E-5,1.711E-6*E17-1.81E-4)
J17          P= IF(E17<125,9.5E-7*E17,I17)
```

```
Equilibrium Spreadsheet
K17         P= IF(F17<300,1.242E-6*F17-3.75E-5,1.711E-6*F17-1.81E-4)
L17         P= IF(F17<125,9.5E-7*F17,K17)
M17         P= IF(G17<300,1.242E-6*G17-3.75E-5,1.711E-6*G17-1.81E-4)
N17         P= IF(G17<125,9.5E-7*G17,M17)
O17         P= J17
Q17         P= (J17-L17)*F6/C17
R17         P= (J17-N17)*F6/D17
S17         P= (Q17+R17)/2
T17         P= U26*O17+U27
U17         P= L17
V17         P= N17
W17         P= (U17+V17)/2
Y17         P= IF(U17<=0,0,U17)
Z17         P= IF(F17<=0,0,Q17)
AA17        P= IF(F17<=0,0,Y17/Z17)
AB17        P= IF(AA17=0,0,Y17)
AC17        P= IF(Y17<=0,AC16-1,AC16)
AD17        P= AB17^2
AE17        P= AA17^2
AF17        P= AB17*AA17
AI17        P= AI7*Y17/(AI8+Y17)
AJ17        P= AH23*AB17+AH24
A18         P= A17+1
C18          = .92
D18          = .397
E18          = 56.5
F18          = 1
G18          = 1
I18         P= IF(E18<300,1.242E-6*E18-3.75E-5,1.711E-6*E18-1.81E-4)
J18         P= IF(E18<125,9.5E-7*E18,I18)
K18         P= IF(F18<300,1.242E-6*F18-3.75E-5,1.711E-6*F18-1.81E-4)
L18         P= IF(F18<125,9.5E-7*F18,K18)
M18         P= IF(G18<300,1.242E-6*G18-3.75E-5,1.711E-6*G18-1.81E-4)
N18         P= IF(G18<125,9.5E-7*G18,M18)
O18         P= J18
Q18         P= (J18-L18)*F6/C18
R18         P= (J18-N18)*F6/D18
S18         P= (Q18+R18)/2
T18         P= U26*O18+U27
U18         P= L18
V18         P= N18
W18         P= (U18+V18)/2
Y18         P= IF(U18<=0,0,U18)
Z18         P= IF(F18<=0,0,Q18)
AA18        P= IF(F18<=0,0,Y18/Z18)
AB18        P= IF(AA18=0,0,Y18)
AC18        P= IF(Y18<=0,AC17-1,AC17)
AD18        P= AB18^2
AE18        P= AA18^2
AF18        P= AB18*AA18
AI18        P= AI7*Y18/(AI8+Y18)
AJ18        P= AH23*AB18+AH24
```

```
Equilibrium Spreadsheet
A19          P= A18+1
C19           = 1
D19           = 1
E19           = 1
F19           = -1
G19           = -1
I19          P= IF(E19<300,1.242E-6*E19-3.75E-5,1.711E-6*E19-1.81E-4)
J19          P= IF(E19<125,9.5E-7*E19,I19)
K19          P= IF(F19<300,1.242E-6*F19-3.75E-5,1.711E-6*F19-1.81E-4)
L19          P= IF(F19<125,9.5E-7*F19,K19)
M19          P= IF(G19<300,1.242E-6*G19-3.75E-5,1.711E-6*G19-1.81E-4)
N19          P= IF(G19<125,9.5E-7*G19,M19)
O19          P= J19
Q19          P= (J19-L19)*F6/C19
R19          P= (J19-N19)*F6/D19
S19          P= (Q19+R19)/2
T19          P= U26*O19+U27
U19          P= L19
V19          P= N19
W19          P= (U19+V19)/2
Y19          P= IF(U19<=0,0,U19)
Z19          P= IF(F19<=0,0,Q19)
AA19         P= IF(F19<=0,0,Y19/Z19)
AB19         P= IF(AA19=0,0,Y19)
AC19         P= IF(Y19<=0,AC18-1,AC18)
AD19         P= AB19^2
AE19         P= AA19^2
AF19         P= AB19*AA19
AI19         P= AI7*Y19/(AI8+Y19)
AJ19         P= AH23*AB19+AH24
A20          P= A19+1
C20           = 1
D20           = 1
E20           = 1
F20           = -1
G20           = -1
I20          P= IF(E20<300,1.242E-6*E20-3.75E-5,1.711E-6*E20-1.81E-4)
J20          P= IF(E20<125,9.5E-7*E20,I20)
K20          P= IF(F20<300,1.242E-6*F20-3.75E-5,1.711E-6*F20-1.81E-4)
L20          P= IF(F20<125,9.5E-7*F20,K20)
M20          P= IF(G20<300,1.242E-6*G20-3.75E-5,1.711E-6*G20-1.81E-4)
N20          P= IF(G20<125,9.5E-7*G20,M20)
O20          P= J20
Q20          P= (J20-L20)*F6/C20
R20          P= (J20-N20)*F6/D20
S20          P= (Q20+R20)/2
T20          P= U26*O20+U27
U20          P= L20
V20          P= N20
W20          P= (U20+V20)/2
Y20          P= IF(U20<=0,0,U20)
Z20          P= IF(F20<=0,0,Q20)
```

```
Equilibrium Spreadsheet
AA20        P= IF(F20<=0,0,Y20/Z20)
AB20        P= IF(AA20=0,0,Y20)
AC20        P= IF(Y20<=0,AC19-1,AC19)
AD20        P= AB20^2
AE20        P= AA20^2
AF20        P= AB20*AA20
AI20        P= AI7*Y20/(AI8+Y20)
AJ20        P= AH23*AB20+AH24
Y21         P= IF(V11<=0,0,V11)
Z21         P= IF(G11<=0,0,R11)
AA21        P= IF(G11<=0,0,Y21/Z21)
AB21        P= IF(AA21=0,0,Y21)
AC21        P= IF(Y21<=0,AC20-1,AC20)
AD21        P= AB21^2
AE21        P= AA21^2
AF21        P= AB21*AA21
AI21        P= AI7*Y21/(AI8+Y21)
AJ21        P= AH23*AB21+AH24
A22         P= "Adsorption vs. Initial Concentration
Y22         P= IF(V12<=0,0,V12)
Z22         P= IF(G12<=0,0,R12)
AA22        P= IF(G12<=0,0,Y22/Z22)
AB22        P= IF(AA22=0,0,Y22)
AC22        P= IF(Y22<=0,AC21-1,AC21)
AD22        P= AB22^2
AE22        P= AA22^2
AF22        P= AB22*AA22
AI22        P= AI7*Y22/(AI8+Y22)
AJ22        P= AH23*AB22+AH24
A23         P= "Adsorption (g cell/ g resin)
Y23         P= IF(V13<=0,0,V13)
Z23         P= IF(G13<=0,0,R13)
AA23        P= IF(G13<=0,0,Y23/Z23)
AB23        P= IF(AA23=0,0,Y23)
AC23        P= IF(Y23<=0,AC22-1,AC22)
AD23        P= AB23^2
AE23        P= AA23^2
AF23        P= AB23*AA23
AG23        P= "   m=
AH23        P= (AC31*AF31-AB31*AA31)/(AC31*AD31-AB31^2)
AI23        P= AI7*Y23/(AI8+Y23)
AJ23        P= AH23*AB23+AH24
A24         P= "Initial Conc. (g cell/ ml)
Y24         P= IF(V14<=0,0,V14)
Z24         P= IF(G14<=0,0,R14)
AA24        P= IF(G14<=0,0,Y24/Z24)
AB24        P= IF(AA24=0,0,Y24)
AC24        P= IF(Y24<=0,AC23-1,AC23)
AD24        P= AB24^2
AE24        P= AA24^2
AF24        P= AB24*AA24
AG24        P= " y-int. =
```

```
Equilibrium Spreadsheet
AH24        P= AA31/AC31-AH23*AB31/AC31
AI24        P= AI7*Y24/(AI8+Y24)
AJ24        P= AH23*AB24+AH24
A25         P= "Adsorption vs. Concentration
Y25         P= IF(V15<=0,0,V15)
Z25         P= IF(G15<=0,0,R15)
AA25        P= IF(G15<=0,0,Y25/Z25)
AB25        P= IF(AA25=0,0,Y25)
AC25        P= IF(Y25<=0,AC24-1,AC24)
AD25        P= AB25^2
AE25        P= AA25^2
AF25        P= AB25*AA25
AI25        P= AI7*Y25/(AI8+Y25)
AJ25        P= AH23*AB25+AH24
Y26         P= IF(V16<=0,0,V16)
Z26         P= IF(G16<=0,0,R16)
AA26        P= IF(G16<=0,0,Y26/Z26)
AB26        P= IF(AA26=0,0,Y26)
AC26        P= IF(Y26<=0,AC25-1,AC25)
AD26        P= AB26^2
AE26        P= AA26^2
AF26        P= AB26*AA26
AG26        P= "  r=
AH26        P= (AC31*AF31-AA31*AB31)/((AC31*AE31-AA31^2)*(AC31*AD31-AB31
               ^2))^.5
AI26        P= AI7*Y26/(AI8+Y26)
AJ26        P= AH23*AB26+AH24
Y27         P= IF(V17<=0,0,V17)
Z27         P= IF(G17<=0,0,R17)
AA27        P= IF(G17<=0,0,Y27/Z27)
AB27        P= IF(AA27=0,0,Y27)
AC27        P= IF(Y27<=0,AC26-1,AC26)
AD27        P= AB27^2
AE27        P= AA27^2
AF27        P= AB27*AA27
AI27        P= AI7*Y27/(AI8+Y27)
AJ27        P= AH23*AB27+AH24
Y28         P= IF(V18<=0,0,V18)
Z28         P= IF(G18<=0,0,R18)
AA28        P= IF(G18<=0,0,Y28/Z28)
AB28        P= IF(AA28=0,0,Y28)
AC28        P= IF(Y28<=0,AC27-1,AC27)
AD28        P= AB28^2
AE28        P= AA28^2
AF28        P= AB28*AA28
AI28        P= AI7*Y28/(AI8+Y28)
AJ28        P= AH23*AB28+AH24
Y29         P= IF(V19<=0,0,V19)
Z29         P= IF(G19<=0,0,R19)
AA29        P= IF(G19<=0,0,Y29/Z29)
AB29        P= IF(AA29=0,0,Y29)
AC29        P= IF(Y29<=0,AC28-1,AC28)
```

Equilibrium Spreadsheet

```
AD29        P= AB29^2
AE29        P= AA29^2
AF29        P= AB29*AA29
AI29        P= AI7*Y29/(AI8+Y29)
AJ29        P= AH23*AB29+AH24
Y30         P= IF(V20<=0,0,V20)
Z30         P= IF(G20<=0,0,R20)
AA30        P= IF(G20<=0,0,Y30/Z30)
AB30        P= IF(AA30=0,0,Y30)
AC30        P= IF(Y30<=0,AC29-1,AC29)
AD30        P= AB30^2
AE30        P= AA30^2
AF30        P= AB30*AA30
AI30        P= AI7*Y30/(AI8+Y30)
AJ30        P= AH23*AB30+AH24
AA31        P= SUM(AA11:AA30)
AB31        P= SUM(AB11:AB30)
AC31        P= AC30
AD31        P= SUM(AD11:AD30)
AE31        P= SUM(AE11:AE30)
AF31        P= SUM(AF11:AF30)
```

APPENDIX 6: Kinetic Spreadsheet

```
Kinetic Spreadsheet
A1          = "Resin: XU -434-200.00
D1          P= "Int. Con.
E1          P= G14
A2          P= "     K1=
B2          = .0088671
D2          P= "    ka=
E2          P= T11
A3          P= "     K2=
B3          = .0000081
D3          P= "  r =
E3          P= F64
N3          P= 1
O3          P= "Kinetic Models
X3          P= "Time(hr.)
Y3          P= "Exp (ave.)
Z3          P= "Exp #1
AA3         P= "Exp #2
AB3         P= "Exp #3
AC3         P= "IRR(ads)
AD3         P= "REV(ads)
AE3         P= "IRR
AF3         P= "Conc.
AG3         P= "Res.
AH3         P= "Ads.
AI3         P= "dC/dt
AJ3         P= "Rev.
AK3         P= "Conc.
AL3         P= "Res.
AM3         P= "Ads.
AN3         P= "dC/dt
A4          P= "----------
B4          P= "-----------------------------------
N4          P= 2
S4          P= "    K1=
T4          P= B2
V4          P= "  Co=
W4          P= G14
X4          P= 0
Y4          P= Q14
Z4          P= N14
AA4         P= O14
AB4         P= P14
AC4         P= AH4
AD4         P= AM4
AF4         P= W4/(9.5E-13)
AG4         P= T4*W5/((9.5E-13)*W6)
AH4         P= (AF4-AF4)*W6*9.5E-13/W5
AI4         P= (T11*AF4*AG4)
AK4         P= W4/(9.5E-13)
AL4         P= T4*W5/((9.5E-13)*W6)
AM4         P= (AF4-AF4)*W6*9.5E-13/W5
AN4         P= U11*AK4*AL4-U13*(AK4-AK4)
```

```
Kinetic Spreadsheet
B5          P= "Sample #
C5           = "   1
D5           = "   2
E5           = "   3
N5          P= 3
S5          P= "    K2=
T5          P= B3
V5          P= "Mass Res.=
W5          P= SUM(C6:E6)/3
X5          P= X4+W8
AC5         P= AH5
AD5         P= AM5
AF5         P= AF4-AI4*W8
AG5         P= AG4-(AF4-AF5)
AH5         P= (AF4-AF5)*W6*9.5E-13/W5
AI5         P= (T11*AF5*AG5)
AK5         P= AK4-AN4*W8
AL5         P= AL4-(AK4-AK5)
AM5         P= (AK4-AK5)*W6*9.5E-13/W5
AN5         P= U11*AK5*AL5-U13*(AK4-AK5)
B6          P= "Mass (g)
C6           = .5
D6           = .498
E6           = .499
N6          P= "ave.
V6          P= "Vol=
W6          P= D7
X6          P= X5+W8
AC6         P= AH6
AD6         P= AM6
AF6         P= AF5-AI5*W8
AG6         P= AG4-(AF4-AF6)
AH6         P= (AF4-AF6)*W6*9.5E-13/W5
AI6         P= (T11*AF6*AG6)
AK6         P= AK5-AN5*W8
AL6         P= AL4-(AK4-AK6)
AM6         P= (AK4-AK6)*W6*9.5E-13/W5
AN6         P= U11*AK6*AL6-U13*(AK4-AK6)
B7          P= "Sample Volume (ml)
D7           = 10
N7          P= "Adsorbance vs. Time
S7          P= "void vol.
T7          P= .6787
X7          P= X6+W8
AC7         P= AH7
AD7         P= AM7
AF7         P= AF6-AI6*W8
AG7         P= AG4-(AF4-AF7)
AH7         P= (AF4-AF7)*W6*9.5E-13/W5
AI7         P= (T11*AF7*AG7)
AK7         P= AK6-AN6*W8
AL7         P= AL4-(AK4-AK7)
```

```
Kinetic Spreadsheet
AM7          P= (AK4-AK7)*W6*9.5E-13/W5
AN7          P= U11*AK7*AL7-U13*(AK4-AK7)
B8           P= "Original Klett
D8            = 104
N8           P= "Time (hr.)
V8           P= "delta time
W8           P= .05
X8           P= X7+W8
AC8          P= AH8
AD8          P= AM8
AF8          P= AF7-AI7*W8
AG8          P= AG4-(AF4-AF8)
AH8          P= (AF4-AF8)*W6*9.5E-13/W5
AI8          P= (T11*AF8*AG8)
AK8          P= AK7-AN7*W8
AL8          P= AL4-(AK4-AK8)
AM8          P= (AK4-AK8)*W6*9.5E-13/W5
AN8          P= U11*AK8*AL8-U13*(AK4-AK8)
A9           P= "Date:
B9           P= "----------------------------------------
I9           P= "Conc. vs. Time
S9           P= "Keq.=
T9           P= 1/(T5/9.5E-13)
V9           P= "d time >6hr.
W9           P= .5
X9           P= X8+W8
Y9           P= Q15
Z9           P= N15
AA9          P= O15
AB9          P= P15
AC9          P= AH9
AD9          P= AM9
AF9          P= AF8-AI8*W8
AG9          P= AG4-(AF4-AF9)
AH9          P= (AF4-AF9)*W6*9.5E-13/W5
AI9          P= (T11*AF9*AG9)
AK9          P= AK8-AN8*W8
AL9          P= AL4-(AK4-AK9)
AM9          P= (AK4-AK9)*W6*9.5E-13/W5
AN9          P= U11*AK9*AL9-U13*(AK4-AK9)
C10          P= " Sample #
G10          P= "ADSORBANCE
I10          P= "Conc. (g cell/ml)
N10          P= "Adsorbance (g cell/ g resin)
S10          P= "Eq. fctr:
T10          P= 1
V10          P= "Adsorbance(g cell/g dry resin)
X10          P= X9+W8
AC10         P= AH10
AD10         P= AM10
AF10         P= AF9-AI9*W8
AG10         P= AG4-(AF4-AF10)
```

```
Kinetic Spreadsheet
AM7          P= (AK4-AK7)*W6*9.5E-13/W5
AN7          P= U11*AK7*AL7-U13*(AK4-AK7)
B8           P= "Original Klett
D8            = 104
N8           P= "Time (hr.)
V8           P= "delta time
W8           P= .05
X8           P= X7+W8
AC8          P= AH8
AD8          P= AM8
AF8          P= AF7-AI7*W8
AG8          P= AG4-(AF4-AF8)
AH8          P= (AF4-AF8)*W6*9.5E-13/W5
AI8          P= (T11*AF8*AG8)
AK8          P= AK7-AN7*W8
AL8          P= AL4-(AK4-AK8)
AM8          P= (AK4-AK8)*W6*9.5E-13/W5
AN8          P= U11*AK8*AL8-U13*(AK4-AK8)
A9           P= "Date:
B9           P= "----------------------------------------
I9           P= "Conc. vs. Time
S9           P= "Keq.=
T9           P= 1/(T5/9.5E-13)
V9           P= "d time >6hr.
W9           P= .5
X9           P= X8+W8
Y9           P= Q15
Z9           P= N15
AA9          P= O15
AB9          P= P15
AC9          P= AH9
AD9          P= AM9
AF9          P= AF8-AI8*W8
AG9          P= AG4-(AF4-AF9)
AH9          P= (AF4-AF9)*W6*9.5E-13/W5
AI9          P= (T11*AF9*AG9)
AK9          P= AK8-AN8*W8
AL9          P= AL4-(AK4-AK9)
AM9          P= (AK4-AK9)*W6*9.5E-13/W5
AN9          P= U11*AK9*AL9-U13*(AK4-AK9)
C10          P= " Sample #
G10          P= "ADSORBANCE
I10          P= "Conc. (g cell/ml)
N10          P= "Adsorbance (g cell/ g resin)
S10          P= "Eq. fctr:
T10          P= 1
V10          P= "Adsorbance(g cell/g dry resin)
X10          P= X9+W8
AC10         P= AH10
AD10         P= AM10
AF10         P= AF9-AI9*W8
AG10         P= AG4-(AF4-AF10)
```

```
Kinetic Spreadsheet
AH10        P= (AF4-AF10)*W6*9.5E-13/W5
AI10        P= (T11*AF10*AG10)
AK10        P= AK9-AN9*W8
AL10        P= AL4-(AK4-AK10)
AM10        P= (AK4-AK10)*W6*9.5E-13/W5
AN10        P= U11*AK10*AL10-U13*(AK4-AK10)
C11         P= "Klett
G11         P= "(g cell/ g resin)
I11         P= "Average
N11         P= " Experimental
Q11         P= "Average
S11         P= "ka=
T11          = F53
U11         P= J53
V11         P= "Adsorbance vs. Time (Model)
X11         P= X10+W8
AC11        P= AH11
AD11        P= AM11
AF11        P= AF10-AI10*W8
AG11        P= AG4-(AF4-AF11)
AH11        P= (AF4-AF11)*W6*9.5E-13/W5
AI11        P= (T11*AF11*AG11)
AK11        P= AK10-AN10*W8
AL11        P= AL4-(AK4-AK11)
AM11        P= (AK4-AK11)*W6*9.5E-13/W5
AN11        P= U11*AK11*AL11-U13*(AK4-AK11)
A12         P= "Time (hr.)
B12         P= "----------------------------
F12         P= "-------------------------
N12         P= " -------------------------
V12         P= "Time (hr.)
X12         P= X11+W8
AC12        P= AH12
AD12        P= AM12
AF12        P= AF11-AI11*W8
AG12        P= AG4-(AF4-AF12)
AH12        P= (AF4-AF12)*W6*9.5E-13/W5
AI12        P= (T11*AF12*AG12)
AK12        P= AK11-AN11*W8
AL12        P= AL4-(AK4-AK12)
AM12        P= (AK4-AK12)*W6*9.5E-13/W5
AN12        P= U11*AK12*AL12-U13*(AK4-AK12)
A13         P= "(hr.)
B13         P= "   1
C13         P= "   2
D13         P= "    3
G13         P= "  1
I13         P= "   2
K13         P= "   3
N13         P= "   1
O13         P= "   2
P13         P= "   3
```

```
Kinetic Spreadsheet
Q13          P= "Ave.
S13          P= "kd =
T13          P= 0
U13          P= J53/(1/(T5/9.5E-13))
X13          P= X12+W8
AC13         P= AH13
AD13         P= AM13
AF13         P= AF12-AI12*W8
AG13         P= AG4-(AF4-AF13)
AH13         P= (AF4-AF13)*W6*9.5E-13/W5
AI13         P= (T11*AF13*AG13)
AK13         P= AK12-AN12*W8
AL13         P= AL4-(AK4-AK13)
AM13         P= (AK4-AK13)*W6*9.5E-13/W5
AN13         P= U11*AK13*AL13-U13*(AK4-AK13)
A14           = 0
B14           = D8
C14           = D8
D14           = D8
F14          P= IF(B14<300,1.242E-6*B14-3.75E-5,1.711E-6*B14-1.81E-4)
G14          P= IF(B14<125,9.5E-7*B14,F14)
H14          P= IF(C14<300,1.242E-6*C14-3.75E-5,1.711E-6*C14-1.81E-4)
I14          P= IF(C14<125,9.5E-7*C14,H14)
J14          P= IF(D14<300,1.242E-6*D14-3.75E-5,1.711E-6*D14-1.81E-4)
K14          P= IF(D14<125,9.5E-7*D14,J14)
N14          P= (G14-G14)*D7/C6
O14          P= (I14-I14)*D7/D6
P14          P= (K14-K14)*D7/E6
Q14          P= SUM(N14:P14)/3
X14          P= X13+W8
Y14          P= Q16
Z14          P= N16
AA14         P= O16
AB14         P= P16
AC14         P= AH14
AD14         P= AM14
AF14         P= AF13-AI13*W8
AG14         P= AG4-(AF4-AF14)
AH14         P= (AF4-AF14)*W6*9.5E-13/W5
AI14         P= (T11*AF14*AG14)
AK14         P= AK13-AN13*W8
AL14         P= AL4-(AK4-AK14)
AM14         P= (AK4-AK14)*W6*9.5E-13/W5
AN14         P= U11*AK14*AL14-U13*(AK4-AK14)
A15           = .25
B15           = 23
C15           = 34
D15           = 31.5
F15          P= IF(B15<300,1.242E-6*B15-3.75E-5,1.711E-6*B15-1.81E-4)
G15          P= IF(B15<125,9.5E-7*B15,F15)
H15          P= IF(C15<300,1.242E-6*C15-3.75E-5,1.711E-6*C15-1.81E-4)
I15          P= IF(C15<125,9.5E-7*C15,H15)
```

Kinetic Spreadsheet

```
J15        P= IF(D15<300,1.242E-6*D15-3.75E-5,1.711E-6*D15-1.81E-4)
K15        P= IF(D15<125,9.5E-7*D15,J15)
N15        P= (G14-G15)*D7/C6
O15        P= (I14-I15)*D7/D6
P15        P= (K14-K15)*D7/E6
Q15        P= SUM(N15:P15)/3
X15        P= X14+W8
AC15       P= AH15
AD15       P= AM15
AF15       P= AF14-AI14*W8
AG15       P= AG4-(AF4-AF15)
AH15       P= (AF4-AF15)*W6*9.5E-13/W5
AI15       P= (T11*AF15*AG15)
AK15       P= AK14-AN14*W8
AL15       P= AL4-(AK4-AK15)
AM15       P= (AK4-AK15)*W6*9.5E-13/W5
AN15       P= U11*AK15*AL15-U13*(AK4-AK15)
A16         = .5
B16         = 5
C16         = 13
D16         = 12
F16        P= IF(B16<300,1.242E-6*B16-3.75E-5,1.711E-6*B16-1.81E-4)
G16        P= IF(B16<125,9.5E-7*B16,F16)
H16        P= IF(C16<300,1.242E-6*C16-3.75E-5,1.711E-6*C16-1.81E-4)
I16        P= IF(C16<125,9.5E-7*C16,H16)
J16        P= IF(D16<300,1.242E-6*D16-3.75E-5,1.711E-6*D16-1.81E-4)
K16        P= IF(D16<125,9.5E-7*D16,J16)
N16        P= (G14-G16)*D7/C6
O16        P= (I14-I16)*D7/D6
P16        P= (K14-K16)*D7/E6
Q16        P= SUM(N16:P16)/3
X16        P= X15+W8
AC16       P= AH16
AD16       P= AM16
AF16       P= AF15-AI15*W8
AG16       P= AG4-(AF4-AF16)
AH16       P= (AF4-AF16)*W6*9.5E-13/W5
AI16       P= (T11*AF16*AG16)
AK16       P= AK15-AN15*W8
AL16       P= AL4-(AK4-AK16)
AM16       P= (AK4-AK16)*W6*9.5E-13/W5
AN16       P= U11*AK16*AL16-U13*(AK4-AK16)
A17         = .75
B17         = 3
C17         = 7
D17         = 5
F17        P= IF(B17<300,1.242E-6*B17-3.75E-5,1.711E-6*B17-1.81E-4)
G17        P= IF(B17<125,9.5E-7*B17,F17)
H17        P= IF(C17<300,1.242E-6*C17-3.75E-5,1.711E-6*C17-1.81E-4)
I17        P= IF(C17<125,9.5E-7*C17,H17)
J17        P= IF(D17<300,1.242E-6*D17-3.75E-5,1.711E-6*D17-1.81E-4)
K17        P= IF(D17<125,9.5E-7*D17,J17)
```

```
Kinetic Spreadsheet
N17          P= (G14-G17)*D7/C6
O17          P= (I14-I17)*D7/D6
P17          P= (K14-K17)*D7/E6
Q17          P= SUM(N17:P17)/3
S17          P= "Prediction using Rev. Rxn.
X17          P= X16+W8
AC17         P= AH17
AD17         P= AM17
AF17         P= AF16-AI16*W8
AG17         P= AG4-(AF4-AF17)
AH17         P= (AF4-AF17)*W6*9.5E-13/W5
AI17         P= (T11*AF17*AG17)
AK17         P= AK16-AN16*W8
AL17         P= AL4-(AK4-AK17)
AM17         P= (AK4-AK17)*W6*9.5E-13/W5
AN17         P= U11*AK17*AL17-U13*(AK4-AK17)
A18           = 1
B18           = 1
C18           = 3
D18           = 2
F18          P= IF(B18<300,1.242E-6*B18-3.75E-5,1.711E-6*B18-1.81E-4)
G18          P= IF(B18<125,9.5E-7*B18,F18)
H18          P= IF(C18<300,1.242E-6*C18-3.75E-5,1.711E-6*C18-1.81E-4)
I18          P= IF(C18<125,9.5E-7*C18,H18)
J18          P= IF(D18<300,1.242E-6*D18-3.75E-5,1.711E-6*D18-1.81E-4)
K18          P= IF(D18<125,9.5E-7*D18,J18)
N18          P= (G14-G18)*D7/C6
O18          P= (I14-I18)*D7/D6
P18          P= (K14-K18)*D7/E6
Q18          P= SUM(N18:P18)/3
S18          P= "  a =
T18          P= 1
X18          P= X17+W8
AC18         P= AH18
AD18         P= AM18
AF18         P= AF17-AI17*W8
AG18         P= AG4-(AF4-AF18)
AH18         P= (AF4-AF18)*W6*9.5E-13/W5
AI18         P= (T11*AF18*AG18)
AK18         P= AK17-AN17*W8
AL18         P= AL4-(AK4-AK18)
AM18         P= (AK4-AK18)*W6*9.5E-13/W5
AN18         P= U11*AK18*AL18-U13*(AK4-AK18)
A19           = 1.5
B19           = 0
C19           = .5
D19           = 0
F19          P= IF(B19<300,1.242E-6*B19-3.75E-5,1.711E-6*B19-1.81E-4)
G19          P= IF(B19<125,9.5E-7*B19,F19)
H19          P= IF(C19<300,1.242E-6*C19-3.75E-5,1.711E-6*C19-1.81E-4)
I19          P= IF(C19<125,9.5E-7*C19,H19)
J19          P= IF(D19<300,1.242E-6*D19-3.75E-5,1.711E-6*D19-1.81E-4)
```

```
Kinetic Spreadsheet
K19          P= IF(D19<125,9.5E-7*D19,J19)
N19          P= (G14-G19)*D7/C6
O19          P= (I14-I19)*D7/D6
P19          P= (K14-K19)*D7/E6
Q19          P= SUM(N19:P19)/3
S19          P= "  b =
T19          P= ((T4*W5/W6)-W4)/9.5E-13+1/(T5/9.5E-13)
X19          P= X18+W8
Y19          P= Q17
Z19          P= N17
AA19         P= O17
AB19         P= P17
AC19         P= AH19
AD19         P= AM19
AF19         P= AF18-AI18*W8
AG19         P= AG4-(AF4-AF19)
AH19         P= (AF4-AF19)*W6*9.5E-13/W5
AI19         P= (T11*AF19*AG19)
AK19         P= AK18-AN18*W8
AL19         P= AL4-(AK4-AK19)
AM19         P= (AK4-AK19)*W6*9.5E-13/W5
AN19         P= U11*AK19*AL19-U13*(AK4-AK19)
A20           = 2
B20           = 0
C20           = 1
D20           = 0
F20          P= IF(B20<300,1.242E-6*B20-3.75E-5,1.711E-6*B20-1.81E-4)
G20          P= IF(B20<125,9.5E-7*B20,F20)
H20          P= IF(C20<300,1.242E-6*C20-3.75E-5,1.711E-6*C20-1.81E-4)
I20          P= IF(C20<125,9.5E-7*C20,H20)
J20          P= IF(D20<300,1.242E-6*D20-3.75E-5,1.711E-6*D20-1.81E-4)
K20          P= IF(D20<125,9.5E-7*D20,J20)
N20          P= (G14-G20)*D7/C6
O20          P= (I14-I20)*D7/D6
P20          P= (K14-K20)*D7/E6
Q20          P= SUM(N20:P20)/3
S20          P= "  c =
T20          P= -(W4/T5)
X20          P= X19+W8
AC20         P= AH20
AD20         P= AM20
AF20         P= AF19-AI19*W8
AG20         P= AG4-(AF4-AF20)
AH20         P= (AF4-AF20)*W6*9.5E-13/W5
AI20         P= (T11*AF20*AG20)
AK20         P= AK19-AN19*W8
AL20         P= AL4-(AK4-AK20)
AM20         P= (AK4-AK20)*W6*9.5E-13/W5
AN20         P= U11*AK20*AL20-U13*(AK4-AK20)
A21           = 3
B21           = 1
C21           = 3
```

```
Kinetic Spreadsheet
D21           = 2
F21          P= IF(B21<300,1.242E-6*B21-3.75E-5,1.711E-6*B21-1.81E-4)
G21          P= IF(B21<125,9.5E-7*B21,F21)
H21          P= IF(C21<300,1.242E-6*C21-3.75E-5,1.711E-6*C21-1.81E-4)
I21          P= IF(C21<125,9.5E-7*C21,H21)
J21          P= IF(D21<300,1.242E-6*D21-3.75E-5,1.711E-6*D21-1.81E-4)
K21          P= IF(D21<125,9.5E-7*D21,J21)
N21          P= (G14-G21)*D7/C6
O21          P= (I14-I21)*D7/D6
P21          P= (K14-K21)*D7/E6
Q21          P= SUM(N21:P21)/3
S21          P= "Predict
T21          P= V24
U21          P= "exp.
V21          P= Q23
X21          P= X20+W8
AC21         P= AH21
AD21         P= AM21
AF21         P= AF20-AI20*W8
AG21         P= AG4-(AF4-AF21)
AH21         P= (AF4-AF21)*W6*9.5E-13/W5
AI21         P= (T11*AF21*AG21)
AK21         P= AK20-AN20*W8
AL21         P= AL4-(AK4-AK21)
AM21         P= (AK4-AK21)*W6*9.5E-13/W5
AN21         P= U11*AK21*AL21-U13*(AK4-AK21)
A22           = 4
B22           = 0
C22           = 0
D22           = 0
F22          P= IF(B22<300,1.242E-6*B22-3.75E-5,1.711E-6*B22-1.81E-4)
G22          P= IF(B22<125,9.5E-7*B22,F22)
H22          P= IF(C22<300,1.242E-6*C22-3.75E-5,1.711E-6*C22-1.81E-4)
I22          P= IF(C22<125,9.5E-7*C22,H22)
J22          P= IF(D22<300,1.242E-6*D22-3.75E-5,1.711E-6*D22-1.81E-4)
K22          P= IF(D22<125,9.5E-7*D22,J22)
N22          P= (G14-G22)*D7/C6
O22          P= (I14-I22)*D7/D6
P22          P= (K14-K22)*D7/E6
Q22          P= SUM(N22:P22)/3
W22          P= "(gcell/g dry res.)
X22          P= X21+W8
AC22         P= AH22
AD22         P= AM22
AF22         P= AF21-AI21*W8
AG22         P= AG4-(AF4-AF22)
AH22         P= (AF4-AF22)*W6*9.5E-13/W5
AI22         P= (T11*AF22*AG22)
AK22         P= AK21-AN21*W8
AL22         P= AL4-(AK4-AK22)
AM22         P= (AK4-AK22)*W6*9.5E-13/W5
AN22         P= U11*AK22*AL22-U13*(AK4-AK22)
```

```
Kinetic Spreadsheet
A23          = 5
B23          = 0
C23          = 0
D23          = 0
F23         P= IF(B23<300,1.242E-6*B23-3.75E-5,1.711E-6*B23-1.81E-4)
G23         P= IF(B23<125,9.5E-7*B23,F23)
H23         P= IF(C23<300,1.242E-6*C23-3.75E-5,1.711E-6*C23-1.81E-4)
I23         P= IF(C23<125,9.5E-7*C23,H23)
J23         P= IF(D23<300,1.242E-6*D23-3.75E-5,1.711E-6*D23-1.81E-4)
K23         P= IF(D23<125,9.5E-7*D23,J23)
N23         P= (G14-G23)*D7/C6
O23         P= (I14-I23)*D7/D6
P23         P= (K14-K23)*D7/E6
Q23         P= SUM(N23:P23)/3
X23         P= X22+W8
AC23        P= AH23
AD23        P= AM23
AF23        P= AF22-AI22*W8
AG23        P= AG4-(AF4-AF23)
AH23        P= (AF4-AF23)*W6*9.5E-13/W5
AI23        P= (T11*AF23*AG23)
AK23        P= AK22-AN22*W8
AL23        P= AL4-(AK4-AK23)
AM23        P= (AK4-AK23)*W6*9.5E-13/W5
AN23        P= U11*AK23*AL23-U13*(AK4-AK23)
A24          = 24
B24          = 0
C24          = 0
D24          = 0
F24         P= IF(B24<300,1.242E-6*B24-3.75E-5,1.711E-6*B24-1.81E-4)
G24         P= IF(B24<125,9.5E-7*B24,F24)
H24         P= IF(C24<300,1.242E-6*C24-3.75E-5,1.711E-6*C24-1.81E-4)
I24         P= IF(C24<125,9.5E-7*C24,H24)
J24         P= IF(D24<300,1.242E-6*D24-3.75E-5,1.711E-6*D24-1.81E-4)
K24         P= IF(D24<125,9.5E-7*D24,J24)
N24         P= (G14-G24)*D7/C6
O24         P= (I14-I24)*D7/D6
P24         P= (K14-K24)*D7/E6
Q24         P= SUM(N24:P24)/3
S24         P= "Fin. Ads.
T24         P= (-T19+(T19^2-4*T18*T20)^.5)/(2*T18)
U24         P= (W4-(9.5E-13*T24))*W6/W5
V24         P= IF(U24*W5>W4*W6,0,U24)
X24         P= X23+W8
Y24         P= Q18
Z24         P= N18
AA24        P= O18
AB24        P= P18
AC24        P= AH24
AD24        P= AM24
AF24        P= AF23-AI23*W8
AG24        P= AG4-(AF4-AF24)
```

```
Kinetic Spreadsheet
AH24        P= (AF4-AF24)*W6*9.5E-13/W5
AI24        P= (T11*AF24*AG24)
AK24        P= AK23-AN23*W8
AL24        P= AL4-(AK4-AK24)
AM24        P= (AK4-AK24)*W6*9.5E-13/W5
AN24        P= U11*AK24*AL24-U13*(AK4-AK24)
T25         P= (-T19-(T19^2-4*T18*T20)^.5)/(2*T18)
U25         P= (W4-(9.5E-13*T25))*W6/W5
V25         P= IF(U25*W5>W4*W6,0,U25)
X25         P= X24+W8
AC25        P= AH25
AD25        P= AM25
AF25        P= AF24-AI24*W8
AG25        P= AG4-(AF4-AF25)
AH25        P= (AF4-AF25)*W6*9.5E-13/W5
AI25        P= (T11*AF25*AG25)
AK25        P= AK24-AN24*W8
AL25        P= AL4-(AK4-AK25)
AM25        P= (AK4-AK25)*W6*9.5E-13/W5
AN25        P= U11*AK25*AL25-U13*(AK4-AK25)
F26         P= "Calculating ka Irr. Rxn. 2nd Order
K26         P= "Calculating ka Rev. Rxn. 2nd Order
X26         P= X25+W8
AC26        P= AH26
AD26        P= AM26
AF26        P= AF25-AI25*W8
AG26        P= AG4-(AF4-AF26)
AH26        P= (AF4-AF26)*W6*9.5E-13/W5
AI26        P= (T11*AF26*AG26)
AK26        P= AK25-AN25*W8
AL26        P= AL4-(AK4-AK26)
AM26        P= (AK4-AK26)*W6*9.5E-13/W5
AN26        P= U11*AK26*AL26-U13*(AK4-AK26)
A27         P= "Calculating Rxn. Order Cells
F27         P= " B0=
G27         P= T4*C6/((9.5E-13)*D7)
H27         P= T4*D6/((9.5E-13)*D7)
I27         P= T4*E6/((9.5E-13)*D7)
J27         P= " a=
K27         P= G28*G27
L27         P= H28*H27
M27         P= I28*I27
X27         P= X26+W8
AC27        P= AH27
AD27        P= AM27
AF27        P= AF26-AI26*W8
AG27        P= AG4-(AF4-AF27)
AH27        P= (AF4-AF27)*W6*9.5E-13/W5
AI27        P= (T11*AF27*AG27)
AK27        P= AK26-AN26*W8
AL27        P= AL4-(AK4-AK27)
AM27        P= (AK4-AK27)*W6*9.5E-13/W5
```

Kinetic Spreadsheet

```
AN27        P= U11*AK27*AL27-U13*(AK4-AK27)
A28         P= "dC/dt
B28         P= "C ave
C28         P= "C
F28         P= "A0 =
G28         P= G14/9.5E-13
H28         P= I14/9.5E-13
I28         P= K14/9.5E-13
J28         P= "b =
K28         P= -(G27+G28+(1/(1/(T5/9.5E-13))))
L28         P= -(H27+H28+(1/(1/(T5/9.5E-13))))
M28         P= -(I27+I28+(1/(1/(T5/9.5E-13))))
X28         P= X27+W8
AC28        P= AH28
AD28        P= AM28
AF28        P= AF27-AI27*W8
AG28        P= AG4-(AF4-AF28)
AH28        P= (AF4-AF28)*W6*9.5E-13/W5
AI28        P= (T11*AF28*AG28)
AK28        P= AK27-AN27*W8
AL28        P= AL4-(AK4-AK28)
AM28        P= (AK4-AK28)*W6*9.5E-13/W5
AN28        P= U11*AK28*AL28-U13*(AK4-AK28)
A29         P= (K15-K14)/(9.5E-13*(A15-A14))
B29         P= (K14+K15)/(2*9.5E-13)
C29         P= K14/9.5E-13
E29         P= AI130
G29         P= (1/(G27-G28))
H29         P= (1/(H27-H28))
I29         P= (1/(I27-I28))
J29         P= "q =
K29         P= 4*K27-K28^2
L29         P= 4*L27-L28^2
M29         P= 4*M27-M28^2
X29         P= X28+W8
AC29        P= AH29
AD29        P= AM29
AF29        P= AF28-AI28*W8
AG29        P= AG4-(AF4-AF29)
AH29        P= (AF4-AF29)*W6*9.5E-13/W5
AI29        P= (T11*AF29*AG29)
AK29        P= AK28-AN28*W8
AL29        P= AL4-(AK4-AK29)
AM29        P= (AK4-AK29)*W6*9.5E-13/W5
AN29        P= U11*AK29*AL29-U13*(AK4-AK29)
A30         P= (K16-K15)/(9.5E-13*(A16-A15))
B30         P= (K15+K16)/(2*9.5E-13)
C30         P= K15/9.5E-13
E30         P= 1
F30         P= A14
G30         P= LN(((G27-(G14-G14)/9.5E-13)*G28)/((G28-(G14-G14)/9.5E-1
               3)*G27))*G29
```

```
Kinetic Spreadsheet
H30         P= LN(((H27-(I14-I14)/9.5E-13)*H28)/((H28-(I14-I14)/9.5E-13)
               *H27))*H29
I30         P= LN(((I27-(K14-K14)/9.5E-13)*I28)/((I28-(K14-K14)/9.5E-13)
               *I27))*I29
K30         P= (1/(-K29)^.5)*(LN((2*(G14-G14)/9.5E-13+K28-(-K29)^.5)/(2*
               (G14-G14)/9.5E-13+K28+(-K29)^.5))-K37)
L30         P= (1/(-L29)^.5)*(LN((2*(I14-I14)/9.5E-13+L28-(-L29)^.5)/(2*
               (I14-I14)/9.5E-13+L28+(-L29)^.5))-L37)
M30         P= (1/(-M29)^.5)*(LN((2*(K14-K14)/9.5E-13+M28-(-M29)^.5)/(2*
               (K14-K14)/9.5E-13+M28+(-M29)^.5))-M37)
X30         P= X29+W8
AC30        P= AH30
AD30        P= AM30
AF30        P= AF29-AI29*W8
AG30        P= AG4-(AF4-AF30)
AH30        P= (AF4-AF30)*W6*9.5E-13/W5
AI30        P= (T11*AF30*AG30)
AK30        P= AK29-AN29*W8
AL30        P= AL4-(AK4-AK30)
AM30        P= (AK4-AK30)*W6*9.5E-13/W5
AN30        P= U11*AK30*AL30-U13*(AK4-AK30)
A31         P= (K17-K16)/(9.5E-13*(A17-A16))
B31         P= (K16+K17)/(2*9.5E-13)
C31         P= K16/9.5E-13
E31         P= E30+1
F31         P= A15
G31         P= LN(((G27-(G14-G15)/9.5E-13)*G28)/((G28-(G14-G15)/9.5E-13)
               *G27))*G29
H31         P= LN(((H27-(I14-I15)/9.5E-13)*H28)/((H28-(I14-I15)/9.5E-13)
               *H27))*H29
I31         P= LN(((I27-(K14-K15)/9.5E-13)*I28)/((I28-(K14-K15)/9.5E-13)
               *I27))*I29
K31         P= (1/(-K29)^.5)*(LN((2*(G14-G15)/9.5E-13+K28-(-K29)^.5)/(2*
               (G14-G15)/9.5E-13+K28+(-K29)^.5))-K37)
L31         P= (1/(-L29)^.5)*(LN((2*(I14-I15)/9.5E-13+L28-(-L29)^.5)/(2*
               (I14-I15)/9.5E-13+L28+(-L29)^.5))-L37)
M31         P= (1/(-M29)^.5)*(LN((2*(K14-K15)/9.5E-13+M28-(-M29)^.5)/(2*
               (K14-K15)/9.5E-13+M28+(-M29)^.5))-M37)
X31         P= X30+W8
AC31        P= AH31
AD31        P= AM31
AF31        P= AF30-AI30*W8
AG31        P= AG4-(AF4-AF31)
AH31        P= (AF4-AF31)*W6*9.5E-13/W5
AI31        P= (T11*AF31*AG31)
AK31        P= AK30-AN30*W8
AL31        P= AL4-(AK4-AK31)
AM31        P= (AK4-AK31)*W6*9.5E-13/W5
AN31        P= U11*AK31*AL31-U13*(AK4-AK31)
A32         P= (K18-K17)/(9.5E-13*(A18-A17))
B32         P= (K17+K18)/(2*9.5E-13)
C32         P= K17/9.5E-13
```

```
Kinetic Spreadsheet
E32          P= E31+1
F32          P= A16
G32          P= LN(((G27-(G14-G16)/9.5E-13)*G28)/((G28-(G14-G16)/9.5E-13)
                *G27))*G29
H32          P= LN(((H27-(I14-I16)/9.5E-13)*H28)/((H28-(I14-I16)/9.5E-13)
                *H27))*H29
I32          P= LN(((I27-(K14-K16)/9.5E-13)*I28)/((I28-(K14-K16)/9.5E-13)
                *I27))*I29
K32          P= (1/(-K29)^.5)*(LN((2*(G14-G16)/9.5E-13+K28-(-K29)^.5)/(2*
                (G14-G16)/9.5E-13+K28+(-K29)^.5))-K37)
L32          P= (1/(-L29)^.5)*(LN((2*(I14-I16)/9.5E-13+L28-(-L29)^.5)/(2*
                (I14-I16)/9.5E-13+L28+(-L29)^.5))-L37)
M32          P= (1/(-M29)^.5)*(LN((2*(K14-K16)/9.5E-13+M28-(-M29)^.5)/(2*
                (K14-K16)/9.5E-13+M28+(-M29)^.5))-M37)
X32          P= X31+W8
AC32         P= AH32
AD32         P= AM32
AF32         P= AF31-AI31*W8
AG32         P= AG4-(AF4-AF32)
AH32         P= (AF4-AF32)*W6*9.5E-13/W5
AI32         P= (T11*AF32*AG32)
AK32         P= AK31-AN31*W8
AL32         P= AL4-(AK4-AK32)
AM32         P= (AK4-AK32)*W6*9.5E-13/W5
AN32         P= U11*AK32*AL32-U13*(AK4-AK32)
A33          P= (K19-K18)/(9.5E-13*(A19-A18))
B33          P= (K18+K19)/(2*9.5E-13)
C33          P= K18/9.5E-13
E33          P= E32+1
F33          P= A17
G33          P= LN(((G27-(G14-G17)/9.5E-13)*G28)/((G28-(G14-G17)/9.5E-13)
                *G27))*G29
H33          P= LN(((H27-(I14-I17)/9.5E-13)*H28)/((H28-(I14-I17)/9.5E-13)
                *H27))*H29
I33          P= LN(((I27-(K14-K17)/9.5E-13)*I28)/((I28-(K14-K17)/9.5E-13)
                *I27))*I29
K33          P= (1/(-K29)^.5)*(LN((2*(G14-G17)/9.5E-13+K28-(-K29)^.5)/(2*
                (G14-G17)/9.5E-13+K28+(-K29)^.5))-K37)
L33          P= (1/(-L29)^.5)*(LN((2*(I14-I17)/9.5E-13+L28-(-L29)^.5)/(2*
                (I14-I17)/9.5E-13+L28+(-L29)^.5))-L37)
M33          P= (1/(-M29)^.5)*(LN((2*(K14-K17)/9.5E-13+M28-(-M29)^.5)/(2*
                (K14-K17)/9.5E-13+M28+(-M29)^.5))-M37)
X33          P= X32+W8
AC33         P= AH33
AD33         P= AM33
AF33         P= AF32-AI32*W8
AG33         P= AG4-(AF4-AF33)
AH33         P= (AF4-AF33)*W6*9.5E-13/W5
AI33         P= (T11*AF33*AG33)
AK33         P= AK32-AN32*W8
AL33         P= AL4-(AK4-AK33)
AM33         P= (AK4-AK33)*W6*9.5E-13/W5
```

```
Kinetic Spreadsheet
AN33        P= U11*AK33*AL33-U13*(AK4-AK33)
E34         P= E33+1
F34         P= A18
G34         P= LN(((G27-(G14-G18)/9.5E-13)*G28)/((G28-(G14-G18)/9.5E-13)
               *G27))*G29
H34         P= LN(((H27-(I14-I18)/9.5E-13)*H28)/((H28-(I14-I18)/9.5E-13)
               *H27))*H29
I34         P= LN(((I27-(K14-K18)/9.5E-13)*I28)/((I28-(K14-K18)/9.5E-13)
               *I27))*I29
K34         P= (1/(-K29)^.5)*(LN((2*(G14-G18)/9.5E-13+K28-(-K29)^.5)/(2*
               (G14-G18)/9.5E-13+K28+(-K29)^.5))-K37)
L34         P= (1/(-L29)^.5)*(LN((2*(I14-I18)/9.5E-13+L28-(-L29)^.5)/(2*
               (I14-I18)/9.5E-13+L28+(-L29)^.5))-L37)
M34         P= (1/(-M29)^.5)*(LN((2*(K14-K18)/9.5E-13+M28-(-M29)^.5)/(2*
               (K14-K18)/9.5E-13+M28+(-M29)^.5))-M37)
X34         P= X33+W8
Y34         P= Q19
Z34         P= N19
AA34        P= O19
AB34        P= P19
AC34        P= AH34
AD34        P= AM34
AF34        P= AF33-AI33*W8
AG34        P= AG4-(AF4-AF34)
AH34        P= (AF4-AF34)*W6*9.5E-13/W5
AI34        P= (T11*AF34*AG34)
AK34        P= AK33-AN33*W8
AL34        P= AL4-(AK4-AK34)
AM34        P= (AK4-AK34)*W6*9.5E-13/W5
AN34        P= U11*AK34*AL34-U13*(AK4-AK34)
X35         P= X34+W8
AC35        P= AH35
AD35        P= AM35
AF35        P= AF34-AI34*W8
AG35        P= AG4-(AF4-AF35)
AH35        P= (AF4-AF35)*W6*9.5E-13/W5
AI35        P= (T11*AF35*AG35)
AK35        P= AK34-AN34*W8
AL35        P= AL4-(AK4-AK35)
AM35        P= (AK4-AK35)*W6*9.5E-13/W5
AN35        P= U11*AK35*AL35-U13*(AK4-AK35)
X36         P= X35+W8
AC36        P= AH36
AD36        P= AM36
AF36        P= AF35-AI35*W8
AG36        P= AG4-(AF4-AF36)
AH36        P= (AF4-AF36)*W6*9.5E-13/W5
AI36        P= (T11*AF36*AG36)
AK36        P= AK35-AN35*W8
AL36        P= AL4-(AK4-AK36)
AM36        P= (AK4-AK36)*W6*9.5E-13/W5
AN36        P= U11*AK36*AL36-U13*(AK4-AK36)
```

```
Kinetic Spreadsheet
K37         P= LN((K28-(-K29)^.5)/(K28+(-K29)^.5))
L37         P= LN((L28-(-L29)^.5)/(L28+(-L29)^.5))
M37         P= LN((M28-(-M29)^.5)/(M28+(-M29)^.5))
O37         P= "Kinetic Data
X37         P= X36+W8
AC37        P= AH37
AD37        P= AM37
AF37        P= AF36-AI36*W8
AG37        P= AG4-(AF4-AF37)
AH37        P= (AF4-AF37)*W6*9.5E-13/W5
AI37        P= (T11*AF37*AG37)
AK37        P= AK36-AN36*W8
AL37        P= AL4-(AK4-AK37)
AM37        P= (AK4-AK37)*W6*9.5E-13/W5
AN37        P= U11*AK37*AL37-U13*(AK4-AK37)
X38         P= X37+W8
AC38        P= AH38
AD38        P= AM38
AF38        P= AF37-AI37*W8
AG38        P= AG4-(AF4-AF38)
AH38        P= (AF4-AF38)*W6*9.5E-13/W5
AI38        P= (T11*AF38*AG38)
AK38        P= AK37-AN37*W8
AL38        P= AL4-(AK4-AK38)
AM38        P= (AK4-AK38)*W6*9.5E-13/W5
AN38        P= U11*AK38*AL38-U13*(AK4-AK38)
A39         P= "ln(-(dC/dt)) vs. ln(C)
O39         P= "Time (hr.)
Q39         P= "Exp 1
R39         P= "Exp 2
S39         P= "Exp 3
T39         P= "IRR(ads.)
U39         P= "REV(ads.)
X39         P= X38+W8
AC39        P= AH39
AD39        P= AM39
AF39        P= AF38-AI38*W8
AG39        P= AG4-(AF4-AF39)
AH39        P= (AF4-AF39)*W6*9.5E-13/W5
AI39        P= (T11*AF39*AG39)
AK39        P= AK38-AN38*W8
AL39        P= AL4-(AK4-AK39)
AM39        P= (AK4-AK39)*W6*9.5E-13/W5
AN39        P= U11*AK39*AL39-U13*(AK4-AK39)
A40         P= "ln(-(dC/dt))
B40         P= "  ln(C)
E40         P= E33
F40         P= SUM(F30:F33)
G40         P= SUM(G30:G33)
H40         P= SUM(H30:H33)
I40         P= SUM(I30:I33)
K40         P= SUM(K30:K33)
```

Kinetic Spreadsheet

```
L40         P= SUM(L30:L33)
M40         P= SUM(M30:M33)
O40         P= X4
Q40         P= Z4
R40         P= AA4
S40         P= AB4
T40         P= AC4
U40         P= AD4
X40         P= X39+W8
AC40        P= AH40
AD40        P= AM40
AF40        P= AF39-AI39*W8
AG40        P= AG4-(AF4-AF40)
AH40        P= (AF4-AF40)*W6*9.5E-13/W5
AI40        P= (T11*AF40*AG40)
AK40        P= AK39-AN39*W8
AL40        P= AL4-(AK4-AK40)
AM40        P= (AK4-AK40)*W6*9.5E-13/W5
AN40        P= U11*AK40*AL40-U13*(AK4-AK40)
A41         P= LN(-A29)
B41         P= LN(B29)
C41         P= LN(C29)
D41         P= 1
F41         P= F30^2
G41         P= F30*G30
H41         P= F30*H30
I41         P= F30*I30
K41         P= F30*K30
L41         P= F30*L30
M41         P= F30*M30
O41         P= X9
Q41         P= Z9
R41         P= AA9
S41         P= AB9
T41         P= AC9
U41         P= AD9
X41         P= X40+W8
AC41        P= AH41
AD41        P= AM41
AF41        P= AF40-AI40*W8
AG41        P= AG4-(AF4-AF41)
AH41        P= (AF4-AF41)*W6*9.5E-13/W5
AI41        P= (T11*AF41*AG41)
AK41        P= AK40-AN40*W8
AL41        P= AL4-(AK4-AK41)
AM41        P= (AK4-AK41)*W6*9.5E-13/W5
AN41        P= U11*AK41*AL41-U13*(AK4-AK41)
A42         P= LN(-A30)
B42         P= LN(B30)
C42         P= LN(C30)
D42         P= 2
F42         P= F31^2
```

Kinetic Spreadsheet

```
G42         P= F31*G31
H42         P= F31*H31
I42         P= F31*I31
K42         P= F31*K31
L42         P= F31*L31
M42         P= F31*M31
O42         P= X14
Q42         P= Z14
R42         P= AA14
S42         P= AB14
T42         P= AC14
U42         P= AD14
X42         P= X41+W8
AC42        P= AH42
AD42        P= AM42
AF42        P= AF41-AI41*W8
AG42        P= AG4-(AF4-AF42)
AH42        P= (AF4-AF42)*W6*9.5E-13/W5
AI42        P= (T11*AF42*AG42)
AK42        P= AK41-AN41*W8
AL42        P= AL4-(AK4-AK42)
AM42        P= (AK4-AK42)*W6*9.5E-13/W5
AN42        P= U11*AK42*AL42-U13*(AK4-AK42)
A43         P= LN(-A31)
B43         P= LN(B31)
C43         P= LN(C31)
D43         P= 3
F43         P= F32^2
G43         P= F32*G32
H43         P= F32*H32
I43         P= F32*I32
K43         P= F32*K32
L43         P= F32*L32
M43         P= F32*M32
O43         P= X19
Q43         P= Z19
R43         P= AA19
S43         P= AB19
T43         P= AC19
U43         P= AD19
X43         P= X42+W8
AC43        P= AH43
AD43        P= AM43
AF43        P= AF42-AI42*W8
AG43        P= AG4-(AF4-AF43)
AH43        P= (AF4-AF43)*W6*9.5E-13/W5
AI43        P= (T11*AF43*AG43)
AK43        P= AK42-AN42*W8
AL43        P= AL4-(AK4-AK43)
AM43        P= (AK4-AK43)*W6*9.5E-13/W5
AN43        P= U11*AK43*AL43-U13*(AK4-AK43)
A44         P= LN(-A32)
```

Kinetic Spreadsheet

```
B44         P= LN(B32)
C44         P= LN(C32)
D44         P= 4
F44         P= F33^2
G44         P= F33*G33
H44         P= F33*H33
I44         P= F33*I33
K44         P= F33*K33
L44         P= F33*L33
M44         P= F33*M33
O44         P= X24
Q44         P= Z24
R44         P= AA24
S44         P= AB24
T44         P= AC24
U44         P= AD24
X44         P= X43+W8
Y44         P= Q20
Z44         P= N20
AA44        P= O20
AB44        P= P20
AC44        P= AH44
AD44        P= AM44
AF44        P= AF43-AI43*W8
AG44        P= AG4-(AF4-AF44)
AH44        P= (AF4-AF44)*W6*9.5E-13/W5
AI44        P= (T11*AF44*AG44)
AK44        P= AK43-AN43*W8
AL44        P= AL4-(AK4-AK44)
AM44        P= (AK4-AK44)*W6*9.5E-13/W5
AN44        P= U11*AK44*AL44-U13*(AK4-AK44)
A45         P= LN(-A33)
B45         P= LN(B33)
C45         P= LN(C33)
D45         P= 5
F45         P= F34^2
G45         P= F34*G34
H45         P= F34*H34
I45         P= F34*I34
K45         P= F34*K34
L45         P= F34*L34
M45         P= F34*M34
O45         P= X34
Q45         P= Z34
R45         P= AA34
S45         P= AB34
T45         P= AC34
U45         P= AD34
X45         P= X44+W8
AC45        P= AH45
AD45        P= AM45
AF45        P= AF44-AI44*W8
```

Kinetic Spreadsheet

```
AG45        P= AG4-(AF4-AF45)
AH45        P= (AF4-AF45)*W6*9.5E-13/W5
AI45        P= (T11*AF45*AG45)
AK45        P= AK44-AN44*W8
AL45        P= AL4-(AK4-AK45)
AM45        P= (AK4-AK45)*W6*9.5E-13/W5
AN45        P= U11*AK45*AL45-U13*(AK4-AK45)
D46         P= 6
O46         P= X44
Q46         P= Z44
R46         P= AA44
S46         P= AB44
T46         P= AC44
U46         P= AD44
X46         P= X45+W8
AC46        P= AH46
AD46        P= AM46
AF46        P= AF45-AI45*W8
AG46        P= AG4-(AF4-AF46)
AH46        P= (AF4-AF46)*W6*9.5E-13/W5
AI46        P= (T11*AF46*AG46)
AK46        P= AK45-AN45*W8
AL46        P= AL4-(AK4-AK46)
AM46        P= (AK4-AK46)*W6*9.5E-13/W5
AN46        P= U11*AK46*AL46-U13*(AK4-AK46)
D47         P= 7
O47         P= X54
T47         P= AC54
U47         P= AD54
X47         P= X46+W8
AC47        P= AH47
AD47        P= AM47
AF47        P= AF46-AI46*W8
AG47        P= AG4-(AF4-AF47)
AH47        P= (AF4-AF47)*W6*9.5E-13/W5
AI47        P= (T11*AF47*AG47)
AK47        P= AK46-AN46*W8
AL47        P= AL4-(AK4-AK47)
AM47        P= (AK4-AK47)*W6*9.5E-13/W5
AN47        P= U11*AK47*AL47-U13*(AK4-AK47)
D48         P= 8
O48         P= X64
Q48         P= Z64
R48         P= AA64
S48         P= AB64
T48         P= AC64
U48         P= AD64
X48         P= X47+W8
AC48        P= AH48
AD48        P= AM48
AF48        P= AF47-AI47*W8
AG48        P= AG4-(AF4-AF48)
```

```
Kinetic Spreadsheet
AG51          P= AG4-(AF4-AF51)
AH51          P= (AF4-AF51)*W6*9.5E-13/W5
AI51          P= (T11*AF51*AG51)
AK51          P= AK50-AN50*W8
AL51          P= AL4-(AK4-AK51)
AM51          P= (AK4-AK51)*W6*9.5E-13/W5
AN51          P= U11*AK51*AL51-U13*(AK4-AK51)
A52           P= SUM(A41:A44)
B52           P= SUM(B41:B44)
C52           P= SUM(C41:C44)
D52           P= D44
F52           P= "slope=
G52           P= (E40*G51-F40*G40)/(E40*F51-F40^2)
H52           P= (E40*H51-F40*H40)/(E40*F51-F40^2)
I52           P= (E40*I51-F40*I40)/(E40*F51-F40^2)
J52           P= "  = ka =
K52           P= (E40*K51-F40*K40)/(E40*F51-F40^2)
L52           P= (E40*L51-F40*L40)/(E40*F51-F40^2)
M52           P= (E40*M51-F40*M40)/(E40*F51-F40^2)
O52           P= X104
Q52           P= Z104
R52           P= AA104
S52           P= AB104
T52           P= AC104
X52           P= X51+W8
AC52          P= AH52
AD52          P= AM52
AF52          P= AF51-AI51*W8
AG52          P= AG4-(AF4-AF52)
AH52          P= (AF4-AF52)*W6*9.5E-13/W5
AI52          P= (T11*AF52*AG52)
AK52          P= AK51-AN51*W8
AL52          P= AL4-(AK4-AK52)
AM52          P= (AK4-AK52)*W6*9.5E-13/W5
AN52          P= U11*AK52*AL52-U13*(AK4-AK52)
A53           P= "Y^2
B53           P= "X^2
D53           P= "X*Y
F53     ETR   P= (I52+H52+G52)/3
J53     ETR   P= (K52+M52+L52)/3
X53           P= X52+W8
AC53          P= AH53
AD53          P= AM53
AF53          P= AF52-AI52*W8
AG53          P= AG4-(AF4-AF53)
AH53          P= (AF4-AF53)*W6*9.5E-13/W5
AI53          P= (T11*AF53*AG53)
AK53          P= AK52-AN52*W8
AL53          P= AL4-(AK4-AK53)
AM53          P= (AK4-AK53)*W6*9.5E-13/W5
AN53          P= U11*AK53*AL53-U13*(AK4-AK53)
A54           P= A41^2
```

Kinetic Spreadsheet

```
AH48        P= (AF4-AF48)*W6*9.5E-13/W5
AI48        P= (T11*AF48*AG48)
AK48        P= AK47-AN47*W8
AL48        P= AL4-(AK4-AK48)
AM48        P= (AK4-AK48)*W6*9.5E-13/W5
AN48        P= U11*AK48*AL48-U13*(AK4-AK48)
O49         P= X74
T49         P= AC74
U49         P= AD74
X49         P= X48+W8
AC49        P= AH49
AD49        P= AM49
AF49        P= AF48-AI48*W8
AG49        P= AG4-(AF4-AF49)
AH49        P= (AF4-AF49)*W6*9.5E-13/W5
AI49        P= (T11*AF49*AG49)
AK49        P= AK48-AN48*W8
AL49        P= AL4-(AK4-AK49)
AM49        P= (AK4-AK49)*W6*9.5E-13/W5
AN49        P= U11*AK49*AL49-U13*(AK4-AK49)
D50         P= " ka =
O50         P= X84
Q50         P= Z84
R50         P= AA84
S50         P= AB84
T50         P= AC84
U50         P= AD84
X50         P= X49+W8
AC50        P= AH50
AD50        P= AM50
AF50        P= AF49-AI49*W8
AG50        P= AG4-(AF4-AF50)
AH50        P= (AF4-AF50)*W6*9.5E-13/W5
AI50        P= (T11*AF50*AG50)
AK50        P= AK49-AN49*W8
AL50        P= AL4-(AK4-AK50)
AM50        P= (AK4-AK50)*W6*9.5E-13/W5
AN50        P= U11*AK50*AL50-U13*(AK4-AK50)
F51         P= SUM(F41:F44)
G51         P= SUM(G41:G44)
H51         P= SUM(H41:H44)
I51         P= SUM(I41:I44)
K51         P= SUM(K41:K44)
L51         P= SUM(L41:L44)
M51         P= SUM(M41:M44)
O51         P= X94
T51         P= AC94
U51         P= AD94
X51         P= X50+W8
AC51        P= AH51
AD51        P= AM51
AF51        P= AF50-AI50*W8
```

Kinetic Spreadsheet

```
B54         P= B41^2
C54         P= C41^2
D54         P= A41*B41
G54         P= G30^2
H54         P= H30^2
I54         P= I30^2
K54         P= K30^2
L54         P= L30^2
M54         P= M30^2
X54         P= X53+W8
AC54        P= AH54
AD54        P= AM54
AF54        P= AF53-AI53*W8
AG54        P= AG4-(AF4-AF54)
AH54        P= (AF4-AF54)*W6*9.5E-13/W5
AI54        P= (T11*AF54*AG54)
AK54        P= AK53-AN53*W8
AL54        P= AL4-(AK4-AK54)
AM54        P= (AK4-AK54)*W6*9.5E-13/W5
AN54        P= U11*AK54*AL54-U13*(AK4-AK54)
A55         P= A42^2
B55         P= B42^2
C55         P= C42^2
D55         P= A42*B42
G55         P= G31^2
H55         P= H31^2
I55         P= I31^2
K55         P= K31^2
L55         P= L31^2
M55         P= M31^2
X55         P= X54+W8
AC55        P= AH55
AD55        P= AM55
AF55        P= AF54-AI54*W8
AG55        P= AG4-(AF4-AF55)
AH55        P= (AF4-AF55)*W6*9.5E-13/W5
AI55        P= (T11*AF55*AG55)
AK55        P= AK54-AN54*W8
AL55        P= AL4-(AK4-AK55)
AM55        P= (AK4-AK55)*W6*9.5E-13/W5
AN55        P= U11*AK55*AL55-U13*(AK4-AK55)
A56         P= A43^2
B56         P= B43^2
C56         P= C43^2
D56         P= A43*B43
G56         P= G32^2
H56         P= H32^2
I56         P= I32^2
K56         P= K32^2
L56         P= L32^2
M56         P= M32^2
X56         P= X55+W8
```

Kinetic Spreadsheet

```
AC56        P= AH56
AD56        P= AM56
AF56        P= AF55-AI55*W8
AG56        P= AG4-(AF4-AF56)
AH56        P= (AF4-AF56)*W6*9.5E-13/W5
AI56        P= (T11*AF56*AG56)
AK56        P= AK55-AN55*W8
AL56        P= AL4-(AK4-AK56)
AM56        P= (AK4-AK56)*W6*9.5E-13/W5
AN56        P= U11*AK56*AL56-U13*(AK4-AK56)
A57         P= A44^2
B57         P= B44^2
C57         P= C44^2
D57         P= A44*B44
G57         P= G33^2
H57         P= H33^2
I57         P= I33^2
K57         P= K33^2
L57         P= L33^2
M57         P= M33^2
X57         P= X56+W8
AC57        P= AH57
AD57        P= AM57
AF57        P= AF56-AI56*W8
AG57        P= AG4-(AF4-AF57)
AH57        P= (AF4-AF57)*W6*9.5E-13/W5
AI57        P= (T11*AF57*AG57)
AK57        P= AK56-AN56*W8
AL57        P= AL4-(AK4-AK57)
AM57        P= (AK4-AK57)*W6*9.5E-13/W5
AN57        P= U11*AK57*AL57-U13*(AK4-AK57)
A58         P= A45^2
B58         P= B45^2
C58         P= C45^2
D58         P= A45*B45
G58         P= G34^2
H58         P= H34^2
I58         P= I34^2
K58         P= K34^2
L58         P= L34^2
M58         P= M34^2
X58         P= X57+W8
AC58        P= AH58
AD58        P= AM58
AF58        P= AF57-AI57*W8
AG58        P= AG4-(AF4-AF58)
AH58        P= (AF4-AF58)*W6*9.5E-13/W5
AI58        P= (T11*AF58*AG58)
AK58        P= AK57-AN57*W8
AL58        P= AL4-(AK4-AK58)
AM58        P= (AK4-AK58)*W6*9.5E-13/W5
AN58        P= U11*AK58*AL58-U13*(AK4-AK58)
```

Kinetic Spreadsheet

```
X59         P= X58+W8
AC59        P= AH59
AD59        P= AM59
AF59        P= AF58-AI58*W8
AG59        P= AG4-(AF4-AF59)
AH59        P= (AF4-AF59)*W6*9.5E-13/W5
AI59        P= (T11*AF59*AG59)
AK59        P= AK58-AN58*W8
AL59        P= AL4-(AK4-AK59)
AM59        P= (AK4-AK59)*W6*9.5E-13/W5
AN59        P= U11*AK59*AL59-U13*(AK4-AK59)
X60         P= X59+W8
AC60        P= AH60
AD60        P= AM60
AF60        P= AF59-AI59*W8
AG60        P= AG4-(AF4-AF60)
AH60        P= (AF4-AF60)*W6*9.5E-13/W5
AI60        P= (T11*AF60*AG60)
AK60        P= AK59-AN59*W8
AL60        P= AL4-(AK4-AK60)
AM60        P= (AK4-AK60)*W6*9.5E-13/W5
AN60        P= U11*AK60*AL60-U13*(AK4-AK60)
X61         P= X60+W8
AC61        P= AH61
AD61        P= AM61
AF61        P= AF60-AI60*W8
AG61        P= AG4-(AF4-AF61)
AH61        P= (AF4-AF61)*W6*9.5E-13/W5
AI61        P= (T11*AF61*AG61)
AK61        P= AK60-AN60*W8
AL61        P= AL4-(AK4-AK61)
AM61        P= (AK4-AK61)*W6*9.5E-13/W5
AN61        P= U11*AK61*AL61-U13*(AK4-AK61)
G62         P= SUM(G54:G57)
H62         P= SUM(H54:H57)
I62         P= SUM(I54:I57)
J62         P= SUM(J54:J57)
K62         P= SUM(K54:K57)
L62         P= SUM(L54:L57)
M62         P= SUM(M54:M57)
X62         P= X61+W8
AC62        P= AH62
AD62        P= AM62
AF62        P= AF61-AI61*W8
AG62        P= AG4-(AF4-AF62)
AH62        P= (AF4-AF62)*W6*9.5E-13/W5
AI62        P= (T11*AF62*AG62)
AK62        P= AK61-AN61*W8
AL62        P= AL4-(AK4-AK62)
AM62        P= (AK4-AK62)*W6*9.5E-13/W5
AN62        P= U11*AK62*AL62-U13*(AK4-AK62)
F63         P= " r=
```

Kinetic Spreadsheet

```
G63     P= (E40*G51-F40*G40)/((E40*F51-F40^2)*(E40*G62-G40^2))^.5
H63     P= (E40*H51-F40*H40)/((E40*F51-F40^2)*(E40*H62-H40^2))^.5
I63     P= (E40*I51-F40*I40)/((E40*F51-F40^2)*(E40*I62-I40^2))^.5
K63     P= (E40*K51-F40*K40)/((E40*F51-F40^2)*(E40*K62-K40^2))^.5
L63     P= (E40*L51-F40*L40)/((E40*F51-F40^2)*(E40*L62-L40^2))^.5
M63     P= (E40*M51-F40*M40)/((E40*F51-F40^2)*(E40*M62-M40^2))^.5
X63     P= X62+W8
AC63    P= AH63
AD63    P= AM63
AF63    P= AF62-AI62*W8
AG63    P= AG4-(AF4-AF63)
AH63    P= (AF4-AF63)*W6*9.5E-13/W5
AI63    P= (T11*AF63*AG63)
AK63    P= AK62-AN62*W8
AL63    P= AL4-(AK4-AK63)
AM63    P= (AK4-AK63)*W6*9.5E-13/W5
AN63    P= U11*AK63*AL63-U13*(AK4-AK63)
A64     P= SUM(A54:A57)
B64     P= SUM(B54:B57)
C64     P= SUM(C54:C57)
D64     P= SUM(D54:D57)
F64     P= (G63+H63+I63)/3
X64     P= X63+W8
Y64     P= Q21
Z64     P= N21
AA64    P= O21
AB64    P= P21
AC64    P= AH64
AD64    P= AM64
AF64    P= AF63-AI63*W8
AG64    P= AG4-(AF4-AF64)
AH64    P= (AF4-AF64)*W6*9.5E-13/W5
AI64    P= (T11*AF64*AG64)
AK64    P= AK63-AN63*W8
AL64    P= AL4-(AK4-AK64)
AM64    P= (AK4-AK64)*W6*9.5E-13/W5
AN64    P= U11*AK64*AL64-U13*(AK4-AK64)
```

APPENDIX 7: Equilibrium Data

Resin: IRA-938 Int./Fin.
Date: 7/27/88 Solution pH6.21/6. K1 .00914
Tris buffer .05M K2 .00002
r .99733

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.396	.4	415	152	154
2	.402	.4	372	110	120
3	.405	.398	302	49.5	54
4	.403	.4	251	26.5	31
5	.405	.403	204	16.5	12.5
6	.405	.397	150	8.5	7.5
7	.407	.404	101	4.2	4.2
8	.393	.397	54	2	3
9	.342	1	34	1	-1
10	1	1	1	-1	-1

Resin: IRA-938 Int./Fin.
Date: 7/21/88 Solution pH6.3/6.2 K1 .01379
Tris buffer .05 or M K2 .00008
r .71365

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.401	.403	445	193	132.5
2	.402	.401	389	135	76
3	.407	.394	335	46	50
4	.403	.409	268	28	29
5	.404	.394	213	26	24
6	.397	.405	150	21.8	18.8
7	.409	.401	103.5	10	15
8	.4	.402	48.5	10	14
9	.401	1	31	4.8	-1
10	1	1	1	-1	-1

Resin: IRA-938 Int./Fin.
Date: 7/20/88 Solution pH6.5/6.5 K1 -.0755
Tris buffer .05 or M K2 -.0009
r -.0853

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.406	.4	444	125.5	148
2	.396	.406	389	53	64
3	.401	.407	325	49	45
4	.399	.408	272	35	37
5	.398	.394	212	27.5	32
6	.396	.405	154	42	30
7	.409	.394	100	21	31
8	.407	.399	51.5	16	12.5
9	.403	1	30	9.5	-1
10	1	1	1	-1	-1

Resin: IRA-938 Int./Fin.
Date: 7/26/88 Solution pH6.64/6. K1 .00903
Tris buffer .05M K2 .00001
r .97761

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.404	.405	427	142	153
2	.397	.4	377	114	116
3	.398	.403	315	82.5	73
4	.393	.412	268	53.5	53
5	.407	.394	212	19	23.5
6	.395	.398	150	7	8
7	.393	.403	102.5	2	3.5
8	.407	.401	54	1	1
9	.367	1	35	.2	-1
10	1	1	1	-1	-1

Resin: IRA-938 Int./Fin.
Date: 7/26/88 Solution pH7.2/7.1 K1 .00719
Tris buffer .05M K2 .00001
r .99806

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.399	.397	440	224	180
2	.398	.404	385	158	123
3	.402	.399	322	44.5	46.5
4	.412	.392	273	32	31
5	.401	.404	206	14	15.5
6	.391	.395	152	7.5	6.5
7	.394	.403	103	4.5	4.5
8	.408	.392	56	3	3
9	.404	1	32	1.5	-1
10	1	1	1	-1	-1

Resin: IRA-938 Int./Fin.
Date: 7/19/88 Solution pH7.0/7.2 K1 .00719
Tris buffer .05 or M K2 .00001
r .99806

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.402	.399	440	244	242
2	.398	.4	380	150	139
3	.398	.396	304	87	56
4	.397	.398	230	22.5	20
5	.4	.403	153	5	7
6	.404	.401	103.5	3	3
7	.405	.407	52	1	2
8	1	1	1	-1	-1
9	1	1	1	-1	-1
10	1	1	1	-1	-1

Resin: IRA-938
Date: 6/29/88 Solution pH 7.32 K1 .01362
Tris buffer .05M K2 .00001
r .93166

Volume of Sample (ml) 8

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.402	.401	440	15	28
2	.402	.398	385	21.5	13.5
3	.399	.401	346	10.5	8
4	.4	.402	298	6	5
5	.401	.4	246	7	4
6	.404	.401	199	4	3
7	.399	.399	156	2	1
8	.4	1	125	3	-1
9	1	1	-1	-1	-1
10	1	1	-1	-1	-1

Resin: IRA-938 Int./Fin.
Date: 6/1/89 Solution pH 7.5 K1 .01365
Tris buffer .05M K2 .00001
r .98152

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.398	.403	460	39	30
2	.405	.397	403	30.5	33
3	.395	.402	360	19	17
4	.396	.402	305	8	10
5	.395	.403	254	5	4
6	.402	.399	208	1.5	1.5
7	.396	.402	160	2	1.5
8	.391	.404	120	1.5	2.5
9	.394	.401	87	1	1
10	.398	1	60	.5	-1

Resin: IRA-938 Int./Fin.
Date: 7/18/88 Solution pH7.5/7.6 K1 .00847
Tris buffer .05 or M K2 .00001
r .99717

Volume of Sample (ml) 12

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.397	.401	445	286	260
2	.396	.398	386	200	181
3	.398	.396	305	108.5	87
4	.409	.395	238	44.5	35
5	.398	.398	203	22.5	15
6	.396	.404	154	6.5	7
7	.397	.397	101	2	1.5
8	.399	.4	53	.5	1
9	.322	1	29	.1	-1
10	1	1	1	-1	-1

Resin: IRA-938 Int./Fin.
Date: 7/14/89 Solution pH7.5 K1 .00776
Tris buffer .1M K2 .00001
r .99772

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.401	.403	450	232	239
2	.401	.402	395	148	157
3	.395	.407	326	81.5	78
4	.405	.403	258	41.5	36.5
5	.396	.405	202	24.5	17
6	.399	.409	132	2	3
7	.399	.397	87	1	.5
8	.395	1	34	.5	-1
9	.4	1	.00001	0	-1
10	1	1	1	-1	-1

Resin: IRA-938 Int./Fin.
Date: 7/25/88 Solution pH7.65/7. K1 .00882
Tris buffer .05M K2 3.9e-6
r .99940

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.408	.401	445	178	188
2	.401	.401	393	123	112
3	.394	.407	331	51	42
4	.403	.397	275	13	13
5	.402	.409	211	11.5	5.5
6	.406	.393	152	1.5	2.5
7	.395	.401	104	.5	1
8	.394	.399	55	.5	.5
9	.4	1	34	.00001	-1
10	1	1	1	-1	-1

Resin: IRA-938 Int./Fin.
Date: 7/27/88 Solution pH7.71/7. K1 .00845
Tris buffer .05M K2 .00002
r .98786

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.395	.395	440	211	169
2	.399	.396	366	154	137
3	.402	.41	310	61	59
4	.399	.393	248	26	24
5	.395	.406	194	16	11
6	.406	.397	152	9.5	10
7	.402	.4	103	4.5	3
8	.396	.396	51	4	3
9	.402	1	32	2	-1
10	1	1	1	-1	-1

Resin: IRA-938 Int./Fin.
Date: 7/28/88 Solution pH7.80/7. K1 .00910
Tris buffer .1M K2 4.8e-7
r .99652

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.395	.399	445	168	29
2	.393	.395	373	104	50
3	.394	.394	305	17.5	8
4	.403	.395	255	3.9	8
5	.405	.395	2206	3.5	1.5
6	.401	.4	154	.5	.5
7	.394	.398	100	.5	.1
8	.406	.401	52.5	.00001	.00001
9	1	1	1	-1	-1
10	1	1	1	-1	-1

Resin: IRA-938 Int./Fin.
Date: 8/2/88 Solution pH7.82/7. K1 .00913
Tris buffer .025M K2 .00001
r .98572

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.399	.396	440	165	177
2	.4	.4	390	114	125
3	.398	.405	308	58	45.5
4	.394	.394	250	41	43
5	.399	.395	204	18	24
6	.405	.409	149	2	3
7	.397	.409	100	1	1
8	.394	.395	554	.00001	.0001
9	.313	1	32.5	.00001	-1
10	1	1	1	-1	-1

Resin: IRA-938 Int./Fin.
Date: 7/28/88 Solution pH8.36/8. K1 .01052
Tris buffer .05M K2 2.4e-6
r .98755

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.401	.409	449	49	126
2	.405	.395	385	37	59
3	.398	.4	307	4.1	7
4	.4	.401	238	3.1	4.5
5	.402	.399	194	2.5	14
6	.403	.399	147	.1	.5
7	.408	.405	99.5	.00001	.00001
8	.396	.407	48	.1	.00001
9	1	1	1	-1	-1
10	1	1	1	-1	-1

Resin: XU-434-200.00 Int./Fin.
Date: 8/4/88 Solution pH6.2/6.5 K1 .00503
Tris buffer .05M K2 .00001
r .99376

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.389	.395	450	331	329
2	.394	.401	382	219	226
3	.406	.39	307	99	112
4	.403	.398	242	44.5	39.5
5	.394	.394	183	17	14
6	.392	.391	136	13	12.5
7	.391	.396	106	14	8
8	.397	.4	46	4.5	3.5
9	.403	1	19	3	-1
10	1	1	1	-1	-1

Resin: XU-434-200.00 Int./Fin.
Date: 8/3/88 Solution pH6.64/6. K1 .00464
Tris buffer .05M K2 1.8e-6
r .99979

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.394	.391	445	-1	-1
2	.395	.393	395	268	264
3	.392	.393	312	-1	-1
4	.392	.39	250	-1	-1
5	.39	.39	200	9	5
6	.395	.394	151	6.5	5.5
7	.389	.393	102	3.5	2.5
8	.396	.39	47	.00001	.5
9	.4	1	27	.5	-1
10	1	1	1	-1	-1

Resin: XU-434-200.00 Int./Fin.
Date: 8/4/88 Solution pH7.1/7.2 K1 .00484
Tris buffer .05M K2 .00001
r .99593

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.394	.39	442	313	325
2	.393	.389	367	237	226
3	.391	.397	307	130.5	102
4	.393	.395	252	46	69
5	.394	.395	192.5	18	23.5
6	.395	.4	153	10	9
7	.397	.392	102	5	5.5
8	.399	.405	57	4	3.5
9	.411	1	26	3	-1
10	1	1	1	-1	-1

Resin: XU-434-200.00 Int./Fin.
Date: 8/160/88 Solution pH7.15/7. K1 .00505
Tris buffer .05M K2 4.2e-7
r .99728

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.393	.398	447	317	324
2	.404	.399	382	201	227
3	.4	.394	308	115	138
4	.392	.395	255	62	58
5	.392	.393	204	16	19.5
6	.393	.397	158	5	4
7	.395	.4	106	3	2.5
8	.397	.397	56.5	1.5	.5
9	.407	1	31.5	.00001	-1
10	1	1	1	-1	-1

Resin: XU-434-200.00 Int./Fin.
Date: 8/8/88 Solution pH7.4/7.5 K1 .00528
Tris buffer .025M K2 4.1e-6
r .99852

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.397	.393	450	317	317
2	.403	.394	395	228	250
3	.394	.402	312	142	122
4	.399	.394	256	12.5	16
5	.393	.393	201	4	3.5
6	.399	.401	156	3.5	3.5
7	.393	.395	108	3.5	3.5
8	.397	.394	58	2.5	2.5
9	.378	1	26	2.5	-1
10	1	1	1	-1	-1

Resin: XU-434-200.00 Int./Fin.
Date: 10/6/88 Solution pH7.5/7.5 K1 .00887
Tris buffer .05M K2 .00001
r .98855

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.397	.402	453	182	181
2	.401	.405	405	149	135
3	.407	.405	345	89	95.5
4	.396	.4	303	64.5	72.5
5	.401	.401	253	9	4.8
6	.403	.4	207	5	6
7	.401	.406	154	2.8	3
8	.403	.401	102	1	.8
9	.395	.402	51.5	.5	1
10	.397	1	1	-1	-1

Resin: XU-434-200.00 Int./Fin.
Date: 6/1/89 Solution pH 7.5 K1 .00892
Tris buffer .05M K2 2.4e-6
r .99763

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.393	.4	466	193	220
2	.397	.404	385	114	71
3	.394	.395	338	61	64
4	.403	.399	294	18	11
5	.4	.41	242	2.5	3
6	.404	.392	204	1	1.5
7	.394	.391	160	1	1.5
8	.409	.41	122	1	.00001
9	.4	.405	89	.5	.00001
10	.403	1	62	.5	-1

Resin: XU-434-200.00 Int./Fin.
Date: 11/30/88 Solution pH7.52/7. K1 .00605
Tris buffer .05M K2 .00001
r .99797

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.401	.397	450	284	288
2	.4	.406	392	226	222
3	.4056	.406	352	173	153
4	.404	.404	289	82.5	72
5	.404	.404	218	20.5	25
6	.401	.404	173	5	8
7	.398	.41	147	5	7
8	.4	.399	108	6	7
9	.395	.398	61	3	3
10	.395	.394	1	-1	-1

Resin: XU-434-200.00 Int./Fin.
Date: 8/10/88 Solution pH7.67/7. K1 .00827
Tris buffer .15M K2 .00001
r .96116

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.394	.397	442	-1	-1
2	.395	.394	387	161	141
3	.397	.395	301	43	41.5
4	.403	.401	254	26	26
5	.401	.401	203	4.5	6.75
6	.404	.401	150.5	4	4
7	.401	.395	103.5	3.8	3.8
8	.398	.401	54.5	3	2.5
9	.405	1	20	3	-1
10	1	1	1	-1	-1

Resin: XU-434-200.00 Int./Fin.
Date: 8/8/88 Solution pH7.74/7. K1 .00491
Tris buffer .1M K2 .00001
r .91988

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.396	.4	450	312	314
2	.403	.97	395	199	205
3	.393	.392	308	92	88
4	.393	.39	246	65	64.2
5	.4	.398	203	19	17.5
6	.392	.395	153	9.5	9
7	.392	.396	103	5	5
8	.397	.398	55	3.5	3
9	.32	1	27	3	-1
10	1	1	1	-1	-1

Resin: XU-434-200.00 Int./Fin.
Date: 7/13/88 Solution pH7.9/7.7 K1 .00644
Tris buffer .05M K2 3.9e-6
r .99345

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.407	.395	435	269	243
2	.411	.408	382	151	155
3	.41	.413	325	125	111
4	.4	.391	266	54	69
5	.401	.403	210	4	8
6	.402	.396	165	4	2
7	.395	.395	114.5	.5	.5
8	.92	.397	56.5	1	1
9	1	1	1	-1	-1
10	1	1	1	-1	-1

Resin: XU-434-200.00 Int./Fin.
Date: 8/3/88 Solution pH8.35/8. K1 .00550
Tris buffer .05M K2 8.2e-7
r .99905

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.394	.393	445	-1	305
2	.393	.397	395	228	-1
3	.396	.393	308	133	122.5
4	.391	.395	248	6	10.5
5	.399	.397	200	.00001	.00001
6	.393	.39	155	.00001	.00001
7	.393	.395	101.5	.5	.00001
8	.397	.391	54	.00001	.00001
9	1	1	1	-1	-1
10	1	1	1	-1	-1

Resin: XU-434-200.01 Int./Fin.
Date: 9/7/88 Solution pH7.0/6.8 K1 .00533
Tris buffer .05M K2 .00004
r .92006

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	1	1	435	-1	-1
2	.4	.407	387	254	255
3	.398	.41	317	146	144.5
4	.407	.401	248	105.5	73.5
5	.402	.404	197	69	36
6	.397	1	151	-1	30.5
7	.41	.407	103	.00001	-1
8	.406	1	64	24.5	-1
9	.403	1	35.5	.00001	-1
10	1	1	1	-1	-1

Resin: XU-434-200.01 Int./Fin.
Date: 9/22/88 Solution pH7.02/7. K1 .00339
Tris buffer .05M K2 3.6e-7
r .98736

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.4	.405	412	333	317
2	.4	.4	319	182	187
3	.397	.397	164.5	34	-1
4	.404	.406	83	10	-1
5	.403	.394	42.5	4.5	-1
6	.401	.407	0	-1	-1
7	.403	.407	1	-1	-1
8	.396	.398	1	-1	-1
9	.396	.4	1	-1	-1
10	1	1	1	-1	-1

Resin: XU-434-200.01 Int./Fin.
Date: 9/7/88 Solution pH7.6/7.4 K1 .00286
Tris buffer .05M K2 .00001
r .98031

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.399	.402	442	-1	378
2	.399	.409	394	315	313
3	.392	.405	307	220	181
4	.405	.399	255	131	152
5	1	1	199	-1	-1
6	.406	.397	150	19	14
7	.394	.404	102	26	39
8	.398	.409	60	-1	8
9	.407	.399	27	6.5	7
10	1	1	1	-1	-1

Resin: XU-434-200.01 Int./Fin.
Date: 9/22/88 Solution pH7.66/7. K1 .00379
Tris buffer .05M K2 1.8e-6
r .99413

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.405	.397	407	315	303
2	.398	.405	275	120	132
3	.4	.404	117	7.2	-1
4	.407	.398	79.5	4	-1
5	.402	.402	42	3.5	-1
6	.393	.399	0	-1	-1
7	.403	.396	1	-1	-1
8	.403	.406	1	-1	-1
9	.401	.4	1	-1	-1
10	1	1	1	-1	-1

Resin: XU-434-200.02 Int./Fin.
Date: 8/23/88 Solution pH7.16/7. K1 .00090
Tris buffer .05M K2 2.9e-7
r .92388

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.397	.405	445	425	420
2	.408	.404	398	380	339
3	.406	.4	315	282	286
4	.408	.406	252	220	214
5	.405	.399	201	174	169
6	.398	.401	145	109	111
7	.402	.402	117	84	79
8	.395	.407	78	39	40.5
9	.405	.403	46.5	14	16
10	.407	1	1	-1	-1

Resin: XU-434-200.02 Int./Fin.
Date: 7/14/88 Solution pH7.5/7.6 K1 .00126
Tris buffer .05M K2 .00001
r .99336

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.406	.399	480	-1	-1
2	.406	.396	430	-1	400
3	.401	.402	377	345	341
4	.399	.4	310	270	264
5	.401	.4	255	208	212
6	.403	.409	199	155	152
7	.406	.399	143	88	90.5
8	.402	.402	112	65	66.5
9	.406	.405	65	26	25
10	.408	.397	29	0	0

Resin: XU-434-200.02 Int./Fin.
Date: 8/23/88 Solution pH7.7/7.5 K1 .00183
Tris buffer .05M K2 .00015
r .85224

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.406	.401	450	412	410
2	.395	.4	393	352	349
3	.4	.397	340	301	300
4	.404	.403	290	258	258.5
5	.405	.397	242	214	212
6	.4	.406	188	159	157.5
7	.402	.401	135	103	97
8	.409	.404	81	54	46
9	.401	.399	17.5	.5	-1
10	.4	1	1	-1	-1

Resin: XU-434-200.02 Int./Fin.
Date: 10/13/88 Solution pH7.76/7. K1 .00267
Tris buffer .05M K2 2.5e-6
r .99407

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.397	.399	447	-1	-1
2	.397	.393	380	315	307
3	.403	.403	345	259	-1
4	.405	.406	292	-1	205
5	.409	.407	246	138	135
6	.403	.404	199	83	80
7	.41	.402	152	26	20.5
8	.408	.403	71	5	5
9	.407	.41	40.5	-1	-1
10	.411	1	1	-1	-1

Resin: XUS-40187.00 Int./Fin.
Date: 8/10/88 Solution pH6.08/6. K1 .00046
Tris buffer .05M K2 .00031
r .46287

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.398	.398	445	440	439
2	.4	.396	395	382	374
3	.401	.401	311	301	294
4	.408	.403	254	240	250
5	.403	.397	204	-1	-1
6	.393	.393	157	-1	-1
7	.395	.403	106	103.5	104
8	.399	.404	55	51	50
9	.405	.402	24	21	19.5
10	1	1	1	-1	-1

Resin: XUS-40187.00 Int./Fin.
Date: 8/9/88 Solution pH6.66/6. K1 .00121
Tris buffer .05M K2 .00022
r .84889

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.397	.391	440	415	417
2	.394	.398	365	340	345
3	.395	.392	307	285	290
4	.407	.396	254	228	232
5	.39	.409	200	183	183
6	.395	.403	155	142	137
7	.395	.405	104	93	92
8	.398	.395	57	44	45.5
9	.4	.406	31	21	21
10	1	1	-1	-1	-1

Resin: XUS-40187.00 Int./Fin.
Date: 7/6/88 Solution pH7.6/7.7 K1 .00466
Tris buffer .05M K2 .00042
r .92337

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.4	.4	442	375	373
2	.402	.4	391	335	335
3	.399	.403	341	284	284
4	.4	.398	278	222	224
5	.403	.4	201	149	153
6	.4	.398	95	66.5	-1
7	.398	.399	1	-1	-1
8	.401	.399	1	-1	-1
9	.399	.399	1	-1	-1
10	.403	.402	1	-1	-1

Resin: XUS-40187.00 Int./Fin.
Date: 8/9/88 Solution pH8.19/7. K1 .00437
Tris buffer .05M K2 .00040
r .77411

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.39	.402	443	377	381
2	.401	.392	390	336	332
3	.402	.4	307	245	242
4	.403	.392	241	187	193
5	.402	.398	190	149	147
6	.398	.402	148	115	113.5
7	.397	.397	105	85	80
8	.395	.399	51.5	36.5	34
9	.397	.395	19	9	9
10	1	1	-1	-1	-1

Resin: XU-40123.00 Int./Fin.
Date: 6/30/88 Solution pH7.6/4.3 K1 -.0013
Tris buffer .05M K2 -.0001
r -.4196

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.4	.399	412	480	485
2	.403	.398	375	442	445
3	.398	.403	326	390	388
4	.399	.4	276	323	321
5	.401	.399	215	238	243
6	.398	.4	174	199	195
7	.402	.4	126	137	139
8	.403	.397	89	83	95
9	.398	.397	42.5	34	40.5
10	.401	.402	23.5	19	22

Resin: XY-40032.00 Int./Fin.
Date: 7/11/88 Solution pH7.5/8.0 K1 .04268
Tris buffer .05M K2 .01205
r -.0451

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.403	.4	480	445	439
2	.401	.407	415	364	364
3	.398	.4	370	323	322
4	.404	.398	305	266	264
5	.4	.401	252	214	216
6	.404	.401	206	178.5	178
7	.401	.4	161	139	138
8	.399	.403	113	94.5	95.5
9	.399	.397	79	68.5	68.5
10	.397	.399	44	40.5	40

Resin: XY-40032.00 Int./Fin.
Date: 7/11/88 Solution pH7.6/8.0 K1 -.0427
Tris buffer .05M K2 -.0121
r -.0451

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.403	.4	480	445	439
2	.401	.407	415	364	364
3	.398	.4	370	323	322
4	.404	.398	305	266	264
5	.4	.401	252	214	216
6	.404	.401	206	178.5	178
7	.401	.4	161	139	138
8	.399	.403	113	94.5	95.5
9	.399	.397	79	68.5	68.5
10	.397	.399	44	40.5	40

Resin: XU-40170.00 Int./Fin.
Date: 7/12/88 Solution pH7.5/7.6 K1 .00096
Tris buffer .05M K2 .00001
r .94647

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.4032	.398	445	425	416
2	.399	.4	380	361	353
3	.398	.402	325	290	292
4	.407	.403	281	240	242
5	.399	.4	220	187	185
6	.405	.403	193	154.5	154
7	.399	.401	157	123	119
8	.4	.398	123	94	93
9	.394	.396	84.5	57	55.5
10	.399	.4	44	24	24

Resin: XY-40013.00 Int./Fin.
Date: 7/13/89 Solution pH7.5/7.5 K1 .00192
Tris buffer .05M K2 3.7e-6
r .93489

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	#2	Initial Klett	Final Klett (24 hrs.) #1	#2
1	.399	.396	439	402	377
2	.395	.394	368	303	315
3	.393	.397	291	218	228
4	.406	1	207	134	-1
5	.403	1	132	66.5	-1
6	.403	1	81	17	-1
7	.39	1	43	10	-1
8	.409	1	24	7	-1
9	.405	1	0	0	-1
10	1	1	1	-1	-1

Resin: XM-1062-18-24 Int./Fin.
Date: 7/13/89 Solution pH7.5/7.5 K1 .00455
Tris buffer .05M K2 .00001
r .98227

Volume of Sample (ml) 9

Sample #	Resin Mass (g) #1	Resin Mass (g) #2	Initial Klett	Final Klett (24 hrs.) #1	Final Klett (24 hrs.) #2
1	.397	.404	440	330	326
2	.4	.398	354	204	153
3	.41	.402	290	73	161
4	.394	1	214	44	-1
5	.397	1	151	12	-1
6	.397	1	99	13	-1
7	.403	1	62	17	-1
8	.395	1	43	2.5	-1
9	.397	1	0	0	-1
10	1	1	1	-1	-1

APPENDIX 8: Kinetic Data

Resin: IRA-938 Int. Con. .0000779
K1= .008819 ka= 0
K2= .0000039 r = 0

Sample #	1	2	3
Mass (g)	.499	.506	.499
Sample Volume (ml)	10		
Original Klett	82		

Date:

Time (hr.)	Sample # Klett 1	2	3
0	82	82	82
.25	47	45	45
.5	35	38	33
.75	28	29	26
1	28	26	25
1.5	15	17	15.5
2	13	13	12
3	7.5	6.5	6.5
4	7	6.5	7
5	4	2	2
24	4	3	3

Resin: IRA-938 Int. Con. .0002333
K1= .008819 ka= 0
K2= .0000039 r = 0

Sample #	1	2	3
Mass (g)	.503	.504	.507
Sample Volume (ml)	10		
Original Klett	218		

Date:

Time (hr.)	Sample # Klett 1	2	3
0	218	218	218
.25	181	181	171
.5	172	167	170
.75	162	159	157
1	155	149	149
1.5	141	134	131
2	126	123	120
3	112	101	99.5
4	93	89.5	86
5	91	82	79.5
24	24	18	17

Resin: XU -434-200.00 Int. Con. .0000779
K1= .0088671 ka= 0
K2= .0000081 r = 0

Sample #	1	2	3
Mass (g)	.508	.505	.497
Sample Volume (ml)	10		
Original Klett	82		

Date:

Time (hr.)	Sample # Klett 1	2	3
0	82	82	82
.25	37	32	40
.5	17	18	24
.75	15	10.5	14
1	12	8	10
1.5	8.5	7.5	6
2	6	3	3.5
3	3	5	5
4	0	0	0
5	0	0	0
24	0	0	0

Resin: XU-434-200.00 Int. Con. .0000988
K1= .0088671 ka= 0
K2= .0000081 r = 0

Sample #	1	2	3
Mass (g)	.5	.498	.499
Sample Volume (ml)	10		
Original Klett	104		

Date:

Time (hr.)	Sample # Klett 1	2	3
0	104	104	104
.25	23	34	31.5
.5	5	13	12
.75	3	7	5
1	1	3	2
1.5	0	.5	0
2	0	1	0
3	1	3	2
4	0	0	0
5	0	0	0
24	0	0	0

Resin: XU-434-200.00 Int. Con. .0001649
K1= .0088671 ka= 0
K2= .0000081 r = 0

Sample #	1	2	3
Mass (g)	.504	.00001	.000001
Sample Volume (ml)		10	
Original Klett		163	

Date:

Time (hr.)	Sample # Klett 1	2	3
0	163	163	163
.25	68		
.5	34.5		
.75	16.5		
1	9		
1.5	3		
2	2		
3	.5		
4	0		
5	0		
24	2		

Resin: XU-434-200.00 Int. Con. .0002308
K1= .0088671 ka= 0
K2= .0000081 r = 0

Sample #	1	2	3
Mass (g)	.502	.504	.506
Sample Volume (ml)		10	
Original Klett		216	

Date:

Time (hr.)	Sample # Klett 1	2	3
0	216	216	216
.25	94	91	91
.5	59	55	54
.75	32	31	32
1	16.5	20	17
1.5	1.5	6	3.5
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
24	4	5	5

Resin: XU-434-200.00 Int. Con. .0002544
K1= .0088671 ka= 0
K2= .0000081 r = 0

Sample #	1	2	3
Mass (g)	.505	.00001	.000001
Sample Volume (ml)		10	
Original Klett		235	

Date:

Time (hr.)	Sample # Klett		
(hr.)	1	2	3
0	235	235	235
.25	142		
.5	103		
.75	71		
1	49		
1.5	28		
2	3.5		
3	0		
4	0		
5	2		
24	4		

Resin: XU-434-200.00 Int. Con. .0002879
K1= .0088671 ka= 0
K2= .0000081 r = 0

Sample #	1	2	3
Mass (g)	.505	.00001	.000001
Sample Volume (ml)		10	
Original Klett		262	

Date:

Time (hr.)	Sample # Klett		
(hr.)	1	2	3
0	262	262	262
.25	151		
.5	103.5		
.75	72		
1	50		
1.5	26		
2	13		
3	3		
4	1.5		
5	1.5		
24	2.5		

Resin: XU-434-200.00 Int. Con. .0004076
K1= .0088671 ka= 0
K2= .0000081 r = 0

Sample #	1	2	3
Mass (g)	.502	.00001	.000001
Sample Volume (ml)		10	
Original Klett		344	

Date:

Time (hr.)	Sample # Klett 1	2	3
0	344	344	344
.25	240		
.5	179		
.75	136		
1	98		
1.5	59		
2	33.5		
3	14		
4	8		
5	0		
24	34		

Resin: XU-434-200.01 Int. Con. .0000789
K1= .0060509 ka= 0
K2= .0000082 r = 0

Sample #	1	2	3
Mass (g)	.495	.503	.497
Sample Volume (ml)	10		
Original Klett	83		

Date:

Time (hr.)	Sample # Klett 1	2	3
0	83	83	83
.25	45	42	41
.5	23	23	25
.75	11.5	12	10
1	3	3.5	4
1.5	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
24	4.5	3.5	6

Resin: XU-434-200.01 Int. Con. .0000922
K1= .0060509 ka= 0
K2= .0000082 r = 0

Sample #	1	2	3
Mass (g)	.5	.497	.498
Sample Volume (ml)	10		
Original Klett	97		

Date:

Time (hr.)	Sample # Klett 1	2	3
0	97	97	97
.25	47.5	49	48
.5	30	30.5	30
.75	16	19.5	20
1	13.5	13	13
1.5	5	5.5	6
2	3	5	4
3	2	2	2
4	0	1.5	1
5	0	0	0
24	0	0	0

Resin: XU-434-200.01 Int. Con. .0001649
K1= .0060509 ka= 0
K2= .0000082 r = 0

Sample #	1	2	3
Mass (g)	.506	.00001	.000001
Sample Volume (ml)		10	
Original Klett		163	

Date:

Time (hr.)	Sample # Klett 1	2	3
0	163	163	163
.25	105.5		
.5	76		
.75	55		
1	38.5		
1.5	22.5		
2	9		
3	5		
4	4		
5	.5		
24	2		

Resin: XU-434-200.01 Int. Con. .0002308
K1= .0060509 ka= 0
K2= .0000082 r = 0

Sample #	1	2	3
Mass (g)	.504	.509	.504
Sample Volume (ml)		10	
Original Klett		216	

Date:

Time (hr.)	Sample # Klett 1	2	3
0	216	216	216
.25	109.5	108	109
.5	73	70	71
.75	58	52	53
1	45.5	41	43
1.5	27	25	26
2	20	17	18
3	15.5	12	14
4	17	13	14
5	24	16	17
24	59	23	23.5

Resin: XU-434-200.01 Int. Con. .0002544
K1= .0060509 ka= 0
K2= .0000082 r = 0

Sample #	1	2	3
Mass (g)	.501	.00001	.000001
Sample Volume (ml)		10	
Original Klett		235	

Date:

Time (hr.)	Sample # Klett 1	2	3
0	235	235	235
.25	175		
.5	144		
.75	120		
1	100		
1.5	72.5		
2	55.5		
3	31		
4	23		
5	12.5		
24	2		

Resin: XU-434-200.01 Int. Con. .0002879
K1= .0060509 ka= 0
K2= .0000082 r = 0

Sample #	1	2	3
Mass (g)	.495	.00001	.000001
Sample Volume (ml)		10	
Original Klett		262	

Date:

Time (hr.)	Sample # Klett 1	2	3
0	262	262	262
.25	191		
.5	160		
.75	1347		
1	118		
1.5	90		
2	69.5		
3	39		
4	26		
5	19.5		
24	3		

Resin: XU-434-200.01 Int. Con. .0004076
K1= .0060509 ka= 0
K2= .0000082 r = 0

Sample #	1	2	3
Mass (g)	.506	.00001	.000001
Sample Volume (ml)		10	
Original Klett		344	

Date:

Time (hr.)	Sample # Klett 1	2	3
0	344	344	344
.25	278		
.5	239		
.75	206		
1	183		
1.5	145		
2	103		
3	72		
4	60		
5	55		
24	56		

Resin: XU-434-200.02 Int. Con. .0000741
K1= .00266 ka= 0
K2= .0000025 r = 0

Sample #	1	2	3
Mass (g)	.496	.503	.5
Sample Volume (ml)		10	
Original Klett		78	

Date:

Time (hr.)	Sample # Klett		
(hr.)	1	2	3
0	78	78	78
.25	66	65	65.5
.5	61	59	61.5
.75	55	55	54.5
1	49	48	47
1.5	44	44	42.5
2	35	33.5	32
3	26	22	22
4	21	19	19
5	14	13	13
24	9.5	11	12

Resin: XU-434-200.02 Int. Con. .0000789
K1= .00266 ka= 0
K2= .0000025 r = 0

Sample #	1	2	3
Mass (g)	.505	.502	.5
Sample Volume (ml)		10	
Original Klett		83	

Date:

Time (hr.)	Sample # Klett		
(hr.)	1	2	3
0	83	83	83
.25	64	62	60
.5	52	52	50
.75	43	43	41
1	35.5	35	35
1.5	20	23	24.5
2	14	16	17
3	3.5	4	5
4	0	0	0
5	0	0	0
24	8	6.5	6

Resin: XU-434-200.02 Int. Con. .0000988
K1= .00266 ka= 0
K2= .0000025 r = 0

Sample #	1	2	3
Mass (g)	.505	.496	.504
Sample Volume (ml)		10	
Original Klett		104	

Date:

Time (hr.) (hr.)	Sample # Klett 1	2	3
0	104	104	104
.25	81.5	82	82
.5	69.5	69	67
.75	57	59	59
1	46	51	51
1.5	31.5	33	34
2	22	25	25
3	16	20.5	20
4	15	17.5	17
5	16.5	17.5	16.5
24			

Resin: XU-434-200.02 Int. Con. .0001649
K1= .00266 ka= 0
K2= .0000025 r = 0

Sample #	1	2	3
Mass (g)	.504	.00001	.00001
Sample Volume (ml)		10	
Original Klett		163	

Date:

Time (hr.) (hr.)	Sample # Klett 1	2	3
0	163	163	163
.25	.143		
.5	128		
.75	114		
1	103		
1.5	83		
2	70		
3	57		
4	45.5		
5	36.5		
24	17		

Resin: XU-434-200.02 Int. Con. .0002308
K1= .00266 ka= 0
K2= .0000025 r = 0

Sample #	1	2	3
Mass (g)	.503	.504	.497
Sample Volume (ml)		10	
Original Klett		216	

Date:

Time (hr.)	Sample # Klett 1	2	3
0	216	216	216
.25	193	184	187
.5	169	164	167
.75	154	155	155
1	149	146	143
1.5	142	136	136
2	139	132	132.5
3	138	130	127
4	139	131	126
5	136	130	125.5
24	142.5	129	116

Resin: XU-434-200.02 Int. Con. .0002544
K1= .00266 ka= 0
K2= .0000025 r = 0

Sample #	1	2	3
Mass (g)	.505	.00001	.00001
Sample Volume (ml)		10	
Original Klett		235	

Date:

Time (hr.)	Sample # Klett 1	2	3
0	235	235	235
.25	216		
.5	195		
.75	180		
1	168		
1.5	150		
2	135		
3	115		
4	101.5		
5	93		
24	63		

Resin: XU-434-200.02 Int. Con. .0002879
K1= .00266 ka= 0
K2= .0000025 r = 0

Sample #	1	2	3
Mass (g)	.504	.00001	.00001
Sample Volume (ml)		10	
Original Klett		262	

Date:

Time (hr.)	Sample # Klett 1	2	3
0	262	262	262
.25	226		
.5	212		
.75	201		
1	189		
1.5	172		
2	158.5		
3	140		
4	124		
5	116		
24	59		

Resin: XU-434-200.02 Int. Con. .0004076
K1= .00266 ka= 0
K2= .0000025 r = 0

Sample #	1	2	3
Mass (g)	.502	.00001	.00001
Sample Volume (ml)		10	
Original Klett		344	

Date:

Time (hr.)	Sample # Klett 1	2	3
0	344	344	344
.25	318		
.5	300		
.75	286		
1	272		
1.5	245		
2	208		
3	174		
4	143		
5	128		
24	104		

Resin: XU-40091.00 Int. Con. .0000741
K1= ------ ka= 0
K2= ----- r = 0

Sample #	1	2	3
Mass (g)	.499	.5	.505
Sample Volume (ml)		10	
Original Klett		78	

Date:

Time (hr.)	Sample # Klett 1	2	3
0	78	78	78
.25	71	70	70.5
.5	68	68.5	69.5
.75	67.5	67.5	67
1	67	66.5	64
1.5	63	62.5	62.5
2	61	59.5	61
3	60	55	54
4	57	51	50.5
5	48	46	46
24	24	19	20

Resin: XU-40091.00 Int. Con. .0000874
K1= ------ ka= 0
K2= ----- r = 0

Sample #	1	2	3
Mass (g)	.504	.501	.502
Sample Volume (ml)		10	
Original Klett		92	

Date:

Time (hr.)	Sample # Klett 1	2	3
0	92	92	92
.25	77	82	
.5	76	76	
.75	76	76	
1	73.5	71.5	
1.5	73	71	
2	73	67	
3	72	65	
4	68.5	58	
5	58	50	
24	63	50.5	

Resin: XU-40091.00 Int. Con. .0002333
K1= ------ ka= 0
K2= ----- r = 0

Sample #	1	2	3
Mass (g)	.504	.506	.506
Sample Volume (ml)		10	
Original Klett		218	

Date:

Time (hr.)	Sample # Klett 1	2	3
0	218	218	218
.25	239	240	240
.5	241	241	242
.75	242	239	240
1	241	239	239
1.5	236	236	236
2	236	236	235
3	234	234	229
4	230	227	227
5	227	227	221
24	222	215	214

Resin: XU-40091.00 Int. Con. .0004007
K1= ------ ka= 0
K2= ----- r = 0

Sample #	1	2	3
Mass (g)	.504	.501	.499
Sample Volume (ml)		10	
Original Klett		340	

Date:

Time (hr.)	Sample # Klett 1	2	3
0	340	340	340
.25	360	364	355
.5	324	322	321
.75	323	317	317
1	317	314	310
1.5	315	317	310
2	311	306	307
3	309	296	294
4	309	294	296
5	305	293	297
24	300	284	285

Resin: XU-40091.00 Int. Con. .0004007
K1= ------ ka= 0
K2= ----- r = 0

Sample #	1	2	3
Mass (g)	.501	.507	.501
Sample Volume (ml)	10		
Original Klett	340		

Date:

Time (hr.)	Sample # Klett 1	2	3
0	340	340	340
.25	355	345	341
.5	326	327	327
.75	316	317	313
1	317	315	309
1.5	310	307	300
2	307	303	294
3	303	292	289
4	301	296	285
5	300	286	280
24	290	276	268

Resin: XU-40091.00 Int. Con. .0005000
K1= ------ ka= 0
K2= ----- r = 0

Sample #	1	2	3
Mass (g)	.504	.499	.498
Sample Volume (ml)	10		
Original Klett	398		

Date:

Time (hr.)	Sample # Klett 1	2	3
0	398	398	398
.25	353	356	360
.5	354	344	350
.75	353	348	348
1	350	342	342
1.5	347	339	336
2	347	328	330
3	345	326	324
4	342	323	323
5	328	315	315
24	313	283	286

Resin: XU-40091.00 Int. Con. .0005017
K1= ------ ka= 0
K2= ----- r = 0

Sample #	1	2	3
Mass (g)	.505	.5	.504
Sample Volume (ml)		10	
Original Klett		399	

Date:

Time (hr.)	Sample # Klett 1	2	3
0	399	399	399
.25	399	400	400
.5	396	395	395
.75	394	394	390
1	392	388	387
1.5	388	380	380
2	389	375	375
3	386	373	372
4	376	367	366
5	382	364	367
24	363	335	327

Resin: XF-43356.00 Int. Con. .0004640
K1= ------ ka= 0
K2= ----- r = 0

Sample #	1	2	3
Mass (g)	.5	.503	.512
Sample Volume (ml)		10	
Original Klett		377	

Date:

Time (hr.)	Sample # Klett 1	2	3
0	377	377	377
.25	372	372	372
.5	370	371	371
.75	371	371	370
1	369	369	369
1.5	367	367	364
2	3665	366	366
3	365	362	357
4	366	360	356
5	366	356	351
24	457	445	442

Resin: XU-40123.00 Int. Con. .0003028
K1= ------ ka= 0
K2= ----- r = 0

Sample #	1	2	3
Mass (g)	.502	.502	.502
Sample Volume (ml)		10	
Original Klett		274	

Date:

Time (hr.)	Sample # Klett 1	2	3
0	274	274	274
.25	272	272	272
.5	269	265	263
.75	265	260	258
1	266	258	255
1.5	264	255	250
2	263	250	247
3	259	245	240
4	255	242	235
5	252	234	230
24	216	200	196

Resin: XU-40170.00 Int. Con. .0004658
K1= .000961 ka= 0
K2= .0000089 r = 0

Sample #	1	2	3
Mass (g)	.504	.504	.501
Sample Volume (ml)		10	
Original Klett		273	

Date:

Time (hr.)	Sample # Klett		
(hr.)	1	2	3
0	378	378	378
.25	272	272	272
.5	269	265	263
.75	265	260	258
1	266	258	255
1.5	264	255	250
2	263	250	247
3	259	245	240
4	255	242	235
5	252	234	230
24	216	200	196

Resin: XU-40196.01 Int. Con. .0004350
K1= ------ ka= 0
K2= ----- r = 0

	Sample #	1	2	3
	Mass (g)	.508	.504	.502
	Sample Volume (ml)		10	
	Original Klett		360	
Date:				

Time (hr.)	Sample # Klett 1	2	3
0	360	360	360
.25	361	360	361
.5	361	354	360
.75	356	351	352
1	355	345	347
1.5	353	338	338
2	346	334	330
3	346	330	328
4	344	323	321
5	335	319	315
24	315	302	292

Resin: XUS-40187.00 Int. Con. .0004658
K1= .0046641 ka= 0
K2= .0004187 r = 0

Sample #	1	2	3
Mass (g)	.499	.501	.5
Sample Volume (ml)		10	
Original Klett		378	

Date:

Time (hr.)	Sample # Klett 1	2	3
(hr.)			
0	378	378	378
.25	372	372	372
.5	365	365	365
.75	367	364	363
1	362	360	360
1.5	362	360	356
2	359	360	355
3	360	355	350
4	356	348	343
5	346	338	332
24	319	319	315

Resin: XUS-43419.00 Int. Con. .0005
K1= ------ ka= 0
K2= ----- r = 0

Sample #	1	2	3
Mass (g)	.503	.503	.495
Sample Volume (ml)		10	
Original Klett		398	

Date:

Time (hr.)	Sample # Klett		
(hr.)	1	2	3
0	398	398	398
.25	417	402	415
.5	402	417	417
.75	422	422	422
1	420	420	420
1.5	434	430	430
2	445	434	442
3	450	444	444
4	454	445	445
5	457	445	431
24	480	465	460

Resin: XY-40032.00 Int. Con. .0004418
K1= ------ ka= 0
K2= ----- r = 0

Sample #	1	2	3
Mass (g)	.505	.501	.502
Sample Volume (ml)		10	
Original Klett		364	

Date:

Time (hr.)	Sample # Klett		
(hr.)	1	2	3
0	364	364	364
.25	333	321	312
.5	339	325	315
.75	340	323	318
1	340	324	312
1.5	338	321	305
2	336	314	303
3	327	307	294
4	317	303	297
5	309	298	294
24	306	307	297

Resin: XUR-0525-L87-239.02 Int. Con. .0000922

K1= ------ ka= 0

K2= ----- r = 0

Sample #	1	2	3
Mass (g)	.506	.506	.502
Sample Volume (ml)	10		
Original Klett	97		

Date:

Time (hr.)	Sample # Klett 1	2	3
0	97	97	97
.25	77	82	
.5	76	76	
.75	76	76	
1	73.5	71.5	
1.5	73	71	
2	73	67	
3	72	65	
4	68.5	58	
5	58	50	
24	63	50.5	

Resin: XUR-0525-L87-239.02 Int. Con. .0001736

K1= ------ ka= 0

K2= ----- r = 0

Sample #	1	2	3
Mass (g)	.501	.504	.508
Sample Volume (ml)	10		
Original Klett	170		

Date:

Time (hr.)	Sample # Klett 1	2	3
0	170	170	170
.25	162	159	157
.5			
.75	148	149	147
1	146	146.5	143
1.5	142	142	142
2	141	143	138
3	137	139	133
4	134	131.5	132
5	134	133	123
24	207	216	

Resin: XUR-0525-L87-239.03 Int. Con. .0005304
K1= ------ ka= 0
K2= ----- r = 0

	Sample #	1	2	3
	Mass (g)	.503	.509	.498
	Sample Volume (ml)		10	
	Original Klett		400	
Date:				

Time (hr.)	Sample # Klett 1	2	3
0	400	400	400
.25	393	401	393
.5	388	395	387
.75	380	385	380
1	375	374	375
1.5	357	355	355
2	351	343	348
3	332	316	319
4	370	342	347
5	351	317	319
24	313	311	336

Resin: XUR-0525-L87-239.04 Int. Con. .0001736

K1= ------ ka= 0

K2= ----- r = 0

	Sample #	1	2	3
	Mass (g)	.5	.502	.504
	Sample Volume (ml)		10	
	Original Klett		170	
Date:				

Time (hr.)	Sample # Klett 1	2	3
0	170	170	170
.25	156	157	153
.5			
.75	145	157	143
1	141	153	141
1.5	133	147	135.5
2	128	141	128
3	120	126	120
4	117	122	118
5	112	118	116
24	119	136	136

XUR-0525-L87-239. Klett Int 291

	Mass (g)		Klett 1 hr.		Klett 3 hr.	
Sample	A	B	A	B	A	B
.00	.42	.436	289	288	296	292
.01	.509	.502	292	292	292	286
.02	.507	.503	276	271	259	261
.03	.508	.511	151	261	284	284
.04	.507	.502	277	277	259	262
.05	.506	.507	291	294	292	292
.06	.497	.505	295	290	292	292
.07	.512	.502	287	286	278	280
.08	.515	.502	290	288	284	286
.09	.5	.497	296	300	293	300
.10	.503	.505	290	286	285	285

APPENDIX 9: Growth & Production Data

Production #1

Resin:	Mass (g)	Weight %
XU-434-200.00	5.05	4.39
IRA-938	5.066	4.40

Tot. Vol. (ml) 110

	Klett	Volume (ml)
Inoculate	382	10

Free Cell Conc. (Klett)

Time (hr.)	Control	XU-434-200.00	IRA-938
24	411	301	214
48	540	345	290
96	540	295	198

Alpha-amylase (activity units/ml)

Time (hr.)	Control	XU-434-200.00	IRA-938
24	41.76	44.24	37.97
48	83.705	52.17	79.29
96	86.14	84.1	44.65

Production #2

Resin:	Mass (g)	Weight %
XU-434-200.00	5	4.35
IRA-938	5.017	4.36

Tot. Vol. (ml) 110

	Klett	Volume (ml)
Inoculate	155	10

Free Cell Conc. (Klett)

Time (hr.)	Control	XU-434-200.00	IRA-938
24	450	410	221
48	504	385	335
72	480	345	331
96	485	322	237

Alpha-amylase (activity units/ml)

Time (hr.)	Control	XU-434-200.00	IRA-938
24	47.6	52.88	47.4
48	178.1	149.06	179.11
72	200	249.14	265.5
96	323	313	370

Production #3

Resin:	Mass (g)	Weight %
XU-434-200.00	10.092	9.17
IRA-938	10.088	9.16

Tot. Vol. (ml) 100

	Klett	Volume (ml)
Inoculate	392	5

Free Cell Conc. (Klett)

Time (hr.)	Control	XU-434-200.00	IRA-938
24	407	244	15.5
48	432	330	25.5
72	450	255	26

Alpha-amylase (activity units/ml)

Time (hr.)	Control	XU-434-200.00	IRA-938
24	70.63	38.92	94.11
48	319.99	41.8	155.38
72	304.4	41.5	324.78

APPENDIX 10: Recycle Data

Recycle Experiment #1
Adsorption 1

Initial Klett 391
Volume of Solutuion (ml) 9

	Mass Dry Resin (g)		Final Klett	
Sample #	IRA-938	XU-434-200.00	IRA-938	XU-434-200.00
1	.409	.408	107	223
2	.406	.403	103	203
3	.407	.403	125	204
4	.409	.406	120.5	208

Adsorption 2

Initial Klett 395
Volume of Solutuion (ml) 9

	Mass Dry Resin (g)		Final Klett	
Sample #	IRA-938	XU-434-200.00	IRA-938	XU-434-200.00
1	.401	.401	234	347
2	.395	.391	231	336
3	.394	.402	241	347
4	.378	.331	233	341

Adsorption 3

Initial Klett 405
Volume of Solutuion (ml) 9

	Mass Dry Resin (g)		Final Klett	
Sample #	IRA-938	XU-434-200.00	IRA-938	XU-434-200.00
1	.397	.392	238	310
2	.404	.394	216	288
3	.398	.393	226	289
4	.35	.219	238	315

Recycle Experiment #2
Adsorption 1

Initial Klett 405
Volume of Solutuion (ml) 9

Sample #	Mass Dry Resin (g) IRA-938	XU-434-200.00	Final Klett IRA-938	XU-434-200.00
1	.417	.403	191	173
2	.41	.407	187	154
3	.404	.397	153	171
4	.403	.404	191	169

Adsorption 2

Initial Klett 380
Volume of Solutuion (ml) 9

Sample #	Mass Dry Resin (g) IRA-938	XU-434-200.00	Final Klett IRA-938	XU-434-200.00
1	.392	.388	165	241
2	.392	.393	170	241
3	.386	.406	157	240
4	.465	.414	141	239

Recycle Experiment #3
Adsorption 1

Initial Klett 320
Volume of Solutuion (ml) 9

Sample #	Mass Dry Resin (g) IRA-938	XU-434-200.00	Final Klett IRA-938	XU-434-200.00
1	.403	.406	89	133
2	.402	.409	92	127
3	.399	.399	90	114
4	.408	.402	81	117

Adsorption 2

Initial Klett 320
Volume of Solutuion (ml) 9

Sample #	Mass Dry Resin (g) IRA-938	XU-434-200.00	Final Klett IRA-938	XU-434-200.00
1	.406	.395	116.5	170
2	.397	.399	111.5	173
3	.398	.402	114	166
4	.386	.409	111	172

Adsorption 3

Initial Klett 320
Volume of Solutuion (ml) 9

Sample #	Mass Dry Resin (g) IRA-938	XU-434-200.00	Final Klett IRA-938	XU-434-200.00
1	.394	.392	42	78.5
2	.398	.396	44	73
3	.402	.394	42	63
4	.342	.349	47	82

LIST OF REFERENCES

LIST OF REFERENCES

Applebaum, Samuel, B., Demineralization by Ion Exchange in Water Treatment and Chemical Processing of Other Liquids, Academic Press, (1968)

Bailey, J. E., and Ollis, D.F., Biochemical Engineering Fundamentals, McGraw-Hill Book Co., 2nd Ed., (1986)

Bar, Raphael, Gainer, John L., and Kirwan, Donald J., "Ethanol Fermentation by Ionically Adsorbed Zymomomas mobilis: Environmental Effects on Cell Immobilization", Biotech. Bioeng., 19, 1045-1049 (1987)

Bar, Raphael, Gainer, John L., and Kirwan, Donald J., "Immobilization of Acetobacter aceti on Cellulose Ion exchangers: Adsorption Isotherms", Biotech. Bioeng., 18, 1166-1171 (1986)

Barr, M., J. Amer. Pharm. Assoc., 46, 490-492 (1957)

Bird, R. Byron, Stewart, Warren E., and Lightfoot, Edwin N., Transport Phenomena, John Wiley & Sons, (1960)

Campbell, L. L. and Manning, G. B., "Thermostable α-amylase of Bacillus stearothermophilus I. Crystallization and some general properties.", J. Biol. Chem., 236, 2952-2957 (1961)

Chibata, I., Tosa, T., and Sato, T., Applied Microbiol., 27, 878 (1974)

Daniels, Stacey L. and Kempe, Lloyd L., "The Separation of Bacteria by Adsorption onto Ion Exchange Resins", Chemical Engineering Progress Symposium Series: Bioengineering and Food Processing, No. 69, 62, 142-148 (1966)

Daniels, S. L. "The Adsorption of micro-organisms onto solid surfaces".Developments in Industrial Microbiology, 13, 211- 254 (1972)

Dorfner, Konrad, Ion Exchangers Properties and Applications Third Ed., Ann Arbor Science Publishers Inc. (1972)

Durand, G. and Navarro, J.M., "Immobilized Microbial Cells", Process Biochemistry, September, 14-23 (1978)

Gillisen, G., Scholz, H. and Denhert, C. "The treatment of microbial suspensions with ion exchangers", Archiv für Hygiene and Bakteriologie, 154, 145-52 (1961)

Greenberg, Allen, Dow Chemical Company, Midland MI

Groom, Carl A., Daugulis, Andrew J. and White, Bradley N., "Continuous alpha-amylase production using Bacillus amyloquefaciens adsorbed on an ion exchange resin", Appl Micrbiol Biotechnol, 28 8-13 (1988)

Grulke, Eric A., Glassner, David A. and Oriel, Patrick J., "Characterization of an Immobilized Biocatalyst System for Production of Thermostable Amylase", Biotechnology Progress, 5(1) 31-39 (1989)

Hattori, Reiko, "Growth of Escherichia coli on the Surface of an Anion-Exchange Resin in Continuous Flow System", J. Gen. Appl. Microbiol. 18, 319-330 (1972)

Hattori, Reiko, Hattori, Tsutomo, and Furusaka, Choseki, "Growth of Bacteria on the Surface of Anion-Exchange Resin I. Experiment with Batch Culture", J. Gen. Appl. Microbiol. 18, 271-283 (1972)

Hattori, Reiko, Hattori, Tsutomo, and Furusaka, Choseki, "Growth of Bacteria on the Surface of Anion-Exchange Resin II. Electron Microscopic Observation of Adsorbed Cells Growing on Resin Surface by Carbon Replica Method", J. Gen. Appl. Microbiol. 18, 285-293 (1972)

Hattori, Tstomu and Furusaka, Choseki, "Chemical Activities of E. coli Adsorbed on a Resin", J. Biochem., 48(6) 831-837 (1960)

Jarvis, B., Lach, V.H. and Wood, J.M., "Evaluation of the Spiral Plate Maker for the Evaluation of Micro-organisms in Foods", Journal of Applied Bacteriology, 43, 149-157 (1977)

Klein, J. and Vorlop, K. D., "Immobilization Techniques-Cells", Comprehensive Biotechnology The Principles, Applications and Regulation of Biotechnology in Industry, Agriculture and Medicine, 2, Chapter 12, 203-224 (1985)

Klein, J. and Wagner,F.,"Methods for the Immobilization of Microbial Cells", Applied Biochemistry and Bioengineering, 4, 11-51 (1983)

Kolot, Frieda B., "Microbial Carriers-Strategy for Selection", Process Biochemistry, August/September, 2-9 (Part I), October/November, 30-33, 46 (Part II), (1981)

Krug, Thomas A. and Daugulis, Andrew J., "Ethanol Production using Zymomonas mobilis Immobilized on an Ion Exchange Resin", Biotech. Lett. 5, 159-164 (1983)

Kuwajima, Y., Matsui, T. and Kishigami, M., "The Growth-Supporting of some Anion Exchange resins for Phase I Haemophilus pertussis", Japanese Journal of Microbiology, 1(4) 375-381 (1957)

Messing, R. A., and Oppermann, R.A., "Pore Dimensions for Accumulating Biomass. I. Microbes that Reproduce by Fission or by Budding", Biotech. Bioeng., 11, 49-58 (1979)

Niehoff, R. and Echols, W.H., "Comparative Electrophoretic Behavior of Intact Cells and Isolated Cell Walls", Biochimica et Biophysica Acta, 318, 23-32 (1973)

Ollis, David F., "Scanning Microphotometry and Kinetics of Immobilized Cell Systems", Seminar, Michigan State University, March 2, 1989

Oriel, P. J. and Schwacha, A., "Growth on starch and extracellular production of thermostable amylase by Escherichia coli", Enzyme Microbial Tech.., 10, 42-46 (1988)

Oriel, Patrick J., Professor Dept. of Microbiology and Public Health, Michigan State University, Personal Reference

Puck, T.T. & Sagik, B., "Virus and Cell Interaction with Ion Exchangers", Journal of Experimental Medicine, 97, 807-820 (1953)

Rotman, B., "Ion Exchange Resins in Microbiology", Bacteriological Review, 24, 251-260 (1960)

Sato, T., Mori, T., Tosa, T., Chibata, I., Furui, M., Yamashita, K., Sumi, A., Biotech. Bioeng., 17, 1797 (1975)

Tampion J. and Tampion, M. D., "Adsorption", Immobilized cells: principles and applications, Chapt. 5, 135-165 (1987)

Wheaton, R.M. and Seamster, A.H., "A Basic Reference on Ion Exchange; Dow", reprinted from Encyclopedia of Chemical Technology 2nd Ed., 11, 871-899 (1966)

Wood, John M., "The Interaction of Micro-organisms with Ion Exchange Resins". Microbial Adhesion to Surfaces, Chapter 8, pp 163-185, (1980)

Zvaginstev, D.G. and Gusev, V.S., "Concentration and Separation of Bacteria on Dowex Anionite", Microbiology (USSR), 40, 123-126 (1971)

GENERAL REFERENCES

Calvin, C. and Kressman, T.R.E., Ion Exchangers in Organic and Biochemistry, Interscience Publishers, (1957)

Ellwood, D.C. and Melling, J., Adhesion of Microorganisms to Surfaces, Academic Press, (1979)

Mattiasson, Bo, Ed., CRC Immobilized Cells and Organelles, Vol. 1 & 2, CRC press, (1983)

Phillips, C.R. and Poon, Y.C., Immobilization of Cells, Springer-Verlag, (1988)

Savage, D.C. and Fletcher, M., Bacterial Adhesion Mechanisms and Physiological Significance, Plenum Press, (1985)

Suusman, M., "The Virulence Of Escherichia coli: Reviews and Methods", Special Publications of the Society for General Microbiology, 13, Academic Press, (1985)

Venatsubramanian, K. Ed., "Immobilized Cells", ACS Symposium Series 106, (1979)