# THE POTENTIAL OF MICROENCAPSULATED UREASE-ZEOLITE ORAL SORBENT FOR THE REMOVAL OF UREA IN URAEMIA

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the degree of Doctor of Philosophy

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November, 1989

## ABSTRACT

Oral sorbent therapy, as an adjunct or a replacement for dialysis therapy, is one area of research with great potential. If successful it can help kidney failure patients avoid a "life on the machine" existence. For the past twenty years the major problem was in finding an effective oral sorbent for urea. The use of oral microcapsules containing a urease-silica adduct and ion exchanger zirconium phosphate, though successful in reducing urea levels, resulted in a number of problems including a negative calcium balance. In this thesis it is demonstrated that the use of microcapsules containing a urease-zeolite preparation may be a potential route to urea removal. The use of zeolite ion exchangers, and zeolite W in particular, can alleviate the above mentioned problems of zirconium phosphate. In addition, the use of enzyme envelopes on zeolite support, which replaces silica, can reduce the amount of ingested material by at least 25%. The present "in vitro" study shows that the microcapsules remove up to 80% of urea in less than one hour. Preliminary "in vivo" experiments on Sprague-Dawley uraemic rats treated with ingested microcapsules indicate reductions in urea level and a lengthening of survival times compared to controls.

## RESUME

La thérapie par voie buccale, soit en tant qu'adjuvant ou comme remplaçant, est un domaine de la recherche possédant beaucoup de potentiel. Les bons résultats de cette thérapie permettraient aux patients urémiques de vivre autrement que sur un dialyseur. Durant les vingt dernières années, le problème principal a été la recherche d'un methode efficace d'extruction de l'urée par voie buccale. L'utilization des microcapsules contenant une préparation d'uréase et oxide de silicone et d'un échangeur ionique de phosphate de zirconium, avait réduit les niveaux d'urée, mais produisait dans les patients plusieurs effets secondaires, incluant une balance négative de calcium.

Cette thèse démontre que l'administration de microcapsules contenant une préparation d'uréase-zéolithe est une méthode viable d'extraction de l'urée. L'utilization d'échangeurs ioniques de zéolithe, zéolithe W en particulier, peut diminuer les effets secondaires du phosphate de zirconium. En plus, avec l'utilization d'enveloppes enzymatiques sur support zéolithe, qui remplacent les matières d'oxide de silicone, il est possible de réduire la quantité de substance ingérée d'au moins 25%.

La présent étude "in vitro" montre que ces microcapsules éliminent jusqu'a 80% d'urée, en moins d'une heure.

Des expériences préliminaire "in vivo" avec des rats

urémiques traités par voie buccale avec des microcapsules d'uréase-zeolithe indiquent une réduction des niveaux d'urée et une survie prolongée comparé aux contrôles.

## ACKNOWLEDGEMENTS

I am indebted to Dr. T.M.S. Chang, my research supervisor, for his guidance, expertise and encouragement throughout this project.

I am very thankful to Mr. R. Varma and Mr. C. Lister for for their help and friendship.

I am grateful to my friends Harry Wong, Pina Sorrini and to all members of ACORC for their help and encouragement.

Finally, and most of all, I wish to thank my parents and especially my sweetheart Marie-France for their patience and support during this and many other projects.

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# 1. INTRODUCTION

## 1.1 Background

Our body is an isolated system which follows the law of mass action:

In -Out = Accumulation.

When this system is in balance the accumulation term is zero. However, when the balance is offset, as in kidney failure, toxins start accumulating. By strict dietary control, patients live normally until residual kidney function reduces to 5-10% of normal. At this point chronic replacement of renal function is necessary. Haemodialysis, haemofiltration, peritoneal dialysis or other artificial means provide control of water and electrolytes, acid-base balance and excretion of nitrogenous wastes. Water is by far the greater excretory product (0.5-1.5 l/day). This is followed by urea (10-15 g/day) and creatinine (2 g/day). Phosphate, uric acid and other substances are removed in smaller amounts. The importance of "Middle Molecules", molecules in the molecular weight range 300-2000 dalton, is still controversial (Furst, 1976, Chapman, 1980).

At present there's a panoply of theories on what constitutes the uraemic toxin. As Schreiner suggested, it is

unlikely that the "culprit" of uraemia will be found within a single molecular structure (Schreiner, 1979; Ringoir, 1988). When kidney function decreases, numerous metabolites start accumulating in the body. Phosphate, hydrogen ion, uric acid and creatinine are some of these compounds.

Urea is the most abundant of all the accumulating metabolites. We accumulate large quantities of urea, at times 20 or more grams per day. Our body produces urea to reduce the toxicity of metabolites of nitrogen. Urea is the ideal chemical for the expulsion of nitrogen. One molecule of urea expels two molecules of ammonium.

The majority of today's patients suffering from kidney disease is treated by haemodialysis, hence the primary importance of this form of artificial treatment. Abel, Rountree and Turner were the first to realize the potential of using membranes to purify blood. At Johns Hopkins University in 1912 they performed the first application of haemodialysis to an animal (Abel, 1913). Collodion membranes were under intense investigation by physiologists intent on solving the structure of cell membranes. Unfortunately, there were few applications of membrane science. Haemodialysis was not applied to humans until during the second world war in 1943 by Dr. W.J. Kolff (Kolff, 1943, 1944, 1956, 1965). Membrane technology was still in its infancy at the time of Dr.Kolff. Dr.Kolff built his artificial kidney out of

cellophane tubing wound around bulky wooden drums. This primitive dialysis machine kept kidney failure patients alive for some time. A real revolution in kidney failure treatment had begun. Dr.Kolff remembers sending one of his artificial kidneys from Denmark to the Royal Victoria Hospital in Montreal to treat famous hockey players. He reminds us that between 1966 and 1976 over 60,000 patients were treated on haemodialysis (Kolff, 1978).

Haemodialysis did not really become practical until the development of the external arteriovenous cannula system by Dr. B. Scribner (Quinton et al., 1960, 1962; Cole, 1963). The development of the subcutaneous arteriovenous fistula by Brescia and Cimino made vascular access even more conventional (1960). Peritoneal dialysis arrived in 1967 with Henry Tenckoff's development of the inplantable permanent silastic catheter (Tenckhoff, 1968).

Table 1.1 shows the percentage of primary disease resulting in chronic renal failure leading the patient to dialysis (National Dialysis Registry Data file; Bryan, 1976).

**TABLE 1.1.** Percentage of primary disease resulting in renal failure.

| | Percent of Patients |
|---|---|
| Glomerulonephritis | 41.6 |
| Cardiovascular and Hypertension | 13.5 |
| Other urinary tract disease | 10.5 |
| Unknown | 8.4 |
| Congenital Abnormalities | 7.6 |
| Diabetes | 7.2 |
| Kidney Infections | 6.1 |
| Other known | 5.1 |

Glomerulonephritis is the predominant disease causing end-stage renal failure. One third of the number of nephrons can eliminate the normal load of waste products without serious accumulation of any of these in the body fluids. Further reduction in the number of nephrons leads to retention of those waste products that depend on a high glomerular filtration rate for excretion. Death usually ensues when the number of nephrons falls below 5 to 20 percent of normal (Guyton, 1986). Each year many thousands of deaths are prevented through maintenance of life by dialysis or renal transplantation. The majority of patients is treated by dialysis, whereas renal transplantation represents about 10% of the total (Wineman, 1978; Pedrini, 1988).

Figure 1.1 shows the growth of the dialysis population for the period 1970-1978. Up to 1978 the exponential rise indicated the number of patients on dialysis was steadily increasing (Wineman, 1978). Renal transplantation has gained ground in recent years due to the use of cyclosporine and the advent of better surgical techniques.

The dialyser has slowly evolved into a highly sophisticated machine. Alwall devised a dialyser which utilizes cellophane tubing wound around and enclosed by wire screening (Alwall, 1947). MacNeil then applied a parallel flow system in a unit compact enough to carry (MacNeil, 1949). Skeggs

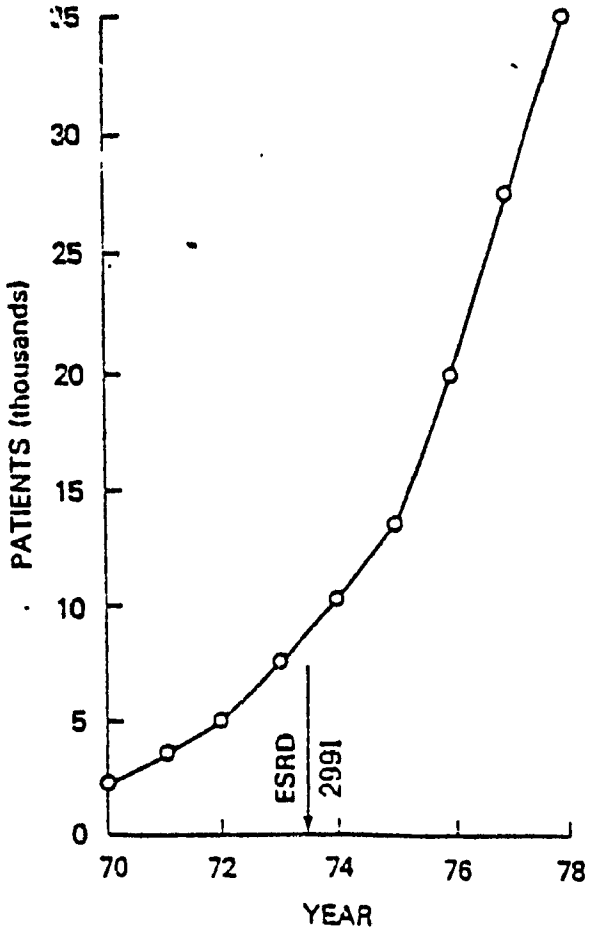



Fig.1.1. U.S. dialysis population 1970-1978 (Wineman, 1978).

further developed the parallel flow system by passing blood between flat sheets of cellophane (Skeggs, 1948). The Guarinos made the dialysate fluid run inside the cellophane tubing with blood passing around the outside in a siliconized glass container (Guarino, 1952). In 1956 Kolff described a simple twin-coil system, prepackaged, sterile and disposable (Kolff, 1956). The parallel plate Kiil dialyser appeared in 1960 (Kiil, 1960). The hollow fibre kidney appeared in 1968 (Stewart, 1968). The coil, plate and the hollow fibre artificial kidneys are now in clinical use.

Unfortunately, not all is well for the artificial kidney. Dialysis treatment is becoming a real drain on governments' financial resources and a greater percentage of the money allocated to the medical pool. The overall costs of the ESRD (End Stage Renal Disease) program in the US were estimated at one billion dollars US for 1978 (ESDR Program, 1977). Annual dialysis costs per patient were US$ 22,464 in 1978; today's figures estimate is about US$ 90,000.

The most common medical complications in dialysis are those associated with the cardiovascular system. Patients may suffer from pericarditis, hypotension, hypertension, and others. For example, hypotension frequently accompanies dialysis as a result of too rapid a removal of fluid. Other complications are those associated with the haematological system, such as anaemia and malfunctions of the coagulation system.

It is also common for uraemic patients to suffer from bone disease, or renal osteodistrophy. This is associated with abnormal calcium and phosphorus metabolism. A secondary reaction of the parathyroid glands produces an excess of parathyroid hormone. There is also an inability to hydroxylate vitamin D into its biological active form, a biochemical process which takes place in healthy kidneys. Current management provides for adequate calcium by dietary control, use of ingestible sorbents to remove phosphate and active metabolites of vitamin D.

Abnormalities of the nervous system have also been noted in dialysis patients. Under extreme cases and when the therapy is inadequate, patients have lost the ability to walk. Another frequent phenomenon is dialysis disequilibrium which causes patients to be disoriented. Cramps, vomiting, anorexia, headache, nausea, restlessness are related disorders. Finally, aluminum toxicity from dialysate water may cause dialysis dementia and death of the patient. Fortunately, its incidence is not common (Ittel, 1987).

Other dysfunctions in dialysis patients include a variety of metabolic derangements, gastrointestinal disturbances, rheumatic complications and endocrine abnormalities such as sexual dysfunctions.

Psychological problems are also common in dialysis patients. It is very difficult to adjust to a "life on the

machine" for both patient and his family. Patients must change their lifestyles to accommodate significant fractions of the week to a machine-related existence. A patient is tied up to a machine eight hours three times a week for the rest of his life.

There is also a perpetual need for skilled personnel. A high incidence of blood access related infections occurs since each dialysis requires regular access to the blood compartment. Blood contamination results from trace metals, carcinogens, plasticizers, etc. found in the dialysate fluid (Daugirdas, 1988; Laurent, 1988; Colton, 1988).

While maintenance of life by dialysis may provide for continued existence, there is immense room for improvement in many if not all cases. Alternative therapies have shown some success over the years. These include dialysate regeneration, haemoperfusion, renal transplantation, intestinal dialysis and dietary maintenance.

In haemodialysis the general trend has been to decrease the size of the dialysis machine so that it may one day become of wearable dimensions (Kolff, 1978). A wearable artificial kidney would allow the patient to conduct a more independent lifestyle and alleviate at least some of the psychological problems mentioned earlier. However, the limit to how small a machine is dictated by the volume of dialysate fluid that is needed to purify the blood on the other side of the

membrane. Water, electrolytes and other waste products can be readily removed by using a combined haemoperfusion-ultrafiltrator system (Chang, 1976). However, this system still requires the removal of urea. Other types of wearable artificial kidneys would also be feasible with the availability of an efficient urea removal system (Kolff, 1976). Chang demonstrated the effectiveness of micro-encapsulated urease enzyme in reducing urea levels in animals (Chang, 1964a, 1966a). The urea removal mechanism was complete with the further addition of a microencapsulated ammonium exchanger, Dowex 50W-12 (Chang, 1966a). Since then, numerous research groups have undertaken studies in this direction for reducing dialysate volume. The commercial Redy system (CCI Life Systems, Van Nuys, California, U.S.A.) employs immobilized urease and an ammonium ion exchanger, zirconium phosphate (Gordon et al, 1969). Other studies aimed at reducing dialysate volume were carried out by Ash et al. who used immobilized urease and zeolite molecular sieves instead of zirconium phosphate (Ash,1980).

Multienzyme systems containing urease, glutamate dehydrogenase and glucose dehydrogenase convert urea and ammonia into non essential amino acids (Cousineau and Chang, 1977; Chang and Malouf, 1978). Further improvement using a multienzyme system including leucine dehydrogenase resulted in the conversion of urea into essential amino acids (Gu and Chang,

1987, 1988, 1989). The ammonium formed from the urease reaction has also been alkalinized into gaseous ammonia for further removal with oxygenators (Piskin and Chang, 1979).

Haemoperfusion, spearheaded by Dr. T.M.S. Chang of McGill University, has offered some promise of success, especially when used in combination with ultrafiltration. The combined haemoperfusion-ultrafiltrator system is now the smallest artificial kidney available (Chang, 1976, figure 1.2). Blood perfused over adsorbents allows the removal of many metabolic products normally excreted in the urine. With activated carbon adsorbent one is able to remove significant quantities of creatinine, uric acid and other toxic metabolites (Chang, 1977). Adsorbents are usually microencapsulated within a polymeric membrane. The use of complex adsorbent systems which employ enzymes such as urease to convert urea to ammonia have also been studied (Chang, 1966a,1967). With this form of therapy it is difficult to control fluid balance as well as mineral and acid-base balance.

Renal transplantation may offer a patient a higher degree of rehabilitation compared to dialysis. Freedom from "life on the machine" is probably the most attractive feature. On the other hand this therapy is not without its complications. Immunosuppressive drugs prevent rejection of the transplanted kidney. Such therapy increases the patient's susceptibility to infections. Unfortunately, within 2 to 3 years the patient

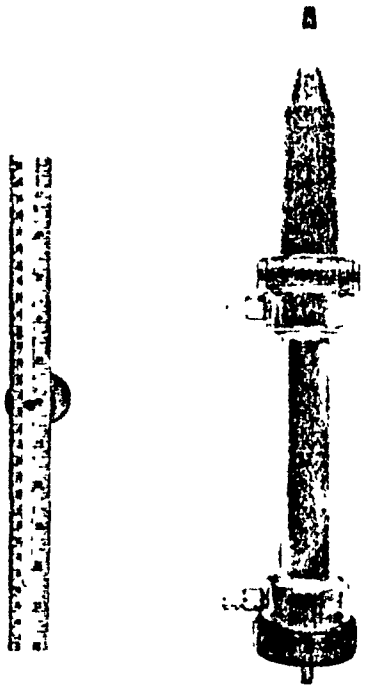


Fig.1.2. Composite artificial kidney (Dialaid), a single unit combining haemodialysis (hollow fibre dialysis membrane) and haemoperfusion (80 gm of 500 Å ultrathin collodion coated BAC-MU spherical petroleum based charcoal). Flow in an antigravity direction with the haemoperfusion section pointing upwards.

who has received a cadaveric donor kidney is normally returned to the dialysis pool. Rejection rates are lower for living-related donor kidneys (NIH Report, 1977).

Intestinal dialysis has been investigated for areas of the world where there might not be any other alternative. The procedure involves the ingestion of large volumes of an isotonic salt solution similar to Ringer's lactate to induce diarrhoea for several hours on the day of treatment. As a result, large quantities of nitrogenous compounds and other uraemic toxins are expelled via the intestinal tract (Young, 1977). However, this approach is not sufficiently effective.

Dietary maintenance applies to chronic renal failure patients whose residual renal function has decreased to a glomerular filtration rate (GFR) of approximately 10 ml/min. The patient is limited to a protein intake of 20 to 40 grams per day. Other investigations involve the use of either keto or hydroxy analogues of amino acids to reduce the amount of waste nitrogen products. A keto-analogue combined with the nitrogen accumulated in kidney failure patients becomes a useful amino acid. Walser used keto-analogues of valine, leucine, isoleucine, methionine and phenylalanine. Some patients improved renal function while others showed no further renal deterioration (Walser, 1974,1975). Giordano has experimented with a low prctein diet enriched with essential amino acids and extra lipids and carbohydrates to

maintain body weight (Giordano, 1972).

Having considered haemodialysis and other viable forms of therapy we undertook to research away from the "life on the machine" approach. To find another way of treating kidney failure patients a bold step seemed appropriate. This bold direction could be the area of ingestible sorbents.

## 1.2. Literature Survey

### 1.2.1 Ingestible sorbents

Ingestible sorbents are now being used for the maintenance of chronic uraemic patients to remove a number of waste products. For example, phosphate adsorbents such as hydrated alumina are routinely used by practically all patients.

Unfortunately, no one has yet found a way to keep nitrogen under control in the form of an oral sorbent. Dr.Kolff was working on intestinal perfusion before devoting himself almost entirely on dialysis. With time, a veil of magic surrounded dialysis to the point where only a few uncompromising spirits kept oral sorbent research alive.

One of these uncompromising spirits was the Greek Dr. Hippocrates Yatzidis.

Activated Charcoal (AC) was used since ancient times as an antidote for poisons. Holt and Holz published in 1963 on the use of an AC oral sorbent for removing poisons (Holt, 1963). Yatzidis introduced activated charcoal in uraemia.

Yatzidis administered between 20 and 50 grams of powdered coconut activated charcoal daily to selected patients for up to 20 months (Yatzidis, 1964, 1976). Despite promising results in vitro, related to creatinine removal, in vivo experiments showed no significant effect. The binding sites of charcoal may have higher affinity for the lipid like substances found in the digestive tract which would prevent any binding to

nitrogenous compounds (Goldenhersh, 1976; Manis, 1977a, 1977b, 1978; Kijma, 1977). Friedman's group was unable to substantiate any worth of charcoal as a nitrogen-extracting ingestible sorbent (Manis, 1977). His data showed unchanging blood urea nitrogen and serum creatinine levels in treated cases and stable faecal nitrogen concentrations. In addition, Denti showed AC to cause a variety of unpleasant disturbances such as nausea, vomiting and constipation (Denti, 1975). Recently, in the USSR, Nikolaev and other groups have found oral activated charcoal very effective (Nikolaev, 1987).

The other uncompromising spirit was the Italian Carmelo Giordano.

Giordano looked at ways to react urea with compounds that are affine to it. He noted that urea reacts with the aldehyde group of formaldehyde to form urea formaldehyde resin. It was then a matter of finding compounds that could be ingested containing aldehyde groups. Giordano envisioned the use of common potato starch, properly oxidized to form the aldehyde moiety to react with urea (oxystarch). He also experimented with cellulose (oxycellulose). Theoretically, a high potential capacity for urea results from one repeat unit of oxystarch binding one mole of urea. In vitro experiments indicated activity at higher temperatures and lower pH.

A 33% drop in blood urea nitrogen (BUN) in patients and a significant increase in faecal nitrogen from 1.4 to 2.5

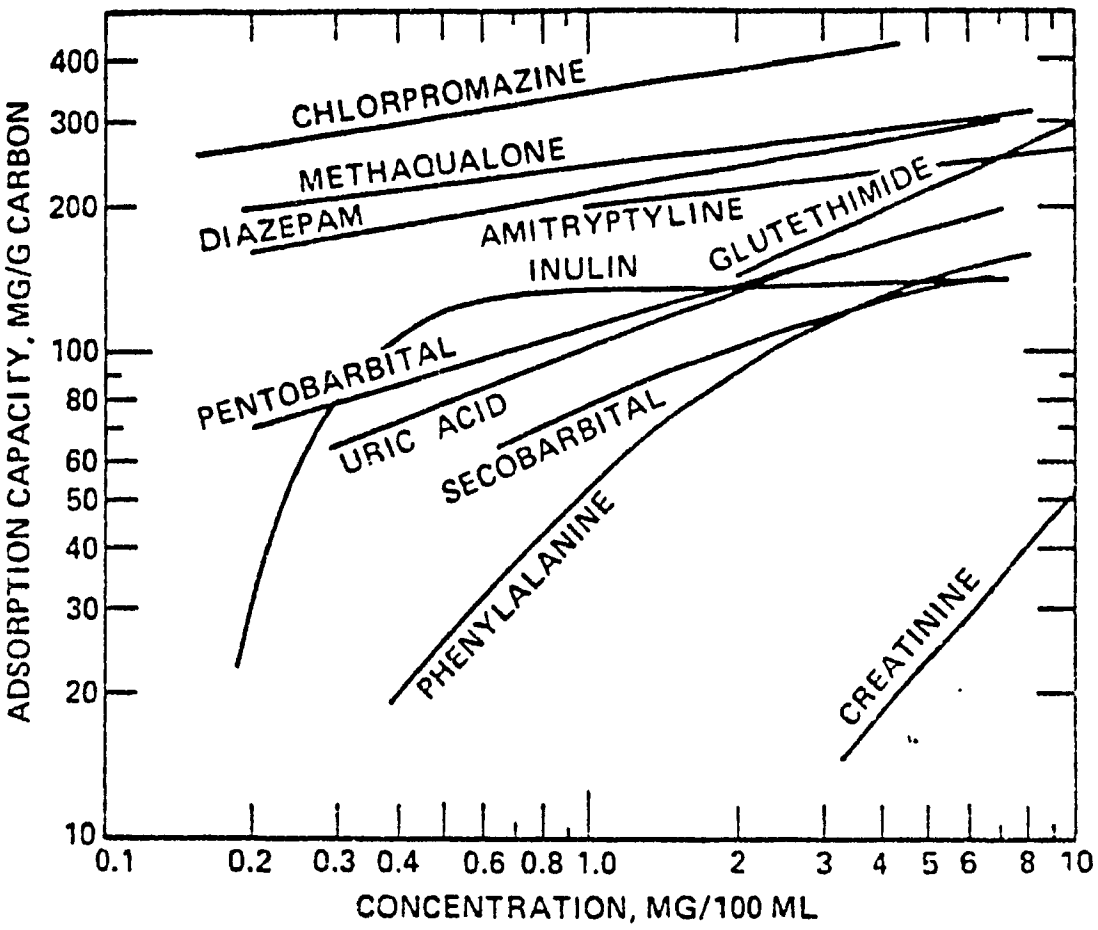



Fig.1.3. Affinity of aqueous solutes for Norit USP activated carbon.

grams per day were hopeful signs of efficacy in vivo (Friedman, 1974; Zeig and Friedman, 1980). Others have contested the validity of these results without the prior reduction of protein intake (Gotch, 1976).

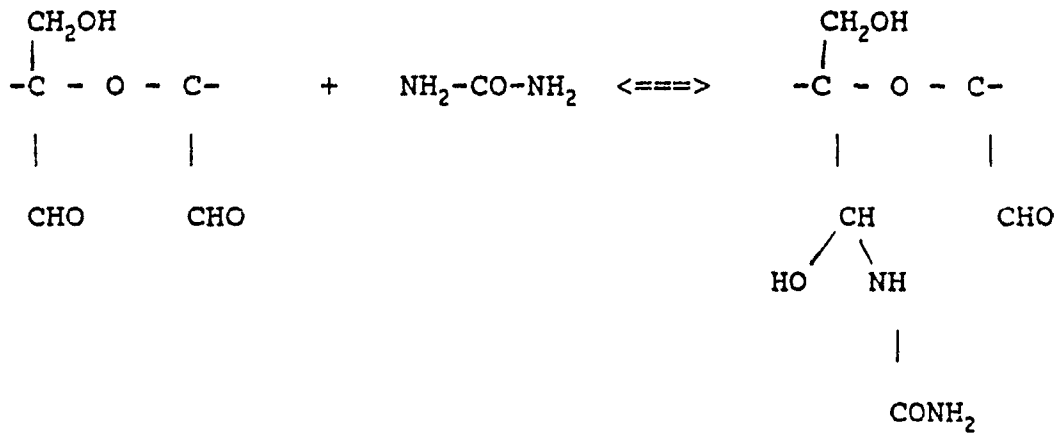
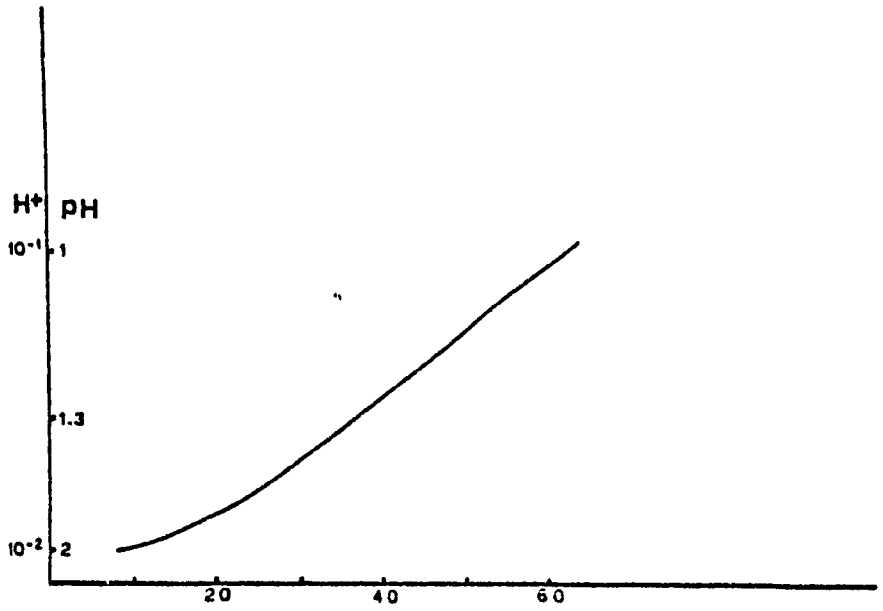



Fig.1.4. Oxystarch binds urea (Meriwether, 1976).

Unlike activated charcoal, which binds at lower temperatures as a result of physical sorption, oxystarch reacts chemically with urea. To obtain a chemical reaction between urea and oxystarch the activation energy barrier must be overcome and this requires higher temperatures. Indeed, urea reacts very favourably at the high temperatures and pressures found industrially to form urea formaldehyde. Unfortunately these conditions are not present in our body.

When tested in vivo oxystarch slowly depolymerizes at intestinal pH 7.4. Its ingestion induces both bowel irritation and diarrhoea. Dr.Friedman, who had initially endorsed oxystarch therapy, finally decided oxystarch to be of no particular benefit, not even for reducing dialysis treatment time (Friedman, 1976). Friedman showed that prolonged survival times after oxystarch or charcoal oral treatment to nephrectomized rats, was not a reduction in uraemia but a result of greater potassium losses and delayed hyperkalemia (Friedman, 1975; Saltzman, 1976; Zeig and Friedman, 1980).

Yatzidis returned with another oral sorbent, Locust Bean Gum (LBG). Locust Bean Gum is a straight chain polymer of mannose derived from the seeds of the carob tree. During World War II locust bean gum was added in bread due to lack of wheat or corn. Hence the claim of its non-toxicity and better digestibility compared to previous sorbents. Calculations revealed locust bean gum to be twice as active as




Oxycellulose binding capacity for urea in acidic media.

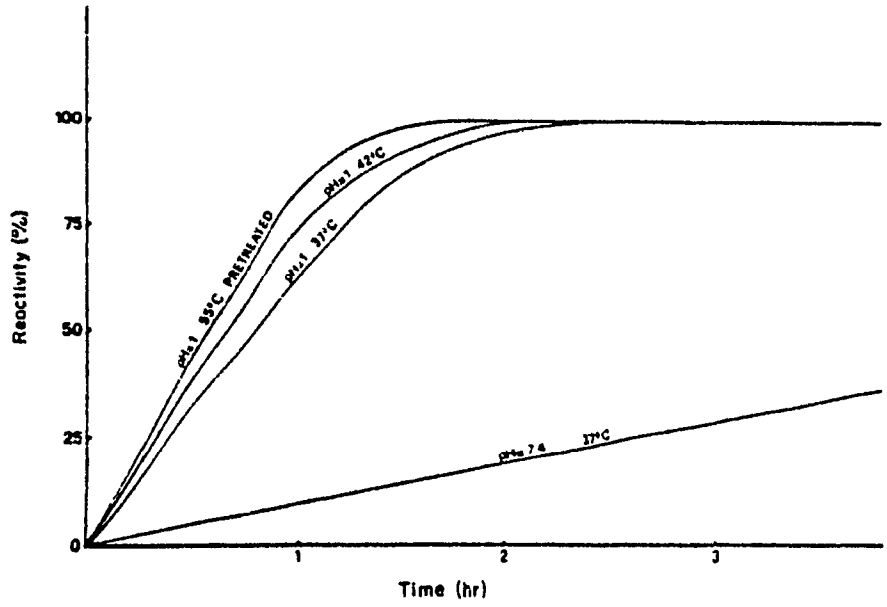



Fig.1.5. Oxycellulose kinetics in various conditions with varying pH and temperature (Giordano, 1980)

oxystarch and four times as charcoal in binding urea (Yatzidis, 1978). Unlike oxystarch there's no measurable hydrolysis or GI absorption. However, in vivo experiments revealed only small decreases in serum phosphate, urea and creatinine. Serious clinical investigations have not been carried out (Zeig and Friedman, 1980).

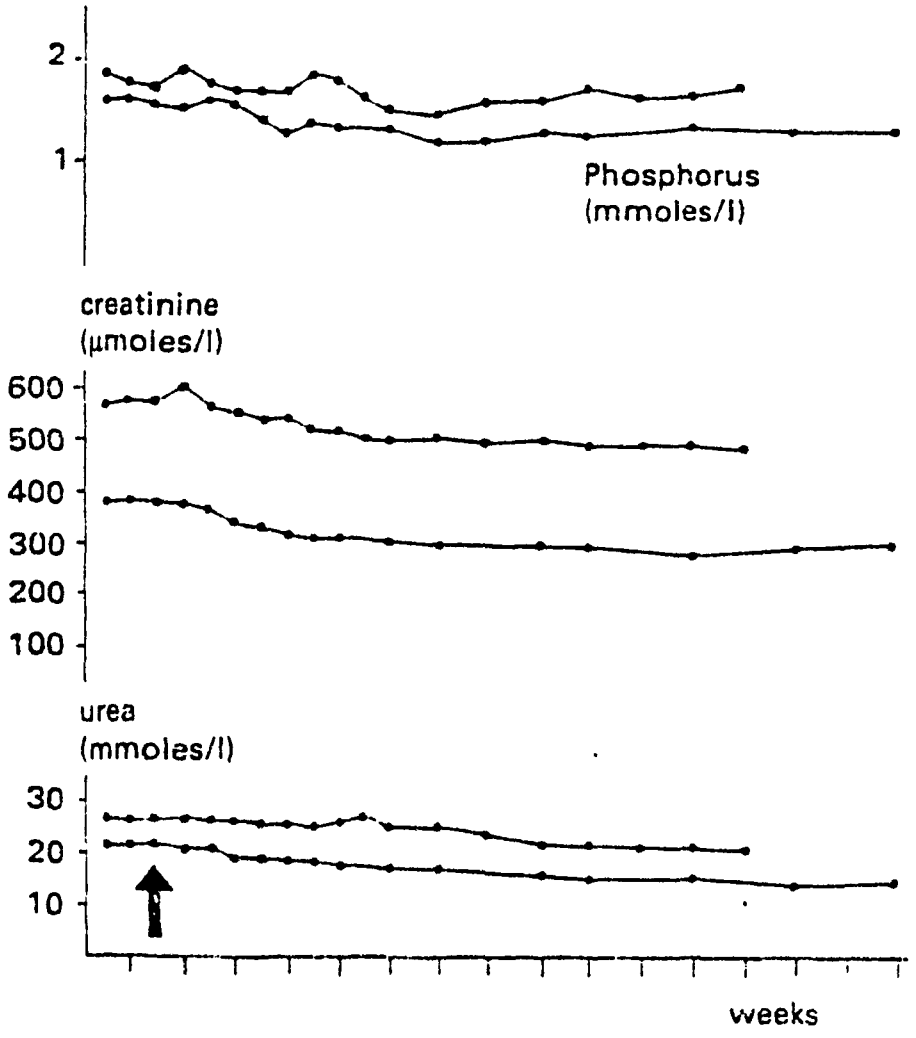



Fig.1.6. Changes of serum creatinine, phosphorus, urea in two uraemic patients on constant diet before and after treatment by oral intake of locust bean gum (Yatzidis, 1978).

A totally different approach to oral sorbent therapy was the use of microencapsulated reactants. Chang was the first to use microencapsulation for medical applications (Chang, 1957). Enzymes, adsorbents, ion exchangers have been microencapsulated (Chang 1963, 1964a, 1964b, 1966a, 1966b). Many are convinced that microencapsulation is the ideal vehicle for administering oral sorbents down the GI tract.

Microencapsulation is desirable for various reasons, namely:

1. To maintain control over the types and sizes of molecules diffusing to the sorbent by using semipermeable membranes.
2. To protect and/or stop release of the capsules' contents for a predetermined and extended period of time -eg. oxidized starch in powder causes severe diarrhoea when administered orally.
3. To maintain special conditions within the capsules' walls which might enhance diffusion concentration gradients.
4. The encapsulated material is not inhibited by food materials which prevent available reaction sites on the adsorbent from being tied up unnecessarily -eg lipids binding to charcoal.
5. Enzymes inside the capsule are protected from proteolytic enzymes in the GI tract.
6. There is limited exposure to the drastic changes in pH along the GI tract, from pH 1-2 in the stomach to pH 7 in the

intestine, because of buffers within the microcapsule.

Microencapsulation has been used for the construction of a type of artificial kidney which was tested for the first time in 1970 on a patient in kidney failure (Chang, 1970b). As the agent for removing toxic substances from the blood, T.M.S. Chang employed microcapsules consisting of tiny pellets of activated charcoal coated with a thin film of collodion. The microcapsules were packed in a chamber connected to the patient's bloodstream. This was effective in removing creatinine, uric acid and other uraemic products. Unfortunately, the capacity of charcoal to remove urea was very low. The next step was to replace the charcoal pellets with immobilized enzymes selected for their ability to remove specific toxic substances. Urease immobilized by physical adsorption on charcoal together with an ammonium binder, zirconium phosphate, is very specific for urea removal and is an integral part of the commercial REDY system (Gordon, 1969).

We now need to describe microencapsulation in relation to enzyme immobilization in general.

### 1.2.2. Enzyme Immobilization

Thousands of different enzymes engineer the complex chemistry of the living cell. Each one of these enzymes is a catalyst for a particular chemical reaction. It is now clear that enzymes could not function if they were simply in the form of enzymes in solution. Native enzymes are fragile materials. They are easily hydrolized and are extremely sensitive to changes in pH and solution composition. There is ample evidence that the great majority of intracellular enzymes functions in an environment either like a gel or adsorbed at interfaces. Enzymes have been reported in a number of cases to be more stable when they are bound to a support. Enzyme immobilization helps us transform fragile native enzyme into more stable entities. The immobilized enzyme can be recycled if necessary, it is not as sensitive to changes in pH or external solution composition and can be protected from various disturbances in the external environment. Native enzymes are generally very expensive and since they dissolve in water they cannot be reused. They are particularly unsuitable for applications where recyclable catalysts would be highly desirable. A column packed with immobilized enzymes can be used repeatedly, and the product that emerges is uncontaminated. Some chemicals can be manufactured with greater ease and fewer reaction steps compared to conventional chemical methods by using immobilized enzymes. The potential of this

technology will be increased even further if the stability of the immobilized enzyme can be improved, if cheaper ways can be developed to isolate enzymes from biological materials on a large scale, and if more progress can be made in enzyme synthesis.

The various immobilization techniques differ in many respects. The isolated enzymes are attached to mechanically stable artificial supports. Among the successful supports are cross-linked dextran gels (Sephadex), cross-linked acrylic polymers (Biogel), polyamino-acids, various kinds of cellulose, ordinary filter paper, glass, etc..

Immobilized enzymes differ by the way they are bound to a fixed support. The principal methods for binding enzymes to supports are: adsorption, covalent chemical linkage, and entrapment of the enzyme within a gel lattice or a microcapsule. Figure 1.7 shows a schematic representation of different types of immobilization methods.

Adsorption

The first method of enzyme immobilization, enzyme adsorption, depends on the ionic, London, Van der Waals, and hydrophobic interaction between the enzyme and its support. It can be generally subdivided into two categories: physical adsorption and ionic binding.

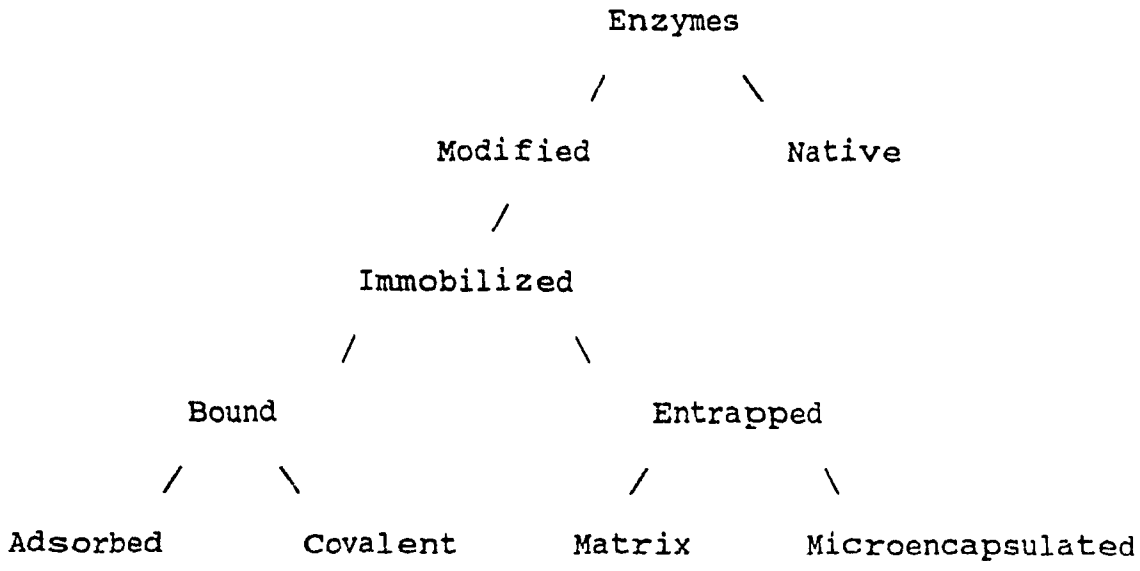



Fig.1.7. Classification of Immobilized Enzymes (Enzyme Eng., ed. L.B. Wingard, J. Wiley and Sons, 1972)

In the physical adsorption method the enzyme protein adsorbs physically on the surface of water-insoluble carriers (figure 1.8a). This method causes little or no destruction of the enzyme active centre and little or no change in the enzyme protein structure. However, the main disadvantage of this method is that enzyme may leak out from the carrier during utilization, as a result of weak binding forces between enzyme protein and carrier. As carriers for this method inorganic materials such as activated carbon, porous glass, acid, clay, bleaching clay, kaolite, alumina, silica gel, bentonite, hydroxylapatite and calcium phosphate gel, as well as natural polymers such as starch and gluten have been employed (Chibata, 1978).

In ionic binding the enzyme is held in place by the attraction of opposite electric charges between the enzyme protein and the ion-exchange residues on the water-insoluble carrier. Physical adsorption may also take place in the binding. As carriers, polysaccharides and synthetic polymers having ion-exchange residues are widely used. Again, this method causes little or no change in the enzyme conformation or destruction of the active sites. Unfortunately, enzyme leakage is also a major problem. Anion exchange carriers such as DEAE-Sephadex, DEAE-cellulose, TEAE-cellulose, ECTEOLA-cellulose and cation exchangers such as Dowex-50 and Amberlite IRC-50 are in general use (Chibata, 1978).

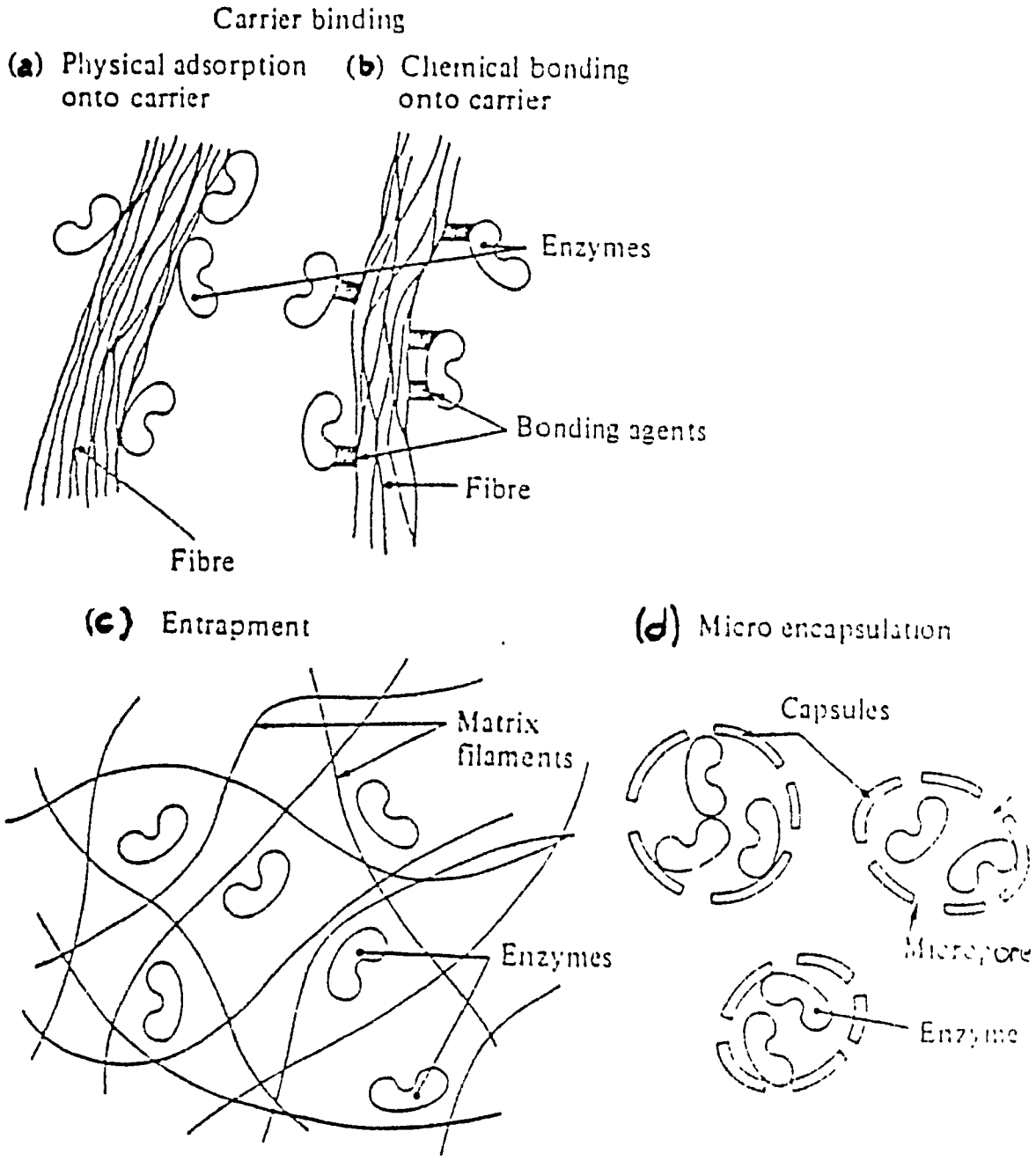



Fig.1.8. Diagrams to show the various methods available for the immobilization of enzymes.

Covalent

The covalent binding method depends on the chemical attachment of an enzyme to a support (figure 1.8b). This method is further subdivided into diazo, peptide and alkylation procedures which vary according to the mode of linkage. For example, the amino, carboxyl, sulfhydril, hydroxyl, imidazole and phenolic functional groups of the enzyme react with the diazonium, acid azide, isocyanate, halide, etc., reactive groups of the carriers. The selection of conditions for immobilization by covalent binding is more difficult than in the cases of physical adsorption or ionic binding. Covalent binding may alter the conformational structure and active centre of the enzyme, resulting in major loss of activity and changes in substrate specificity. On the other hand, the binding force between the enzyme and the carrier is strong and leakage of the enzyme does not occur even in the presence of substrate or salt solutions of high ionic strength (Chibata, 1978).

Examples of covalent attachment are given in figure 1.9.

Matrix Entrapment

The Matrix entrapment method consists of mixing the enzyme in solution with a gel which, as it solidifies, it entraps the enzyme in its interior (figure 1.8c). Alginate, Agar, Polyacrylamide are examples of some of the gels used in the process.

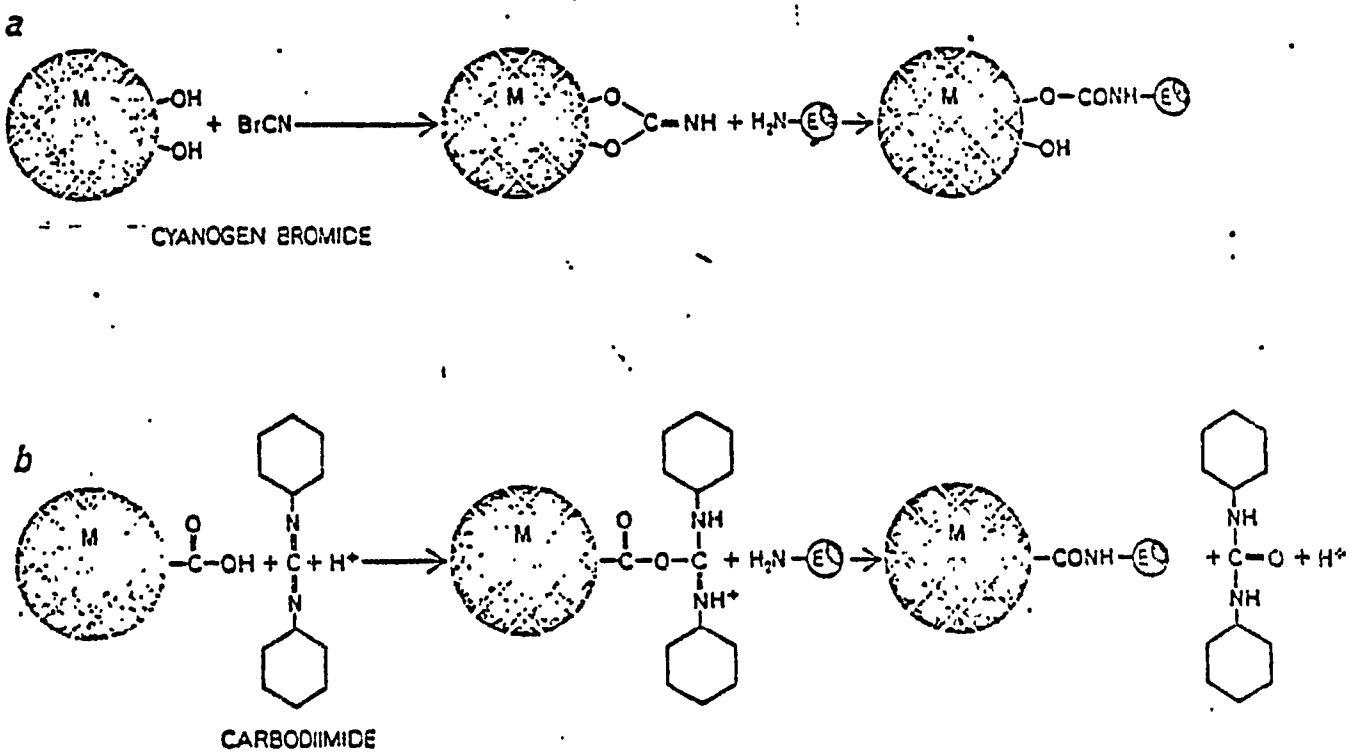



Fig.1.9. The support in (a) is a cross-linked dextran gel (Sephadex) where adjacent hydroxyl groups of the gel can react with cyanogen bromide and combine with amino groups of the enzyme. In (b) the enzyme has been bound to carboxyl groups of the matrix (for example, a cross-linked acrylic polymer) after treatment with a carbodimide (eg.dicyclo-hexyl carbodimide) (Mosbach, 1971)

Microencapsulation

Microencapsulation is the formation of artificial cells which behave in principle much like natural cells. The enzymes are enclosed in microcapsules that allow the substrate to diffuse in and product out (figure 1.8d).

The clinical potential of enzyme technology using immobilized enzymes is substantial. Hundreds of anomalies and diseases can be regarded as inborn errors of metabolism. Of these, a large number represents enzyme-deficiency diseases, in which certain enzymes normally found in the body are either lacking or inactive. A more direct approach is to supply the patient with the missing enzyme. Unfortunately, the foreign protein is immediately eliminated as it produces an adverse immunological reaction in the patient receiving it. On the other hand, if the enzyme is microencapsulated the immunological reaction can be largely prevented. The microcapsules can be exposed directly to blood. They can be packed in an external shunt chamber connected to the circulatory system, as in haemoperfusion. They can also be injected intraperitoneally to act on the extracellular fluid of the patient.

Many enzyme systems have been microencapsulated. Catalase, Asparaginase, Phenylalanine ammonia-lyase, Tyrosinase, and Multienzyme systems are but a few examples of enzymes microencapsulated in this and other laboratories (Catalase: Chang and Poznansky, 1968; Chang, 1972; Poznansky and Chang, 1974.

Asparaginase: Chang, 1971; Chang, 1972; Chong and Chang, 1974, 1977; Mori, 1973. Phenylalanine ammonia-lyase: Bourget and Chang, 1984, 1985. Tyrosinase: Shu and Chang, 1980. Multi-enzymes: Campbell and Chang, 1976, 1977,1978; Chang and Kuntarian, 1978; Grunwald and Chang, 1978, 1979, 1980; Yu and Chang, 1980,1982; Chang, 1985, 1987; Gu and Chang, 1987, 1988, 1989). Enzymes and lipids have also been incorporated into the membrane of the microcapsules to simulate the natural cell (Chang, 1972, 1977; Rosenthal and Chang, 1980). Many other materials beside enzymes including cells, cell extracts and other biologically active materials have been microencapsulated (Chang, 1964, 1966, 1972, 1977, 1984, 1987; Lim, 1980, 1981; Sun, 1981; Leung, 1983; Ergun, 1984; Goosen, 1984; Wong and Chang, 1986; Yuan and Chang, 1986; Sun, 1987a, 1987b, Stevenson, 1988). The latest papers by Chang provide a much more extensive review of the present and future of microencapsulation (Chang, 1985,1987).

In the area of artificial kidney Chang tested separate microcapsules containing urease enzyme and ion exchanger Dowex 50W-12 in a external shunt system in dog animal experiments and found significant reductions in urea levels (Chang, 1966a). He later encapsulated urease and ion exchange Dowex 50W-X8 into each microcapsule (Chang, 1969). Sparks produced nylon microcapsules containing urease enzyme and ion exchanger Amberlite IRC-50, Dowex 50W-X8 (Sparks, 1969).

Chang and Lo incorporated urease in cellulose nitrate (collodion) microcapsules with external ion exchangers zirconium phosphate and Dowex 50W-X12. They demonstrated that this sorbent, when given orally, effectively lowers the blood urea levels in rats (Chang and Lo, 1970). Sparks used ethyl cellulose microcapsules containing oxystarch and activated charcoal. Microencapsulation seemed to be the ideal method for avoiding the hydrolysis of ingested starch and for charcoal the elimination of competitive binding by lipids (Sparks, 1971).

Gardner adopted cellulose acetate butyrate microcapsules containing zirconium phosphate ion exchanger and stabilized urease enzyme (Gardner, 1971, 1975, 1981, 1984).

Asher in 1975 envisioned urease microcapsules contained within liquid membrane capsules. The urea molecule reacts with urease enzyme in the microcapsules to form ammonium which cannot escape the liquid membrane capsule as a result of the ion exchanger nature of the liquid membrane (figure 1.10).

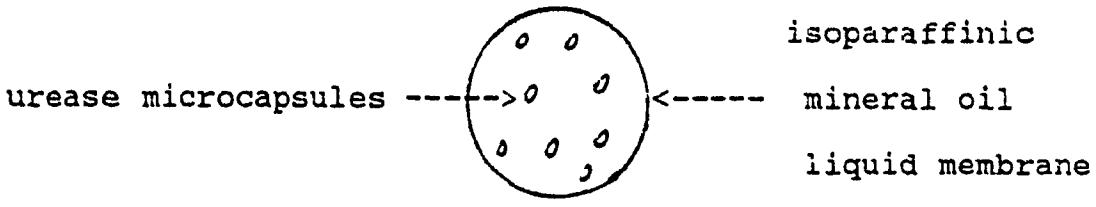



Fig.1.10. Liquid membrane microcapsules

The above are all variations of the same theme: since urea is such an unreactive molecule, react it with the enzyme urease first, which has a very high affinity for the substrate, then trap the product ammonium either with an ion exchanger or with a liquid ion exchanger membrane:

$$NH_2\text{-}CO\text{-}NH_2 + 2\ H_2O \xrightarrow[\text{UREASE}]{} 2\ NH_4^+(aq) + CO_3^=$$

$$X.A + NH_4^+(aq) \xrightarrow[\text{EXCHANGER}]{} X.NH_4 + A^+(aq)$$

Where X represents the ion exchange residue of the ion exchanger and A is the leaving counterion (see Ion Exchange section below).

Microencapsulation can be done by several methods. The interfacial polymerization, phase separation, and liquid membrane and liposome methods are relevant to this thesis.

a) Interfacial Polymerization

The principle behind interfacial polymerization is the polymeric reaction of hydrophillic and hydrophobic monomers at an interface. An hydrophillic solution containing an enzyme and a miscible monomer is emulsified into an organic solvent. An hydrophobic monomer is then added which readily polymerizes with the hydrophillic monomer to form the microcapsule membrane. This procedure uses monomers that polymerize by addition or condensation polymerization. Chang was the first to incorporate enzymes by this procedure (Chang, 1964a,1964b,1966a, 1966b,1969,1971). For example, a solution containing urease enzyme and ethylene diamine is emulsified in 5 times its volume of a chloroform-cyclohexane (1:4) mixture with Span 85 detergent. A polymer membrane readily forms at the interface of microcapsules after the addition of sebacoyl chloride in an equal volume of chloroform/cyclohexane (Chang, 1964a). The main drawback to this procedure is that some enzymes are unstable to the monomers used. Consequently, the choice of monomers is dependent upon the selected enzyme and its intended use.

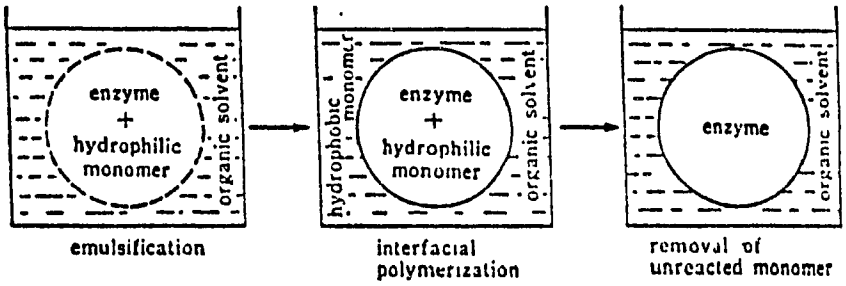



Fig.1.11. Preparation of microcapsules by the interfacial polymerization method.

b) Interfacial Coacervation

Interfacial coacervation involves the precipitation of a polymer out of solution when exposed to a certain concentration of non-solvent. Two variations to this method apply. In the first the enzyme solution is emulsified in a "non-solvent" such as ether. A solvent-polymer solution (eg. ethanol-cellulose nitrate) is added while stirring. The polymer membrane readily forms at the emulsion droplet interface as the polymer precipitates out of solution due to its sudden contact with the non-solvent. Chang's group succeeded in the microencapsulation of asparaginase, urease and catalase (Chang, 1964a,1966b,1968,1971; Poznansky, 1969).

In the second method the enzyme solution is emulsified in a "solvent-polymer" solution (eg. methylene chloride-cellulose

acetate butyrate) and the non-solvent (toluene) is slowly added dropwise to precipitate the polymer at the enzyme droplet interface. Gardner produced very stable urease enzyme microcapsules by this method (Gardner, 1971a,1971b).

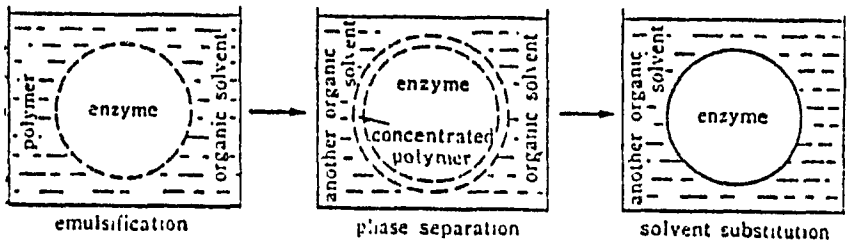



Fig.1.12. Preparation of microcapsules by the interfacial coacervation method

c) Liquid Membrane and Liposome Method

This procedure involves the encapsulation of enzyme solution within an amphipathic liquid-surfactant membrane (liposome). Surfactants and lecithin are widely used to form such membranes. The most significant characteristic of liquid membrane microcapsules is that the permeability of substrate and product across the liquid membrane depends on its solubility in the membrane and not on the membrane pore size as with previous encapsulation methods.

Ammonium ions easily escape out of membranes prepared by either interfacial polymerization or coacervation. Asher

employed liquid membrane microcapsules to trap positively charged ammonium ions within a paraffinic oil capsule (Asher, 1975). Chang's group showed lipid coated nylon membrane microcapsules could slow down the diffusion of small positive ions such as rubidium and sodium (Rosenthal and Chang, 1980).

### 1.2.3. Immobilization of enzymes as envelopes on particles

The most important reason for immobilizing enzymes is their ease of removal from reaction solution by centrifugation or filtration. The majority of immobilizations involve the attachment of the enzyme to a lattice-type matrix, eg. cellulose, polystyrene. Unfortunately, the polymer matrix restricts access of the substrate to the bound enzyme molecule.

The classical consideration of mass transfer in catalytic chemical processes has been successfully applied to immobilized enzymes (Payne, 1981). External and internal diffusional resistances are important. External diffusional limitations originate when substrates flow from the bulk solution to the immobilized biocatalyst's surface across a stagnant boundary layer. Internal diffusional limitations arise inside the immobilized enzyme particle.

The existence of diffusional limitations reduces the catalytic efficiency of immobilized enzymes, especially with larger molecular weight substrates. Diffusional resistances

can be minimized by: (1) decreasing the size and optimizing the geometry of the immobilized enzyme particles, (2) increasing the substrate concentration, (3) enhancing the stirring and flow rate, (4) increasing the porosity and optimizing the enzyme distribution in the beads.

In general, any enzyme can be immobilized with satisfactory retention of its catalytic activity. Although this is valid in the case of low molecular weight substrates, it does not always hold for enzymatic reactions involving high molecular weight substrates. For example, hydrolases covalently attached to solid supports, exhibit much lower enzymatic activity toward polymeric substrates (proteins, polysaccharides, and nucleic acids) than expected on the basis of their reactivity to small substrates. This effect is due to steric hindrances involving the support's surface (Klibanov, 1983). Similarly, interactions between enzymes entrapped in polymeric gels and macromolecules are often severely diminished because of the slow diffusion of the latter in the gel matrices.

To overcome diffusion resistance Haynes introduced enzyme envelopes around colloidal silica particles. The enzymes are fully exposed to the reacting solution (Haynes, 1969). Haynes showed that the optimal amount of immobilized protein is when it forms a monolayer on the surface of the silica. From a given surface area of colloidal silica he calculated the amount of protein which could be adsorbed as a monolayer.

Further layers were not beneficial and a waste of enzyme. The physically absorbed enzyme could then be permanently attached to the colloidal particles by glutaraldehyde crosslinking (figures 1.13 and 1.14).

An outgrowth of Haynes' method was to use carbon, nylon, silica and alumina spherical particles for the preparation of immobilized enzymes by crosslinking with glutaraldehyde (Horvath, 1973). Horvath adopted the name "pellicular immobilized enzymes" because the enzyme is in an active porous medium which forms a thin skin around the support particle (figure 1.15). Although the porous medium adds diffusional resistance, this is counterweighed by the improved stability of the enzyme preparation (Horvath, 1973).

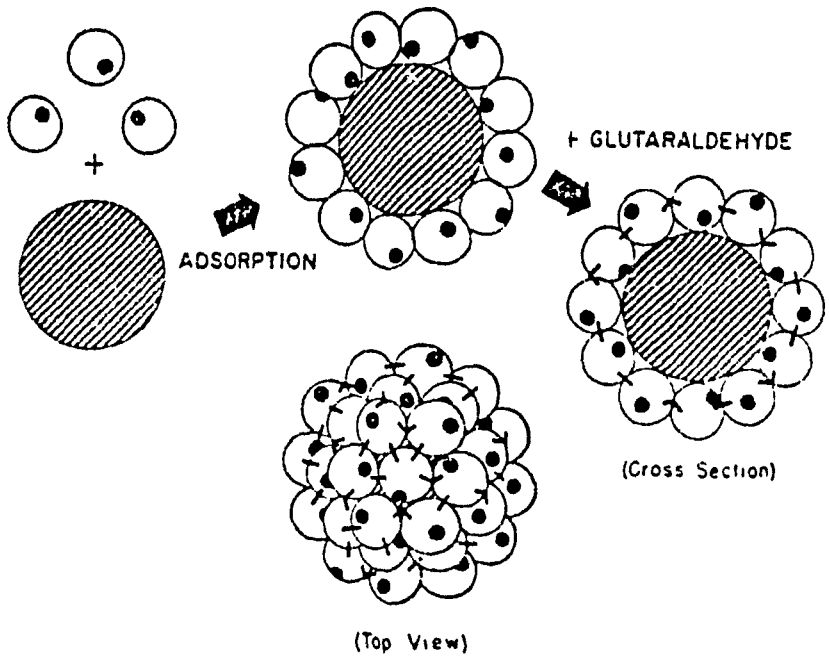



Fig.1.13. Schematic representation of the method for preparing insoluble proteins as envelopes on particles.
The shaded area represents the particle, the dark circles represent the active sites of a protein with biological activity and the bars represent covalent intermolecular crosslinks.

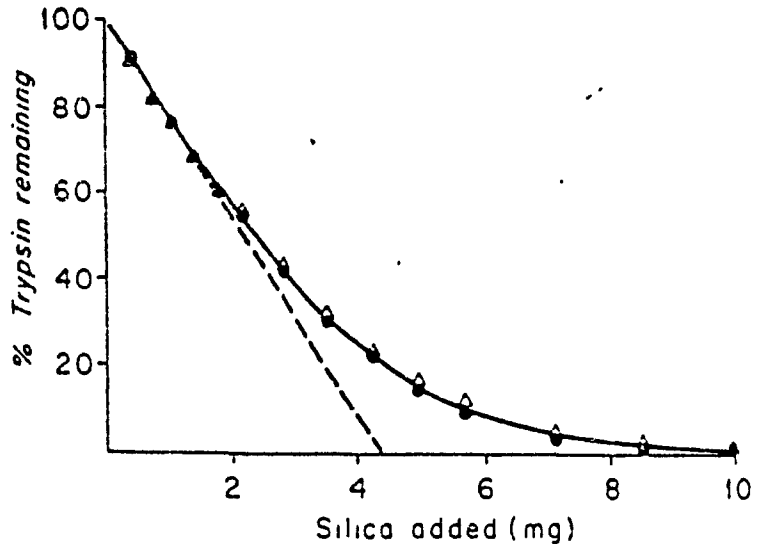



Fig.1.14. Stoichiometry of adsorption of bovine trypsin on silica. Various quantities of silica were added to 1.9 mg of bovine trypsin in a total volume of 3.0 ml of 0.1 M borate, 0.001 M benzamidine, pH 8.5. After mixing and incubation for 15 min. at room temperature, the solutions were centrifuged at 37,000 g for 20 min. The amount of trypsin remaining in the supernatant was determined both by absorbance at 280 mμ, empty triangles, and by activity, full circles.

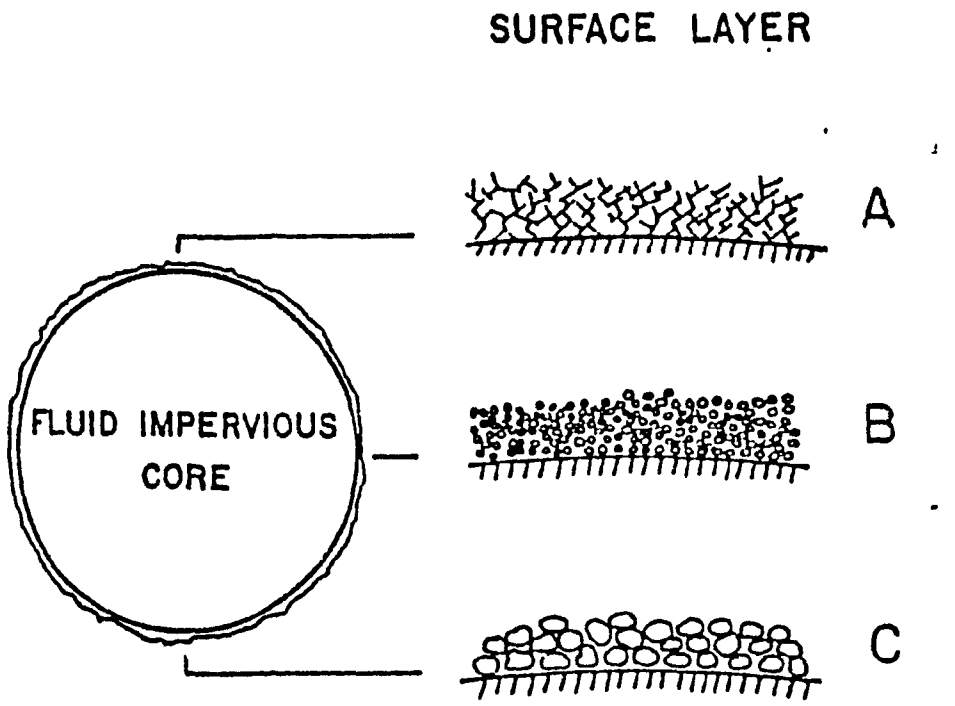



Fig.1.15 Schematic illustration of a pellicular immobilized enzyme particle and the various types of surface layers. A. Crosslinked gel containing covalently bound or entrapped enzyme. B. Small-pore rigid polymeric or inorganic layer with the enzyme covalently bound to or crosslinked onto the surface. C. Large-pore layer impregnated with the immobilized enzyme.

### 1.2.4. Microencapsulated Enzyme Envelopes

Microencapsulating enzyme envelopes is similar in concept to Horvath's procedure since the microcapsule's membrane provides a barrier between the enzyme and the external environment. Gardner reported excellent enzyme stability with this method. He was the first to microencapsulate enzyme envelopes (Gardner, 1971).

Gardner brought microencapsulated oral sorbent therapy further along than other groups. Clinical trials on patients were initiated in 1981 (Kjellstrand et al, 1981). Blood urea nitrogen decreased to 0.60 mg/ml from 0.75 mg/ml within 15 days of treatment.

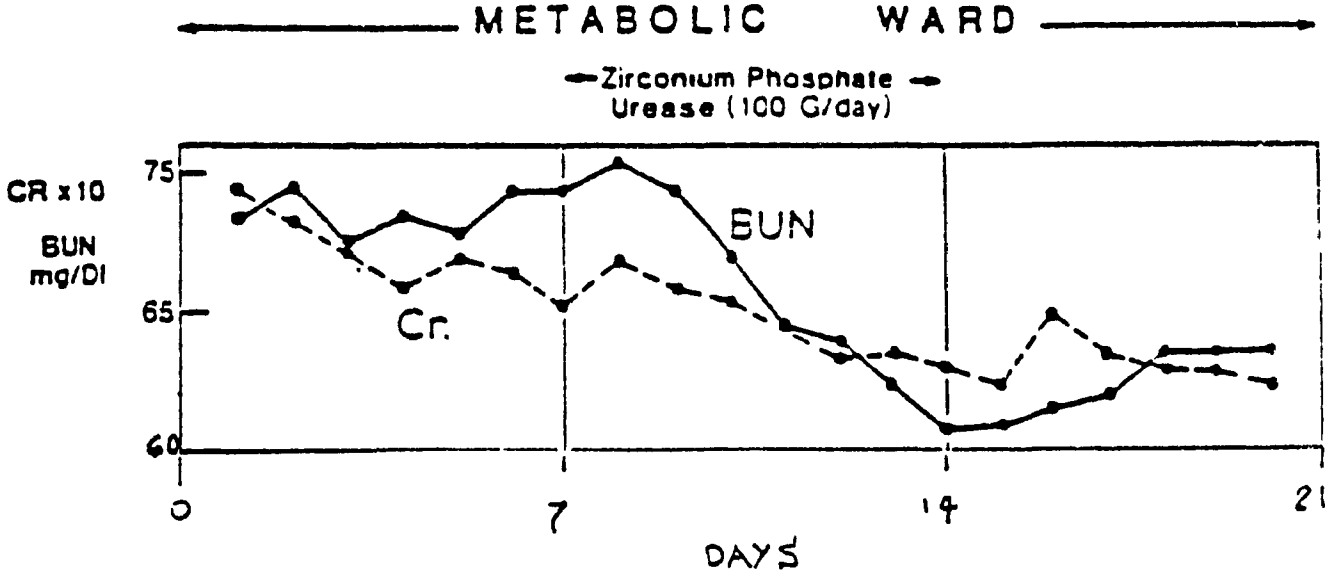



Fig.1.16 Decline in BUN in patient 5 (Kjellstrand et al. 1981).

Unfortunately, patients started complaining of nausea, hard stools, heartburns and hypertension. Two side effects were found to be undesirable:

1. The release of sodium ions from the ZP resin. Patients suffering from kidney failure already have high sodium levels and ZP only augments their hypertension and fluid overload.
2. The uptake of calcium ions may worsen the metabolic bone disease almost universally present in patients (Kjellstrand, 1981).

Zirconium Phosphate resin had to be replaced with something potentially less harmful. Here began our interest in other types of ion exchangers. To better appreciate the nature of ion exchangers let me spend some time in the fundamental area of ion exchange.

### 1.2.5. Ion Exchange

Ion exchangers are a framework carrying a positive or negative electric charge compensated by mobile ions of opposite sign called counter ions. Cation exchangers contain cations, and anion exchangers anions, as counter ions. The counter ions are free to move within the framework and can be exchanged for other ions of the same sign.

To give a simple picture of the ion exchange process, the ion exchanger may be compared to a sponge with counter ions floating in the pores. When the sponge is immersed in a

solution, the counter ions can leave the pores and float outside. However, electroneutrality must be preserved, i.e. the electric surplus charge of the sponge must be compensated at any time by a stoichiometrically equivalent number of counter ions within the pores. Hence, a counter ion can leave the sponge only when, simultaneously, another counter ion enters and takes over the task of contributing its share to the compensation of the framework charge.

According to this simple model, the counter-ion content of the ion exchanger -the so called "ion-exchange capacity" - is a constant which is given by the magnitude of the framework charge and is independent of the nature of the counter ion.

When an ion exchanger in the $Ca^{2+}$ form is placed in a solution of NaCl, counter ion $Ca^{2+}$ will migrate from the exchanger into the solution and counter ion $Na^{+}$ from the solution into the ion exchanger, i.e., an exchange of counter ions takes place. After a certain time, "ion-exchange equilibrium" is attained:

$$\underline{Ca^{2+}} + 2Na^{+} \quad <=====> \quad 2\underline{Na^{+}} + Ca^{2+}$$

where quantities underlined refer to the inside of the ion exchanger.

The cation exchanger, originally in the $Ca^{2+}$ form, is "converted" to the Na+ form. The ion exchanger removes the

$Na^+$ ions from solution and replaces them by $Ca^{2+}$ ions. An ion exchanger containing exchangeable $Ca^{2+}$ ions is said to be in the $Ca^{2+}$ form. Complete exchange to the $Na^+$ form can be achieved by treating the ion exchanger with a sufficient excess of a solution of a sodium salt.

The exchange is stoichiometric and, as a rule, reversible. A cation exchanger which has lost all its $Ca^{2+}$ ions and thus has become "exhausted", can be "regenerated" with a solution of calcium salt such as $CaCl_2$. In regeneration the exchange is reversed, and the ion exchanger is reconverted to the $Ca^{2+}$ form.

Ion exchange resembles sorption in that, in both cases, a dissolved species is taken up by a solid. Ion exchange, in contrast to sorption, is a stoichiometric process. Every ion which is removed from the solution is replaced by an equivalent amount of another ionic species of the same sign. In sorption, on the other hand, a solute is taken up without being replaced by other species. The ion exchanger and the solution contain both counter-ion species $Ca^{2+}$ and $Na^+$. However, the concentration ratio of the two counter ions is not necessarily the same in both cases. Ion exchange is essentially a statistical redistribution of counter ions between the pore liquid and the external solution.

The model also describes "ion-exchange kinetics" correctly. Ion exchange is essentially a diffusion process and has

little, if any, relation to chemical reaction kinetics in the usual sense. Its rate depends on the mobility of the counter ions. However, the simple and well known rate laws of diffusion hold only in exceptional cases.

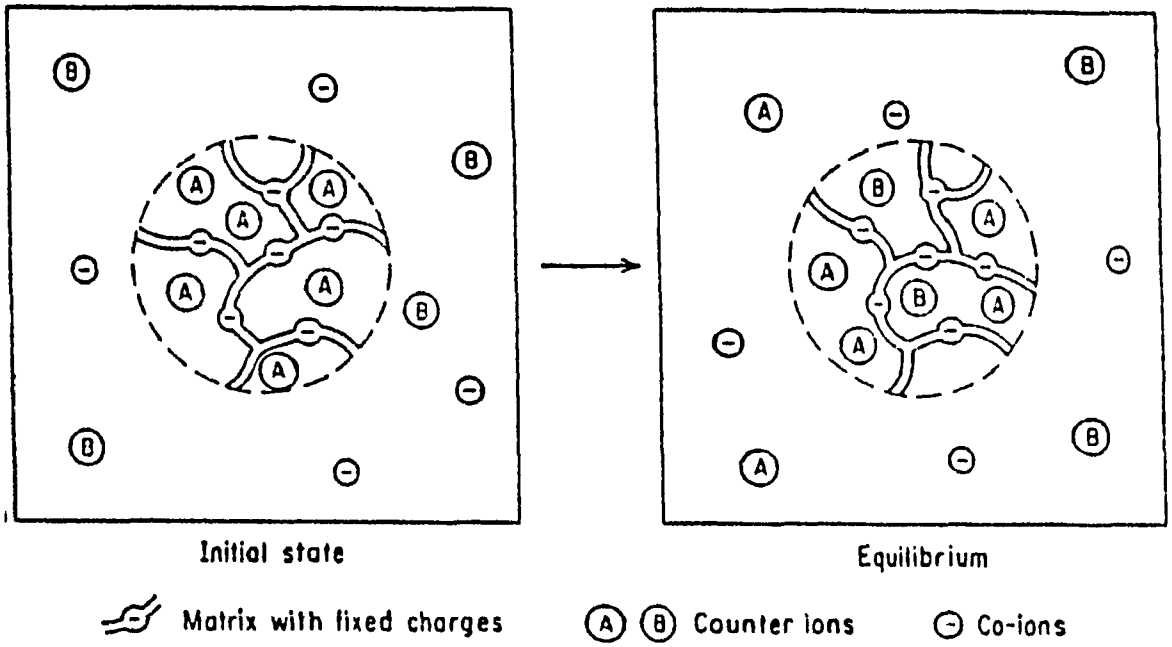



Fig.1.17. Ion Exchange with a solution (schematic). A cation exchanger containing counter ions A is placed in a solution containing counter ions B (left). The counter ions are redistributed by diffusion until equilibrium is attained (right) (Helfferich, 1962).

As a rule, electric forces affect the flux of the ions and cause deviations. The absence of any chemical reaction explains why the heat evolved during an ion-exchange process

is usually small, often less than 2 kcal/mole.

Usually the ion exchanger is selective, i.e. it takes up certain counter ions in preference to others. The preference for one species may have several causes, the most important are:

1. Large counter ions may be sterically excluded from the narrow pores of the ion exchanger.
2. The electrostatic interactions between the charged framework and the counter ions depend on the size and, in particular, on the valence of the counter ion.

These effects depend on the nature of the counter ion and may lead to preferential uptake of a species by the ion exchanger. The ability of the ion exchanger to distinguish between various counter ion species is called "selectivity".

The electrochemical properties of ion exchangers are readily interpreted in terms of this model. Ion exchangers have a high electric conductivity which is due to their high content of mobile ions. The model also shows that the ion exchanger must contain many more counter ions than co-ions. Many electrochemical phenomena in systems with ion-exchanger membranes can be explained with this fact.

**Ion Exchange Membranes**

The term "ion exchanger membrane" may comprise a whole range of materials, including solid films, foils, disks, ribbons,

tubes, plugs, etc., in short anything that may be used as a barrier between two solutions.

For scientific purposes, the so called "homogeneous" membranes are preferred. These are coherent, unsupported gels. For example, a membrane can be formed without much difficulty from the condensation products of phenolsulfonic acid and formaldehyde (Helfferich, 1962) for cation exchanger and of polyethyleneimine and epichlorohydrin for anion-exchanger membranes (Manecke, 1951).

For practical applications, reinforced or "heterogeneous" membranes offer the advantage of higher mechanical strength. These membranes are supported by inert carriers or binders.

Membranes can also be prepared by evaporation and impregnation techniques. The discovery of the ion exchange properties of collodion membranes, made by the evaporation technique, by Teorell and Meyer in 1935 coincided with the synthesis of the first ion exchange resins (Teorell, 1935). The collodion membranes are weak acid cation exchangers. The ion exchange capacity of untreated collodion is very low (of the order of 0.001 meq/g). It can be increased by impregnating with soluble polyelectrolytes. Strong-acid and strong base groups have been introduced into collodion membranes. Good results have been obtained with polystyrenesulfonic acid for cation-exchanger and with poly-2-vinyl-N-methyl-pyridiniumbromide for anion-exchanger membranes (Maclay, 1948;

Neihof, 1954; Gottlieb, 1955). Protamine electrolyte to form anion-exchanger membranes has also been used (Gregor, 1945; Sollner, 1949).

**Ion Exchange Zeolites**

Zeolites have been widely studied for their potential in controlling the large-scale rearrangement and substitution reactions of small hydrocarbons. Interestingly, they have also been used to mimic enzyme function. The analogy between the pores of a zeolite and the binding site of an enzyme is obvious (Herron, 1987). Enzymes and zeolites combined play a role in some industrial operations. For example, mannitol currently commands high prices for pharmaceutical and medical applications while glucose is readily available from corn syrup. The ability of a zeolite to catalyse the hydrogenation of fructose to mannitol, together with immobilized glucose isomerase, produces a one-pot conversion of glucose to mannitol (Ruddleston, 1981).

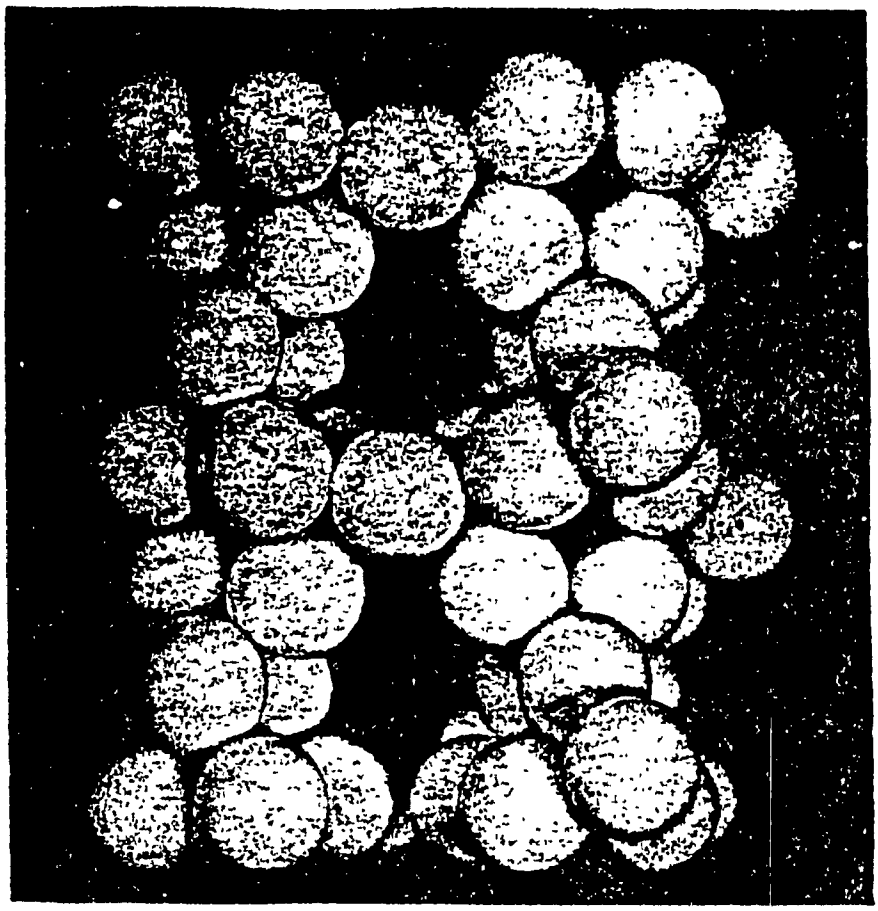


Fig.1.18. A space filling model of zeolite edingtonite shown in the direction of the main channels. The spheres represent oxygen atoms. Each alternate cavity is occupied by a barium atom. The barium ion is coordinated with 6 oxygens and 2 water molecules for a total of 8-fold coordination (Breck, 1984).

Activated carbon, activated alumina, and silica gel do not possess an ordered crystal structure and consequently have non-uniform pores. The pore distribution within the adsorbent may be narrow (20-50 Å) or wide (20 to several thousands Å) as is the case of some activated carbons. Hence, all molecular species, with the exception of high molecular weight polymer materials or proteins, may easily enter the pores. On the other hand, zeolite molecular sieves have pores of uniform size (3Å to 10Å) which are uniquely determined by the unit structure of the crystal. For example, zeolites may be represented by the unit empirical formula:

$$M_{2/n}O.\ Al_2O_3.\ xSiO_2.\ yH_2O$$

where x is equal to or greater than 2, y is the extent of hydration of the unit cell, M is the cation and n is the cation valence. Slight changes in any of these parameters will change the overall crystal shape, its properties (eg. hydrophillic versus hydrophobic) and the pore size accessible to outside molecules. The pore size distributions for a zeolite, a typical silica gel, and activated carbon are illustrated in figure 1.19. Zeolites are called molecular sieves because they exhibit the property of acting as sieves on a molecular scale. Dehydrated crystalline zeolites have a

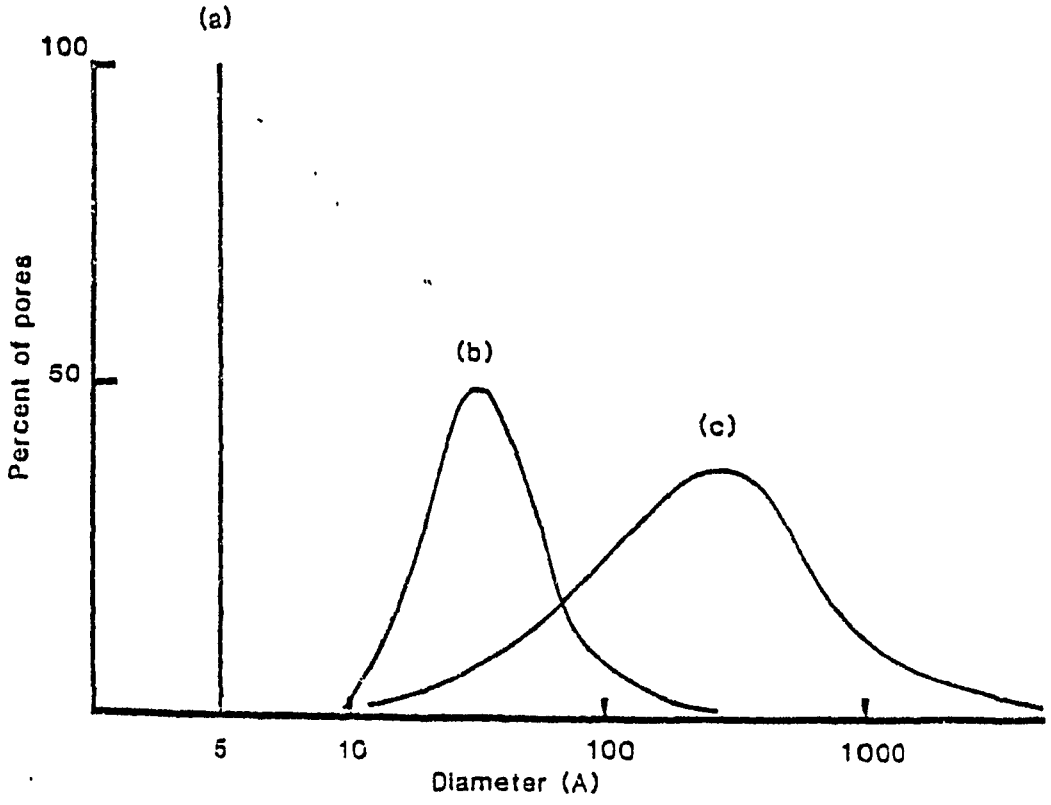



Fig.1.19. Distribution of pore sizes in microporous adsorbents: (a) Dehydrated zeolite, eg. type A; (b) Typical silica gel; (c) Activated carbon (Breck, 1984).

high internal surface area available for adsorption due to the channels of pores which uniformly penetrate the entire volume of the solid. The external surface of the adsorbent particles contributes only a small amount of the total available surface area.

Structurally the zeolites are "framework" aluminosilicates which are based on an infinite extending three dimensional network of alumina ($AlO_4$) and silica ($SiO_4$) tetrahedra linked to each other by sharing all of the oxygens (fig.1.20). The greater the alumina to silica ratio the greater the potential capacity for ion exchange and as a result ammonium exchange.

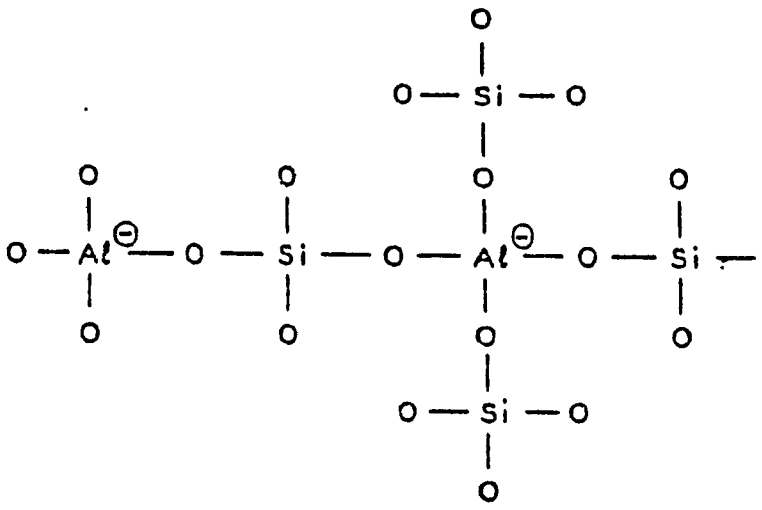


Fig.1.20. Two-dimensional representation of an aluminosilicate framework (Sherry, 1969)

In the late 60's Ames discovered that some zeolites had excellent ammonium selectivities and could be used commercially for the treatment of municipal waste waters (Ames, 1967). In earlier studies a permutite type exchanger and various organic resin ion exchangers were found to have poor selectivity for ammonium ions, resulting in unacceptably low ammonium loadings (Sherman, 1983). Ames used the natural zeolite Clinoptilolite to achieve ammonium removals from waste waters above 95%.

My experience with ammonium ion exchanger zeolites dates back to 1981 when, under the direction of Dr. Ian Cody - a zeolite specialist, we discovered some unusual properties of a steam modified zeolite Y. To produce the hydrogen form we had to routinely ion exchange the zeolite with ammonium chloride. J.D. Sherman of Union Carbide, a friend of Ian Cody, had been reporting on the use of zeolites in dialysis to reduce dialysate volume. The natural thing to do was to extend zeolites to oral sorbent therapy.

## 1.3. Objectives and Strategy

Since urea could potentially be bound physically, chemically or reacted with urease microcapsules it was decided to go by the latter route in light of recent evidence showing very poor performance in either the physical (charcoal) or chemical (oxystarch) methods. In addition, the most recent work by Gardner on the microencapsulated ZPU system had given us some hope to succeed. As well, my supervisor, Dr.Chang, had previously investigated the combination urease microcapsules-zirconium phosphate (Chang and Lo, 1970). Further work on microencapsulated urease and Zirconium Phosphate had been carried out in this laboratory as recently as 1985 (Wolfe, 1985, 1987). Extensive experience on the microencapsulation technique could easily be obtained from this centre. Further improvements on the previous microencapsulated oral sorbents could be implemented in several ways, namely:

1. To investigate zeolite ion exchangers.
2. To experiment with different enzyme immobilization methods beside microencapsulation.
3. To optimize the microencapsulation procedure.
4. To modify the membrane microcapsule.

Important milestones of my work were the formation of enzyme envelopes, the use of ion exchange zeolites, the micro-

encapsulation with a cellulose based polymer and the introduction of electrolytes onto the cellulose membrane by the impregnation method.

Since zeolites have a composition somewhat similar to silica gel, could urease enzyme be immobilized directly on the zeolite without using silica gel as support? Could the surface area provided by the zeolite be sufficient for immobilization, knowing that zeolite consists of particles two orders of magnitude larger than silica gel? Gardner had shown that the urease of the silica immobilized urease system retained about 20% of its initial activity after microencapsulation. Could the zeolite-immobilized urease system retain its activity after microencapsulation involving the use of organic solvents? In addition, could we improve upon the microcapsule membrane by making it a definite barrier to ammonium ions unable to escape the ammonium binder?

These were some of the questions as I proceeded along in search for an efficient urea oral sorbent.

# METHODS

## 2. EXPERIMENTAL

### 2.1 Zeolite Preparation

Zeolites Linde F and W were kindly supplied by Union Carbide, Linde Division, Tarrytown, New York. Typical properties of the as received materials are included in the appendix.

Normally, the zeolites are ion exchanged from the mixed cation (50/50, $K^+$,$Na^+$) to the single sodium form as follows:

a) Spread 100 grams of zeolite onto No.42 Whatman filter paper in a 7 inch diameter Buchner funnel sealed to a 1 litre Erlenmeyer flask. Apply vacuum. Wash the zeolite with 20 litres of 0.5 molar NaCl.

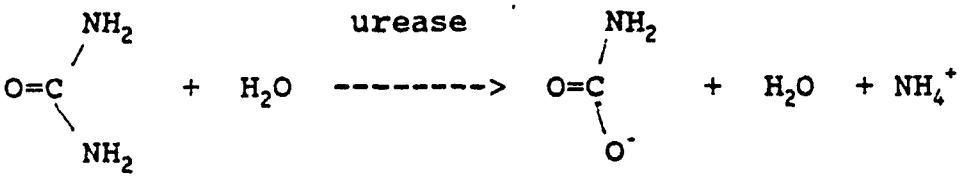
b) Leave the wet zeolite in a vacuum oven with a slight positive air flow at 15 inch Hg vacuum and 100 'C overnight. Dry zeolite will have a talc-like appearance. Store the zeolite in a sealed container to prevent water reabsorption.

The same procedure can be used to obtain the calcium form of the zeolite.

### 2.2. Urease

Jack Bean (Canavalia ensiformis) urease is one of the best sources of commercially available urease which has been crystallized and thoroughly studied. Its molecular weight is 480 000 Dalton. Urease (urea amidohydrolase) catalyses the hydrolysis of urea to ammonium ions.

The mechanism is as follows:




$$\text{----->}\ CO_3^{=} + 2NH_4^{+}$$

Urease activity is determined by coupling the above reaction with glutamate dehydrogenase, and following the oxidation of (NADH + $H^+$) spectrophotometrically at 340 nm (Worthington Manual, p.257, 1977):

$$2NH_4^{+} + 2\ \alpha\text{-ketoglutarate} + 2NADH \text{ -->}$$
$$2\text{glutamate} + 2\ NAD^{+} + 2\ H_2O$$

The decrease in absorbance due to the conversion of NADH (+ $H^+$) to $NAD^+$ is measured at 340 nm. The rate of this conversion is proportional to the amount of urea in the sample for a limited period of time. Native enzyme kinetics were done using a Varian-Cary 219 Spectrophotometer. The urease enzyme was specified as having an activity of 49 units per milligram. One unit results in the oxidation of one micromole of NADH (+

$H^+$) per minute @ 25 °C and pH 7.6 under the specified conditions. The optimum pH for urease in free solution is 6 with a working range of 5 to 8 pH units. Urease is good for 6 to 12 months when refrigerated at -20 °C.

## 2.3. Urease-zeolite sorbent (ZWU)

Urease enzyme was immobilized directly on zeolite ion exchanger as per a modified version of Haynes and Walsh. Haynes used colloidal silica particles as enzyme support (Haynes, 1969). We tested various amounts of urease loadings and glutaraldehyde crosslinking agent to find their optimal concentration for a given amount of zeolite. Urease was varied from 1.5 mg to 100 mg and glutaraldehyde from 1 μL to 220 μL for 500 mg of zeolite. The following is an optimal preparation.

Fifteen milligrams of urease (Sigma, Type IV) and 500 mg of zeolite are suspended in 50 ml of acetate buffer (pH 5.2) under gentle stirring. Seven μL of glutaraldehyde crosslinking reagent are added after 30 minutes while stirring for another 25 minutes. It is important to stir gently to minimize loss of enzyme due to shear. The urease-zeolite sorbent (ZWU) is then precipitated by centrifugation.

## 2.4. ZWU in Oxycellulose matrix

Oxycellulose was purchased from Sigma Chemicals. Two batches with different ketone contents were purchased (Batch A: 10-

16% ketone groups, batch B: 16-22%). Batch B, the most soluble batch, was further used to make the oxycellulose matrix.

Initially, 0.1 grams of oxycellulose are dissolved in 5 ml of phosphate buffer (pH 7.5). The solution is mixed with 0.5 g of urease-zeolite sorbent. Oxycellulose is then reprecipitated by adding 10 ml of 0.1N CaCl2. A white precipitate indicates the formation of calcium crosslinks between the oxycellulose chains.

## 2.5. Microencapsulation

For the removal of urea from the intestinal tract various encapsulation methods were attempted. Alginate and collodion microcapsules containing haemoglobin stabilized urease enzyme and external ammonium selective zeolite were produced and tested. The encapsulated enzyme retains approximately 30% of the activity of the enzyme in free solution. At 4 °C the encapsulated enzyme will last at least three weeks with little loss of activity (Chang, 1972; Wolfe, 1985). When encapsulated the enzyme will function over a much broader pH range, i.e. 4 to 11 (Sundaram, 1973). Diffusion limitations through the microcapsule membrane should not be a problem. The mean pore radius is 18 Å (Chang and Poznansky, 1968b). The molecular radius of urea is 2.5 Å and is little hydrated in aqueous solution, therefore urea diffusion should not be hindered (Colton et al, 1971). The hydrated radii of the products,

$HCO_3^-$, $CO_3^=$ and $NH_4^+$ are 4.0, 5.3 and 1.43 Å respectively (CRC Handbook of Chemistry and Physics). Cellulose acetate butyrate microcapsules containing a urease-zeolite sorbent was one encapsulation method tested extensively in vitro. What follows summarizes experimental procedures involving the manufacture of these microencapsulated systems.

## 2.5.1 Collodion Microcapsules

Reagents

a. Haemoglobin

Dissolve 15 grams of purified bovine haemoglobin (Sigma Grade) in 100 millilitres of 0.1 Molar Tris Buffer (*)(#). Shake for 2 hours in a Multipurpose Rotator (Scientific Industries, Model 151) at 20 RPM and 4 °C. Filter at 4 °C with No.42 Whatman filter paper under a large beaker to limit evaporation. Obtain approximately 10-11 wt% haemoglobin.

Check the haemoglobin concentration using a Beckman D.B.G. spectrophotometer. Well made microcapsules require at least 10 wt% haemoglobin. Standard and Reagent Kits composed of Cyanomethyl haemoglobin are made by Fisher Diagnostic Grade.

(*) For nylon microcapsules Polyethylene Imine (PEI) is used instead.

(#) Add 6.05 grams of TRIS (American Chemicals) to 300 mls of deionized water (pH 10.6). The pH is adjusted to 8.0 using 0.2

molar HCl. The optimum pH is dependent upon the enzyme being used. The final solution is made up to 500 ml with deionized water.

b. Water Saturated Ether (WSE)

Mix 400 ml of anhydrous ether (Anachemia Grade) and 100 ml of deionized water in a separatory funnel for a few minutes. Cautiously reduce the pressure and release the vapour contents into the fumehood after each agitation. Repeat until the pressure is negligible. Let settle and discard the bottom aqueous layer. Store the WSE @ 4 °C in a dark bottle. Discard when yellow.

c. Collodion

Clean and dry a stainless steel tray to a smooth and shiny surface. Remove any static electricity from the tray using an antistatic gun (Zerostat, Discwasher Inc.). Evenly spread 100 ml of collodion solution (Baker Chemicals) on the tray and set it in the fumehood to dry until a thin sheet of cellulose nitrate has detached itself from the tray. Dry the sheets further in an open beaker for at least 24 hours.

Always handle cellulose nitrate with clean and dry hands. Weigh 8 grams into a 250 ml Erlenmeyer flask. Add 105 ml of anhydrous ether (Anachemia Grade) and 35 ml of absolute ethyl alcohol (Commercial Alcohols, Montreal). Add an additional 60

ml of ether dropwise (1 drop per second) from a 60 ml air tight separatory funnel. The resulting collodion solution should be completely clear with no cellulose nitrate precipitate. Store the solution at 4 °C in an aluminum foil or brown bottle.

d. Butyl Benzoate (Kodak Chemicals)

Store the as received Butyl Benzoate reagent in a brown bottle.

e. 50% Tween 20

Mix 400 ml of Tween 20 (Fisher Scientific) with 400 ml of deionized water. Store the solution @ 4 °C.

f. 1% Tween 20

Mix 10 ml of Tween 20 with 990 ml of deionized water. If used for storing microcapsules add 9 grams of NaCl.

Procedure

Collodion microcapsules containing haemoglobin stabilized urease enzyme were prepared according to a modification (Chang, 1985) of the original method (Chang, 1964). The preparation is carried out at 4 °C. Stirring is done on a Jumbo Magnetic Stirrer (Fisher Scientific) at 100 RPM. The RPM is indicated by a digital readout device (Scie-Bec

Electronic, Montreal) as shown in figure 2.1. The stirring bar should preferably be of cylindrical shape and without edges. Sharp edges cut the delicate surface of the freshly prepared collodion microcapsules resulting in a premature release of haemoglobin and a leakage of enzyme. The preparation is as follows:

a) Dissolve 15 milligrams of Jack Bean Urease enzyme (49 units per milligram specific activity, Worthington Grade) with 7.5 ml of haemoglobin solution in a 600 ml beaker.

b) Add 54 ml of water saturated ether (WSE) containing 1% SPAN 85 (Athemix Chemicals). Stir for three seconds at 100 RPM. Immediately add 63 ml of collodion solution while stirring at the same RPM for an additional 2 minutes. Stop stirring and seal the beaker with laboratory film (Parafilm) to minimize ether evaporation. Let sit for another 45 minutes. Now a firm collodion layer is forming around each microcapsule.

c) Remove the ether/collodion supernatant without moving the beaker from on top of the stirrer. Use a large 100 cc syringe to remove the bulk and a smaller disposable pipette for the rest of the supernatant. Add 63 ml of Butyl Benzoate containing 0.63 ml (1%) SPAN 85, then stir at 100 RPM for an additional 2 minutes. Let sit for 45 minutes uncovered.

d) Remove the supernatant as per step c). Add 63 ml of 50% Tween 20 and stir for 1 minute. Add 63 ml of deionized water and continue stirring at the same RPM for an additional 3 minutes.

e) Remove the supernatant as per step c). Wash the microcapsules with 1% Tween 20 until the supernatant is clear and there is no more smell of Butyl Benzoate. Store the microcapsules in saline and 1% Tween 20.

Note: Step b) is the critical step. It is important to stir gently. Avoid moving the beaker containing the newly formed microcapsules unnecessarily. The use of a glass pipette connected to a suction flask may help. In addition, the suction flask serves as a temporary waste container for all the discarded supernatant. In step c) Butyl Benzoate fixes the collodion permanently so that the capsules become more firm and durable.

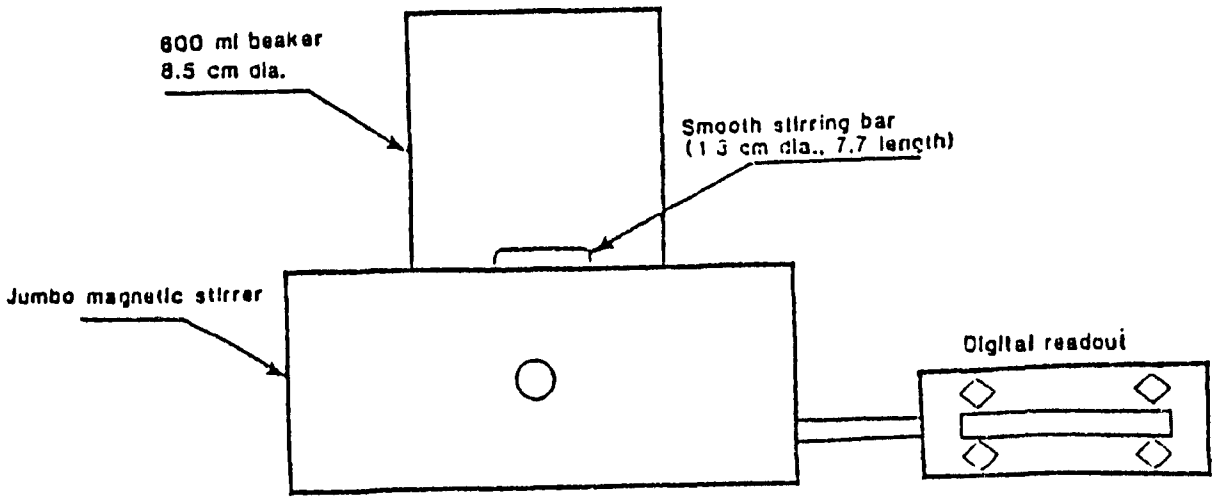



Figure 2.1. Microcapsule Apparatus

## 2.5.2. Other microencapsulation methods

A variety of polymers, including alginate, k-carrageenin, collodion and cellulose propionate were tested for microencapsulating the urease-zeolite adduct (ZWU).

### Alginate

Alginate powder (Keltone HV) was kindly supplied by Kelco (Kelco Specialty, Colloids Ltd., Toronto, Canada). Prepare a 3% wt/wt solution of alginate in deionized water. Suspend 0.5 grams of ZWU in 10 ml of alginate solution. Spherical droplets of this suspension are formed by using an air jet-syringe pump droplet generator (figure 2.2). With this apparatus, the ZWU-sodium alginate suspension is extruded through a needle located inside a sheathed tube through which air flows at a controlled rate. As the extrudate is forced out of the end of the needle by the syringe pump, droplets are pulled off by a rapidly flowing air stream. The droplets gel instantly upon contact with a 1.2 wt% CaCl2 solution. The air flow is adjusted to form spherical microcapsules of relatively large size (500 $\mu$m). Unfortunately, the alginate microcapsules have too much material and therefore are not suitable for use as an oral sorbent. However, the alginate microcapsules can be placed in an external shunt to function as a haemoperfusion device. The large size microcapsule can reduce pressure drop across the chamber to negligible levels (unpublished results).

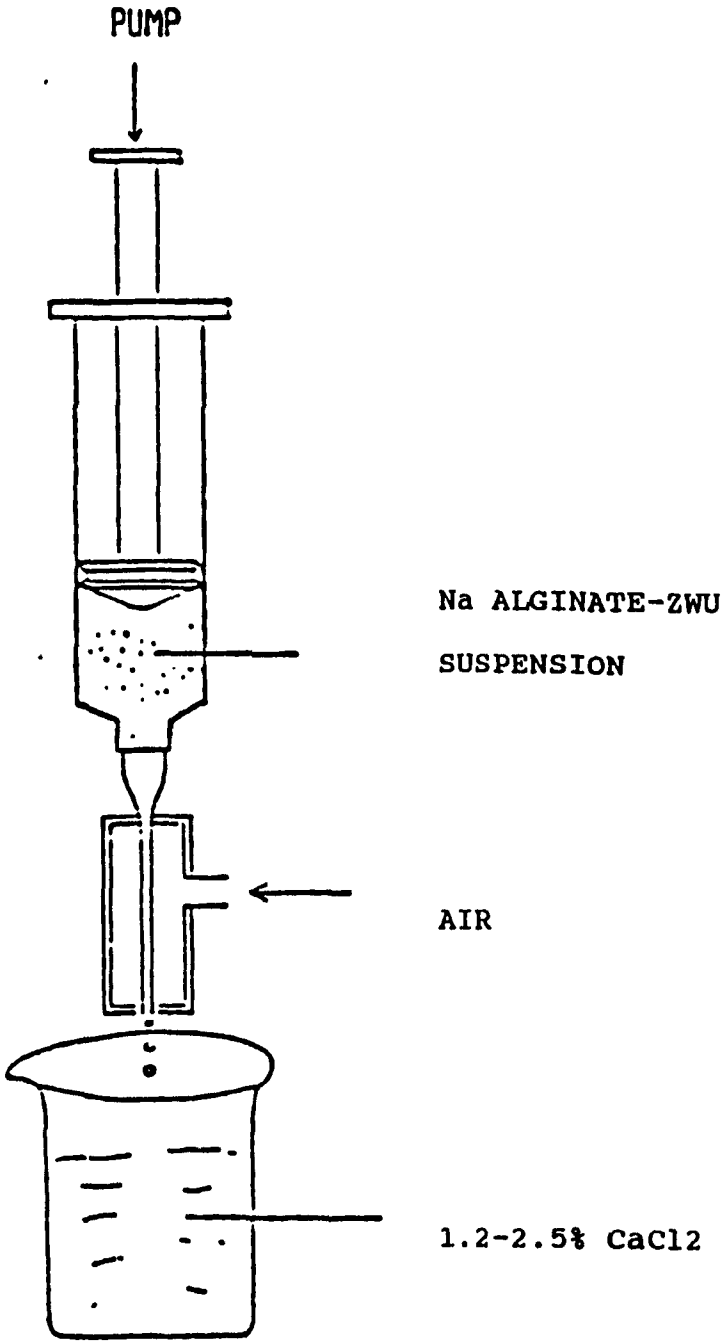



Figure 2.2 Microdroplet forming device

k-Carrageenin

Another bio-polymer, k-Carrageenin, dissolves in saline (0.9 g/dL NaCl). Therefore, k-carrageenin microcapsules could not be used since saline is one medium for testing in vitro. Body fluid also contains 0.9 g/dL of NaCl.

Cellulose Propionate and Collodion

In the use of organic polymers the extremely hydrophillic nature of the zeolite is a limiting factor for finding suitable solvents and non-solvents. Cellulose propionate and collodion could not form stable membranes. For example, collodion added to a suspension of urease-zeolite adduct in ether does not form a stable microcapsule membrane. Unlike urease-collodion the ZWU-collodion microcapsules rupture immediately when immersed in water. In the case of cellulose propionate it was difficult to find the proper non-solvent to precipitate the membrane. Neither hexane nor toluene could be used as non-solvents.

## 2.5.3. Cellulose Acetate Butyrate Sorbent Microcapsules

Gardner was the first to use cellulose acetate butyrate polymer for microencapsulating urease-silica adduct (Garner, 1971). After several attempts we succeeded in microencapsulating urease-zeolite adduct by modifying Gardner's method. The orthogonal method (Kang Fu Gu, 1988) was used to optimize the microcapsule preparation. The variables studied were: the stirring speed, the amount of urease, zeolite, phosphate buffer, polyethylene imine and the application of membrane coatings. The following is an account of the method for producing CAB microcapsules as a result of such optimization studies.

### Microcapsule Preparation

Microcapsules containing the sorbent were made by the phase separation technique (See Literature Section):

a) Sorbent Emulsification

Three millilitres of phosphate buffer (pH 7.5, 1/15 molar), and 50 mg of polyethylene imine (PEI) are added to the ZWU preparation. The sorbent becomes gel-like in appearance. It is then emulsified in 10 ml of an organic polymer solution - 2.5% Cellulose Acetate Butyrate (CAB) (Aldrich) dissolved in 1.5/1 v/v methylene chloride/toluene solvent - at room temperature. The emulsion is in a 25 ml beaker. Stirring is done on a Jumbo Magnetic stirrer at 600 RPM.

b) Membrane Formation

Eight millilitres of toluene are placed in a 30 ml separatory funnel fitted on top of the 25 ml beaker containing the emulsified solution. Toluene non-solvent is added dropwise to precipitate CAB onto the sorbent microdroplets. It is important to add the toluene non-solvent slowly to prevent a sudden precipitation of polymer mass. Five to six drops of toluene per minute is acceptable. At the end of the toluene addition remove the methylene chloride/toluene supernatant with a disposable pipette attached to a suction flask. The microcapsules are then dried overnight under vacuum @ 4 °C to remove extra solvent (crenated microcapsules). The microcapsules can be stored in a sealed jar and used within days of the preparation.

The crenated microcapsules were immersed in distilled water. They were then observed under light microscopy. The microcapsule's sphericity and an average mean diameter were recorded for further analysis.

Important points worth noting are:

1) High stirring speeds maximize the distribution of non-solvent. This avoids the formation of clumps of CAB polymer at the air-liquid interface.

2) High speeds promote the formation of smaller microcapsules which have larger surface area, hence greater reaction rates.

3) The addition of ether as a final step to strengthen the

membrane, as part of Gardner's method (Gardner, 1971), is not part of this procedure. We obtained sufficiently strong membranes after the final drying step. In addition, we wanted to avoid the use of solvents that could inactivate enzyme function, such as ether.

4) The addition of PEI was found to greatly enhance the formation of good microcapsules. The amino groups of polyethylene imine help in the formation of good membranes (Kang Fu Gu, private discussions). The same principle applies to amino groups from protein, such as haemoglobin. The addition of haemoglobin instead of polyethylene imine gave similar results. Polyethylene Imine and haemoglobin are normally used to stabilize enzyme preparations within microcapsules. Haemoglobin is used for collodion microcapsules while haemoglobin or PEI is used for nylon microcapsules. The choice between amino carrying compounds and microencapsulated system is usually a function of the level of purity desired. This is essential in the case of multienzyme systems containing cofactors where polyethylene imine is employed (Grunwald and Chang, 1981).

## 2.6. The addition of coatings

Now that urea sorbent microcapsules have been made we decided to focus on the membrane to improve upon the ammonium capacity. Unfortunately, the membrane by itself did not appear to function as a permselective membrane, despite claims to the contrary (Gardner, 1975). At this point we decided to coat the microcapsules with compounds that could slow down the outward diffusion of ammonium ions. Polyethylene oxide, polyethylene imine, gelatin and lipids were applied as coatings to the CAB microcapsule membrane. Lipid coating (Yu and Chang, 1981) will be explained further to illustrate the general procedure.

A mixture of lecithin and cholesterol in tetradecane -0.7 grams of lecithin and 0.43 grams of cholesterol in 100 ml of tetradecane- is added to the CAB microcapsules in a 200 ml Erlenmeyer flask and the suspension is slowly rotated overnight @ 4 °C on a Fisher Multipurpose rotator. Unfortunately, after many unsuccessful trials at different lecithin to cholesterol ratios, the lipid could not be made to adhere to the microcapsule membrane.

### Strong base type membranes

In a different approach Gottlieb had shown that soluble electrolytes could be introduced and trapped permanently into a cellulose membrane by using an "impregnation" method

(Gottlieb, 1959). He used the positive electrolyte poly-2-vinyl-N-methylpyridinium bromide (2-PVMP) in the production of positively charged anion-exchanger membranes. We opted for methyl iodide instead of bromide to avoid the use of hazardous gases. The preparation of the 2-PVMP electrolyte follows a modified version of Gottlieb (1959) and Gauthier (1985).

Reagents

2-Vinyl Pyridine

Distillation at reduced pressures is carried out with a set-up as shown in figure 2.3. Always wear safety glasses. Examine the glassware carefully to avoid implosions. Lubricate and seal all glass joints against air leaks. A "cow" was used to collect the intermediate fraction of vinylpyridine.

The distilling flask should not be more than half full. The flask in the heating oil bath should be immersed to a depth below the level of the liquid in the flask to avoid bumping problems. A steady stream of argon was allowed to leak through a glass capillary under the surface of the liquid. The argon stream is helpful in reducing the splashing and splattering that inevitably occurs during the course of the operation. The pressure is reduced with a water aspirator before heating is begun.

Leave the release valve open upon evacuation. Then slowly close the release valve. If the liquid contains small quantities of low boiling components, foaming and bumping will almost certainly occur. To avoid it, reopen the release valve

until the foaming abates. A 30 mm reduced pressure was obtained with a glass water aspirator. The pressure is read with a special mercury manometer (Fisher).

In vacuum distillation it is essential to maintain a steady boiling. When the surface is quiet and the system is fully evacuated begin the heating of the oil bath. Maintain a bath temperature as low as possible to provide a controlled rate of distillation. Maintain a slow but steady distillation rate to avoid great differences in pressure between the manometer reading and the reaction vessel. Avoid superheating the vessel by maintaining an oil bath temperature no more than 15-25 °C higher than the head temperature.

The "cow" is an helpful addition to the standard vacuum apparatus since the proper fraction can be collected without disrupting vacuum. In our experiment 2 collecting flasks were used. One is used to collect the first 10% of the distillate containing the low boiling components to discard. The other is to collect the intermediate 80% 2-vinylpyridine fraction. This fraction will be used for subsequent polymerization. The liquid remaining in the distilling flask, containing the high boiling waste and very dark in colour, is also discarded.

Stop the distillation before the reaction vessel becomes completely dry. Without absorption of heat due to vaporization the flask temperature can rise dramatically. Many liquids contain peroxides which become concentrated in highly explosive residues.

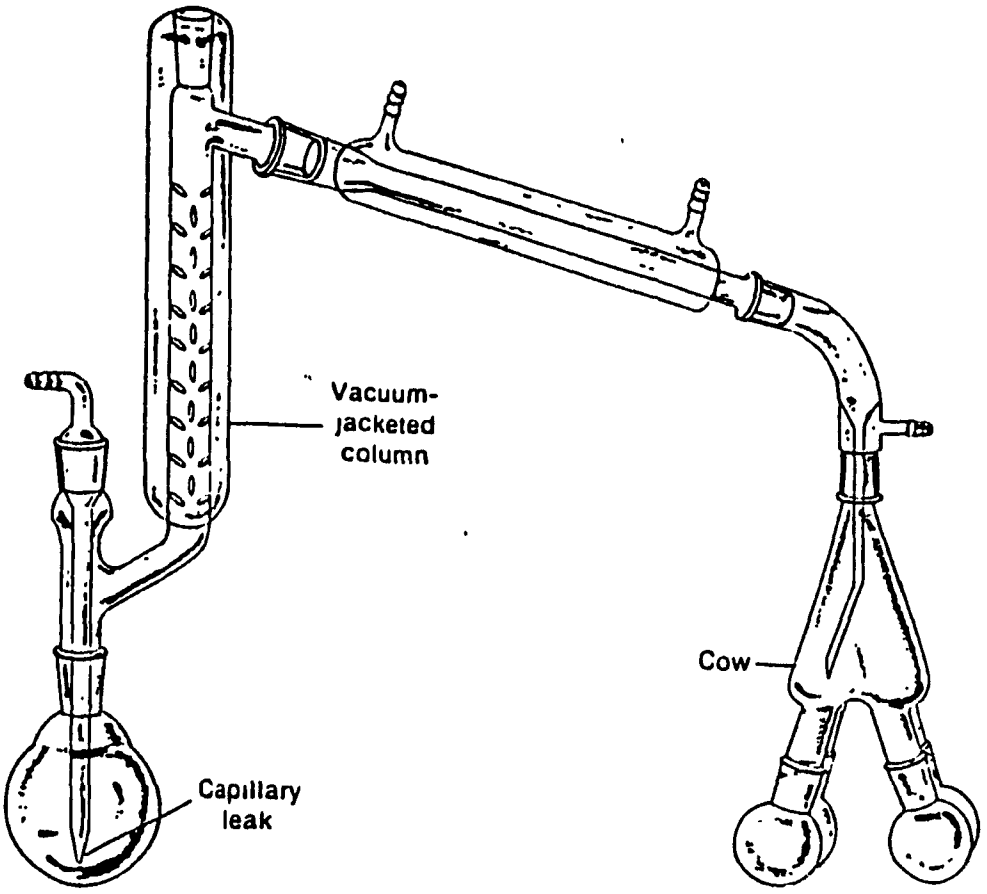



Figure 2.3. Vacuum distillation set-up

Preparation

Dissolve one hundred millilitres of the freshly distilled 2-vinylpyridine in 200 ml of 15% aqueous HCl. Add one gram of ammonium persulfate catalyst and one millilitre of ethyl mercaptan modifier, the latter to obtain polymers of molecular weight sufficiently low for our purpose. The polymerization mixture is kept under argon at 50 °C for 48 hours. The resulting solution is diluted with an equal volume of water.

2N NaOH is added with stirring until the mixture is alkaline. The white gummy mass which separates out is washed with water, redissolved in 1N HCl, filtered and reprecipitated with 2N NaOH under vigorous stirring to yield a finely divided precipitate which is removed from the solution as it is formed. The purified polyvinylpyridine is finally dried under vacuum at room temperature.

The quaternization of polyvinylpyridine is done by a method similar to Gauthier (1985). Add a 10-fold excess of methyl iodide (Warning: cancer causing chemical -extremely toxic) to a 5% solution of polyvinylpyridine in dry dimethylformamide (DMF). The solution is refluxed overnight under nitrogen. If you observe clouding of the solution add absolute ethanol to redissolve any polymer precipitated during the quaternization. The solution, together with a small amount of white precipitate which formed, is poured into 100 parts of rapidly stirred dioxane. The resultant gummy product is redissolved in methyl alcohol, reprecipitated in dioxane, washed with ether, and dried in vacuo.

FTIR analysis was used to determine the extent of quaternization. Films of unquaternized polymer could easily be made with a tool and dye heated press. For the quaternized polymer the KBr method was successful in producing adequate films.

## 2.7. Urea and Ammonium Analysis

Urea and ammonium concentrations were measured with a Technicon Autoanalyzer. The autoanalyzer is used not only to measure $NH_4^+$ and urea, but also other substances such as calcium, sodium, glucose, etc.. The autoanalyzer is a completely automated process that pumps samples and reagents, mixes them, dialyses, heats and finally detects colour in a colorimeter and the results are visualized on a strip chart recorder. Ammonium reacts with sodium phenoxide in the presence of hyperchlorite and nitroprusside to yield a stable blue colour. Urea reacts with diacetyl monoxime in the presence of thiosemicarbazide (TSC) under acidic conditions. TSC intensifies the colour reaction of the products without the need of concentrated acid reagents.

a) Apparatus (see manual for mechanical details)

b) Principle of Operation

Run deionized water through all tubing. Dip the reagent tube into the appropriate reagent bottle, eg. NaOCl, Borate, etc.. Run the reagents for 10-15 minutes until the bubble flow is

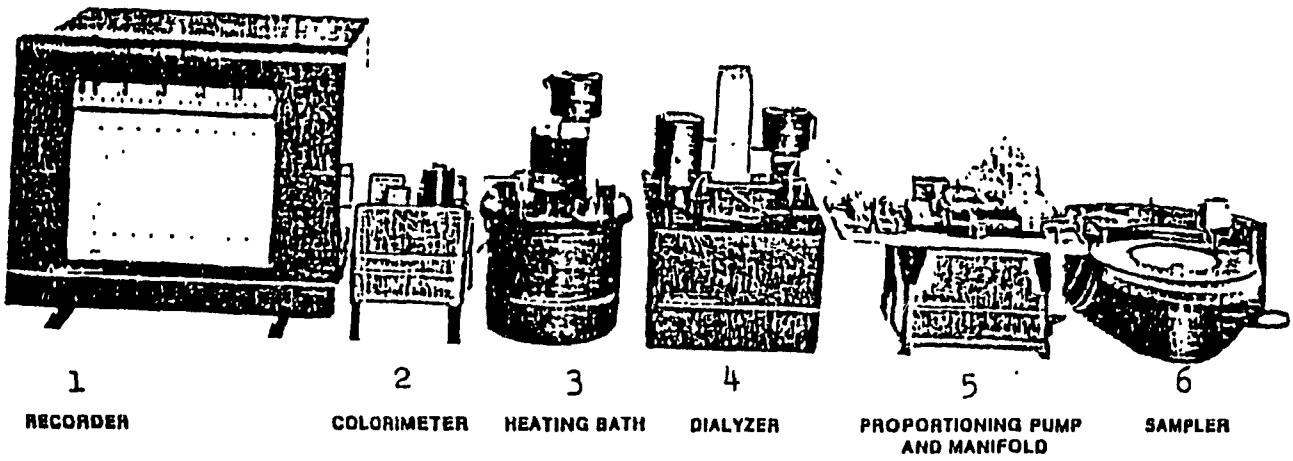



Figure 2.4. The Technicon Autoanalyzer with the six modules interconnected by plastic tubing. The modules are identified as: 1.Recorder. 2.Colorimeter. 3.Heating Bath. 4.Dialyser. 5.Proportioning Pump. 6.Sampler.

uniform throughout, especially out of the colorimetric flow cell. Pinch the flow cell exit tube to free air or trapped dirt. Set the recorder baseline at 100% Transmittance, then block the light beam to adjust for 0% Transmittance.

Run the standards before the samples, with the standard

concentration range as dictated by the sample concentration range. For example, if the predicted sample concentration is around 5 meq/l then 1,2,4,6,8 and 10 meq/l standards could be used to obtain a proper calibration plot.

Run 2 cups for each standard separated by a cup of deionized water. Centre the sampler needle to properly withdraw from the cups. Wait 10-15 minutes for the standard elution curves to appear on the chart recorder. After the last elution curve stop using the reagents. Pass deionized water for 10 minutes and air for 10 minutes in sequence through all tubing. Turn off the recorder, colorimeter and pump. Open the gear pump and release the tension from the plastic tubing.

c) Ammonium Test

The automated procedure for the determination of ammonium ion is based on the Berthelot reaction: the formation of a blue coloured compound (endophenols) occurs when a solution of an ammonium salt is added to sodium phenoxide (phenol) followed by the addition of sodium hypochlorite. A solution of sodium borate (borate buffer) is added to the sample stream to eliminate the precipitation of the hydroxides of calcium and magnesium. A flow diagram of the autoanalyzer for ammonium is shown in figure 2.5.

Operating Conditions

-colorimeter filter at 550 nm, aperture #2

-water bath @ 60 °C

-sampler speed at 30 samples per hour, 1/2 wash ratio

Standards

Prepare a set of 6 ammonium chloride standards (eg. 0.5, 1, 2, 4, 6, 8 meq/l ) in phosphate buffer. For calibration use 2 cups per standard and average out the elution curve values.

Reagents

- Phenol

Add 44.4 ml of 90% liquid phenol (Fisher Scientific) and 0.4 grams of sodium nitroprusside to a 2 litre volumetric flask. Make up to 2 litres with deionized water. Stir at high speed with the magnetic stirrer, then filter by vacuum using No.42 Whatman filter paper. The solution can be stored for 3 to 4 weeks.

- Sodium Hypochlorite

Add 14.4 grams of sodium hydroxide pellets and 40 ml of 5% sodium hypochlorite (Javel, Fisher Scientific) to a 2 litre volumetric flask. Use Javel only if it is yellow in colour. Fill with deionized water. Stir until all has dissolved. Filter by vacuum using No.42 Whatman filter paper.

-Borate Buffer

Mix 2 litres of solution A, 400 ml of solution B and 1600 ml of deionized water. Adjust to pH 8.5 by adding extra solution

B. Vacuum filter with No.42 Whatman filter paper. Conserve at 4 °C. Store the solution for 2-3 months.

Solution A: Add 4.0 grams of potassium chloride (KCl) and 12.4 grams of boric acid ($H_3BO_3$) to 2 litres of deionized water.

Solution B: Add 4.0 grams of sodium hydroxide (NaOH) pellets to 1 litre of deionized water.

- Water (+)

  Use 500 ml of deionized water with 0.5 ml of BRIJ reagent (BRIJ-35, Fisher Scientific, Technicon No.T21-0110).

- Water (-)

  Deionized water

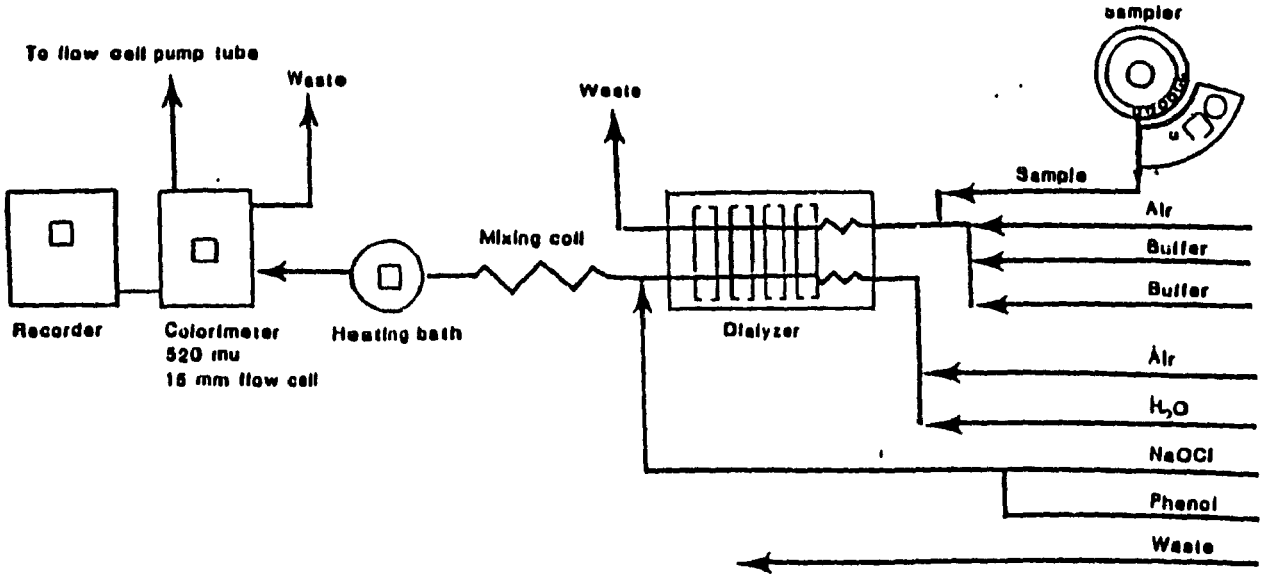



Figure 2.5. Ammonium flow diagram (range 0.5-8 meq/l). Delivery volumes of reagents and samples are determined by the tube size through the proportioning pump.

d) Urea Test

Urea reacts with diacetyl monoxime in the presence of thiosemicarbazide (TSC) under acidic conditions. TSC intensifies the colour reaction. A flow diagram of the autoanalyzer for urea is shown in figure 2.6.

Operating Conditions

-colorimeter filter at 520 nm, aperture #2

-water bath @ 95 °C

-sampler speed at 60 per hour, 2/1 wash ratio

Standards

Prepare a set of standards consisting of 0.2, 0.4, 0.6, 0.8 and 1.0 mg/dl BUN. Fill and position the standards on the sampler wheel, 2 cups for each standard separated by a deionized water cup.

Reagents

-Saline

Add 9 grams of salt (NaCl) and 0.5 ml of BRIJ-35 to a one litre volumetric flask. Fill to the mark with deionized water.

-BUN Colour

The BUN Colour is available commercially (American Chemicals). The solution is composed of 67 ml of diacetyl

monoxime (25 g/l), 67 ml of thiosemicarbazide (5 g/l) and one litre of deionized water with 0.5 ml of BRIJ.

BUN Acid was also commercially available (American Chemicals). Its composition is 1 ml of stock ferric chloride - 85% phosphoric acid solution (15 grams of ferric chloride, 300 ml of 85 % phosphoric acid and 450 ml of deionized water) and 999 ml of 20% sulphuric acid.

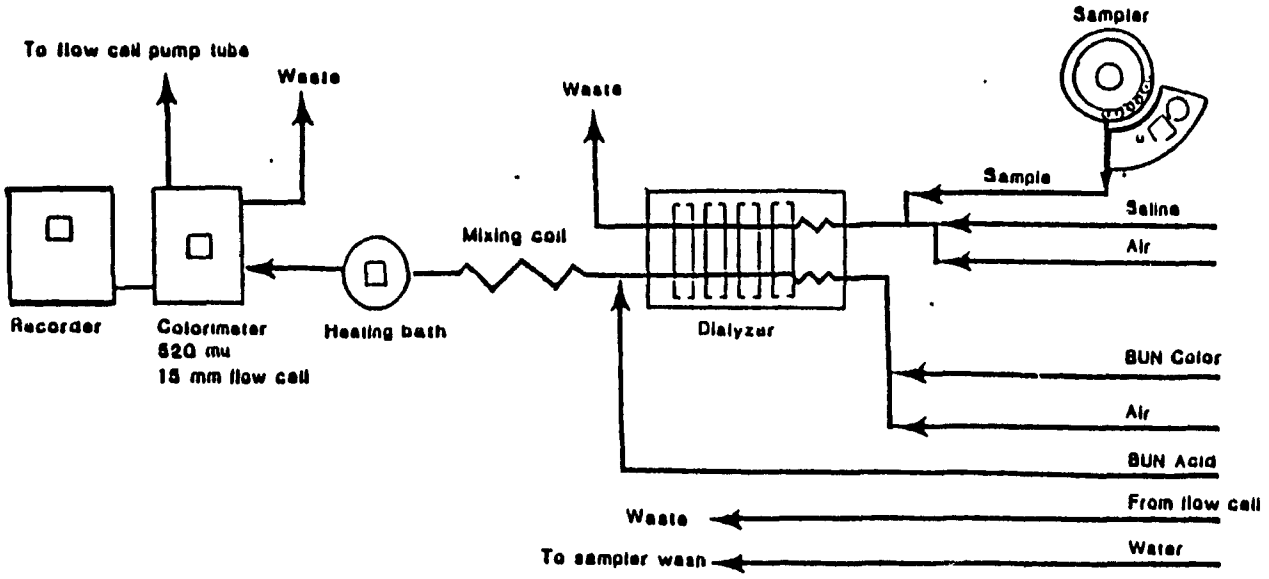



Figure 2.6. Urea flow diagram

## 2.8. In vitro studies

a) Zeolite kinetic studies

Add 0.5 g of dried zeolite powder to a 50 ml Erlenmeyer flask containing 15 ml of 71 meq/L ammonium chloride in deionized water, saline (175 meq/L NaCl) or intestinal solution. The simulated intestinal solution contains the following ions in addition to 71 meq/l $NH_4^+$: calcium 63 meq/l, magnesium 50 meq/l, potassium 90 meq/l and sodium 31 meq/l (Dempsey, 1958). Incubate at 120 RPM and 37 °C. Collect 1 ml samples at 2, 5, 10, 15, 20, 30, 60 minutes and 24 hours. Analyze for ammonium using the Technicon Autoanalyzer. Calcium is analyzed using a Multistat III Plus Autoanalyzer (see Instrumental Laboratory manual). Sodium and potassium analysis are done by standard mass spectroscopy.

b) Zeolite capacity

Add 0.5 g of zeolite to 15 ml of ammonium chloride solutions in sodium chloride while maintaining a constant total ion concentration of 0.2N. Equilibrate the samples for 24 hours in an incubator at 120 RPM and 37 °C. Measure the equilibrium ammonium concentration using the autoanalyzer. The zeolite capacity is computed as follows:

$$A = \frac{Co - C^*}{W} \times V$$

where

A = Zeolite capacity, meq NH4/g zeolite

Co = Initial ammonium concentration, meq/l

C* = Equilibrium ammonium concentration, meq/l

W = Weight of zeolite, grams

V = Volume of ammonium chloride solution, litres

c) Cellulose Acetate Butyrate (CAB) microcapsules

Cellulose acetate butyrate microcapsules formed from 0.5 grams of zeolite are added to 15 ml of 100 mg/dl BUN solution and incubated at 37 °C and 120 RPM for 24 hours. Samples are taken at 5 minutes intervals for 1/2 hour, then at 1 and 24 hours. Urea and ammonium concentrations are measured with the Technicon Autoanalyzer.

## 2.9. In vivo studies

Initially, young male white Sprague-Dawley rats weighing 250-300 grams were rendered uraemic by 5/6 nephrectomy using the arteriole ligation method. The rats are anaesthetized with an intra-peritoneal (ip) injection of 0.8 ml/100 g body weight somnitol (1,3,5 Sodium Pentobarbital, MCAT Manufacturing). Hair is shaved from the right lower side of the abdominal area. A 1 inch long incision through the skin and muscle

layers is made to expose the upper and lower intestinal tract, liver and right kidney. Saline is used to prevent drying of the exposed areas.

Multiple fat layers around the kidney and arterial branches are removed very delicately to avoid rupturing the small arterial and venous branches and the fine ureter at the end of the calyx. Three of the 5 visible arterial branches of the renal artery are ligated with surgical thread to obstruct blood flow to 2/3 of the right kidney. The ligated kidney will turn dark blue in the obstructed areas and remain deep red where blood is flowing, a distinction clearly visible to the naked eye (figure 2.7).

After ligation of the right kidney the muscle and skin incision ends are sewed and stapled back together. Sterile cat gut suture is used for closing the incision in the muscle layers. Silk surgical thread and/or metal staples are used for the skin layer.

The rat is returned to its cage and left there for seven days before operating the left kidney. This second operation is simpler because the ureter is very easy to locate and ligate. Tail blood samples can be withdrawn for BUN and ammonium analysis one day after.

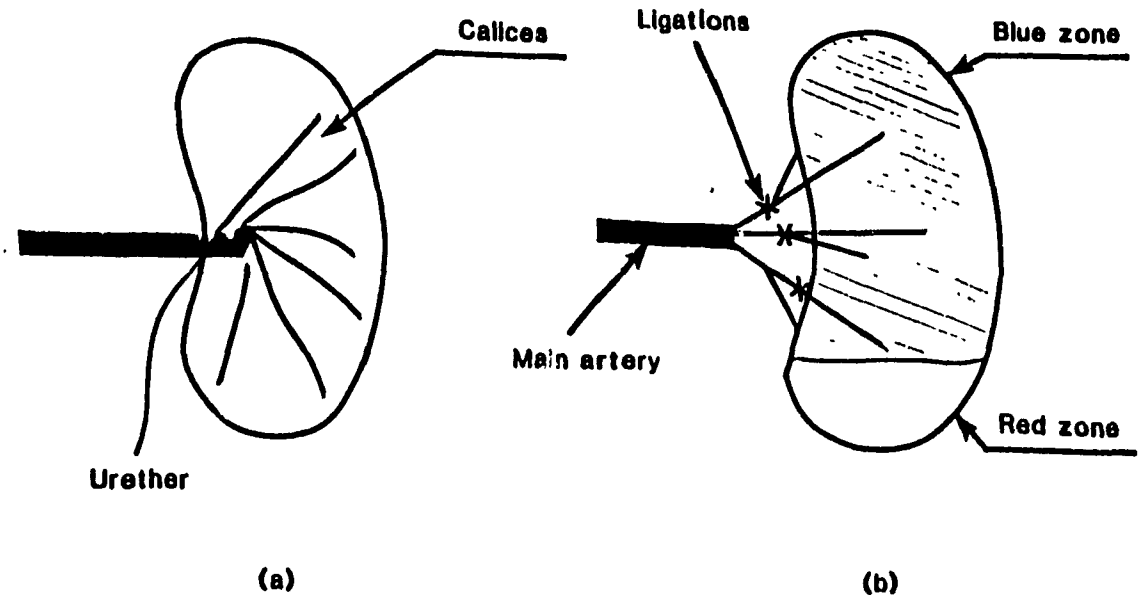



Figure 2.7 (a) The Kidney; (b) Ligation Targets

The success rate for the present operation is 50%, i.e. one of every two rats dies from complications. The average time spent to perform the surgical operation is 6 hours. The rat survival rate is one month after the surgical operation. Eventually, we ordered uraemic rats from Hilltop (Hilltop Laboratories, Scottdale, PA) since their uraemic rat model for chronic renal failure (Platt, 1952) was quite suitable for our work. The purchased uraemic rats were classified as

CVF (Certified Viral Free) male Sprague-Dawley rats weighing about 200 grams. Control rats were fed 20 grams of rat chow diet daily to maintain constant nitrogen intake. Their water intake was not measured nor limited. Treated rats were given an additional one gram daily of microencapsulated sorbent as solid and in liquid suspension form via a stomach tube. The liquid suspension consists of one part microcapsule, two part water mixture in a 3 ml total volume. In one set of experiments with collodion microcapsules the sorbent is administered for two to three consecutive days. The rats are then fed normally for another three days before treating again. This experiment will show any effect of the sorbent upon blood urea values. In experiments with solid microcapsules (crenated CAB microcapsules) the treated rat is fed the microcapsules indefinitely to test differences in survival rates versus controls.

One millilitre blood samples are taken at the same time of the day to minimize daily variations in BUN. The samples are left at room temperature for 30 minutes then centrifuged to separate plasma from red blood cells. The plasma is retained for further urea and ammonia analysis with the autoanalyzer as described previously. Any plasma sample not analyzed immediately is stored @ -20 °C.

# RESULTS

## 3. RESULTS

In 1970 Chang and Lo administered an inorganic ion exchanger zirconium phosphate to reduce urea levels in rats. One year later Gardner experimented on a urease-zirconium phosphate microcapsule "cocktail" to act as an oral sorbent to remove urea in dogs (Gardner et al, 1971, 1975). The urease-sorbent approach was able to reduce urea levels in patients (Kjellstrand et al, 1981). Unfortunately, the patients started complaining of nausea, hard stools, heart burns, hypertension and showing a negative calcium balance. Some of these complications may be due to the ion exchanger zirconium phosphate. Zirconium phosphate hydrolyses at intestinal pH and has a strong affinity for calcium ions. The present study in this thesis is to use a different ammonium adsorbent, a zeolite. Different approaches to immobilizing urease were also studied.

### 3.1 Zeolite ion exchangers

The disappearance of ammonium ions from a 15 ml solution containing an initial concentration of 71 meq/l ammonium chloride is shown in figure 3.1. Different zeolites such as zeolites X, Y and A were tested for their ability to uptake ammonium ions from deionized water. Their performance is very similar (figure 3.1a). In another experiment zeolites X, Y, A and zeolite W were placed in saline solution (0.9 gm/dL NaCl) (figure 3.1b). Clearly, zeolite W outperforms the other

zeolites in ammonium selectivity. The ammonium concentration is reduced to 15 meq/l whereas the other zeolites can only ion exchange down to 40 meq/l.

Unless stated otherwise all kinetics plots were done in a 50 ml Erlenmeyer flask with a zeolite concentration of 0.5 grams in 15 ml of solution incubated @ 37 °C and 120 RPM. The 37 °C temperature and the 120 RPM agitation values simulate intestinal conditions. The zeolite concentration corresponds to what may be present in the intestinal segment at any one time after ingestion. Assuming a daily intake of 200 grams of zeolite, say 50 grams four times a day, the concentration of zeolite in the intestine (the volume of the intestine is 1.2 L) would be 50 g/1.2 L = 41.6 g/L or 625 mg/15 ml or approximately the chosen 500 mg of zeolite/15 ml solution. The effect of zeolite concentration on ammonium uptake is shown in figure 3.2. Keeping the amount of zeolite constant at 0.5 grams, the effect of solution volume is shown in figure 3.3. Beyond 100 ml solution volume there is negligible difference in ammonium uptake.

The effect of contact solution is shown in figure 3.4 for ammonium selective zeolites F and W. The simulated intestinal solution contains the following ions in addition to 71 meq/l $NH_4^+$: calcium 63 meq/l, magnesium 50 meq/l, potassium 90 meq/l and sodium 31 meq/l (Dempsey, 1958). There is no significant

difference between plots for both zeolites, saline providing a slightly better rate of uptake for zeolite W.

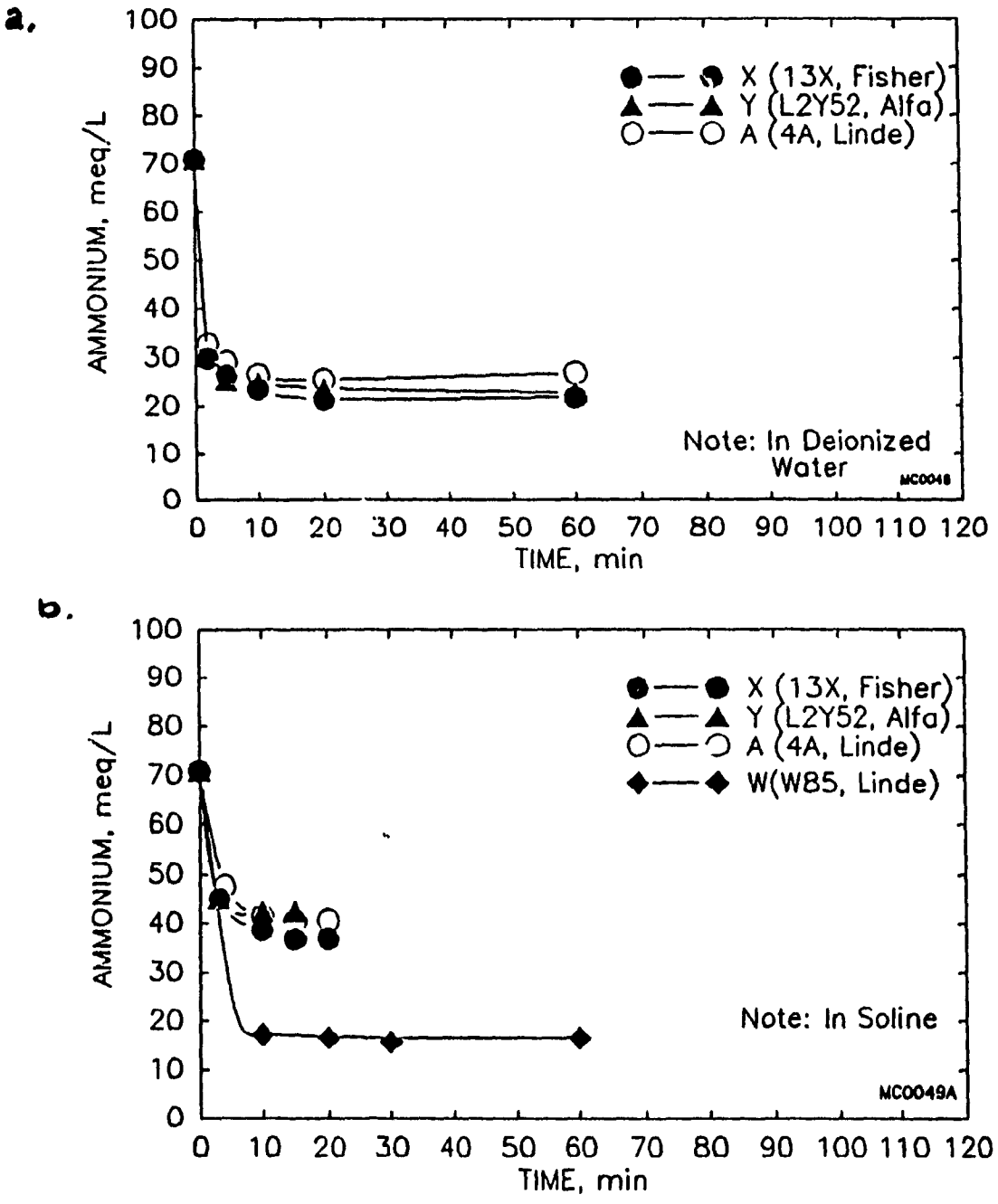



Figure 3.1. ADSORPTION RATES FOR ZEOLITES X, Y, A AND W.

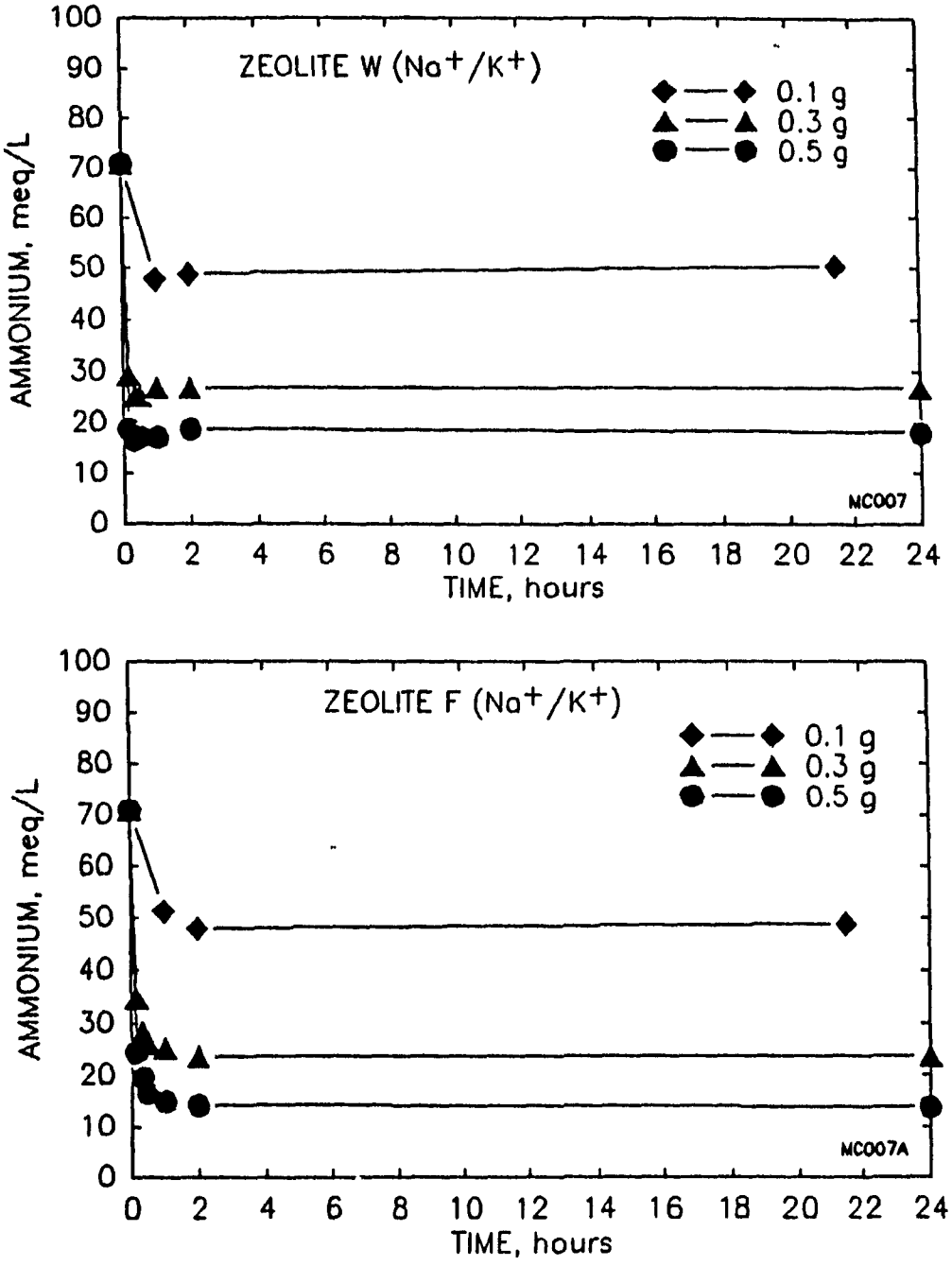



**FIGURE 3.2. ZEOLITE CONCENTRATION EFFECTS. Add indicated amount of zeolite to 15 ml of ammonium chloride solution ($NH_4Cl$, 71 meq/L initial concentration) in saline (NaCl, 0.9 g/dL). Incubate at 37 °C and 120 RPM.**

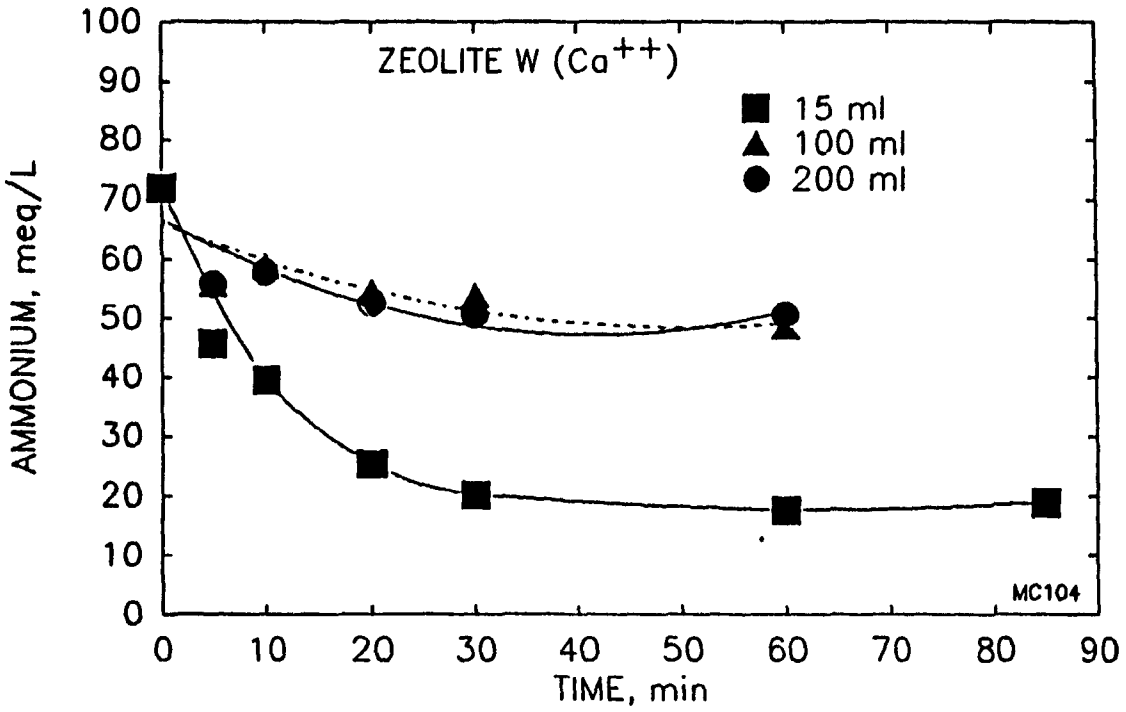



**FIGURE 3.3. SOLUTION VOLUME EFFECTS. Add indicated volume of solution to 0.5 g of zeolite W in the Calcium form. Incubate at 37 °C and 120 RPM.**

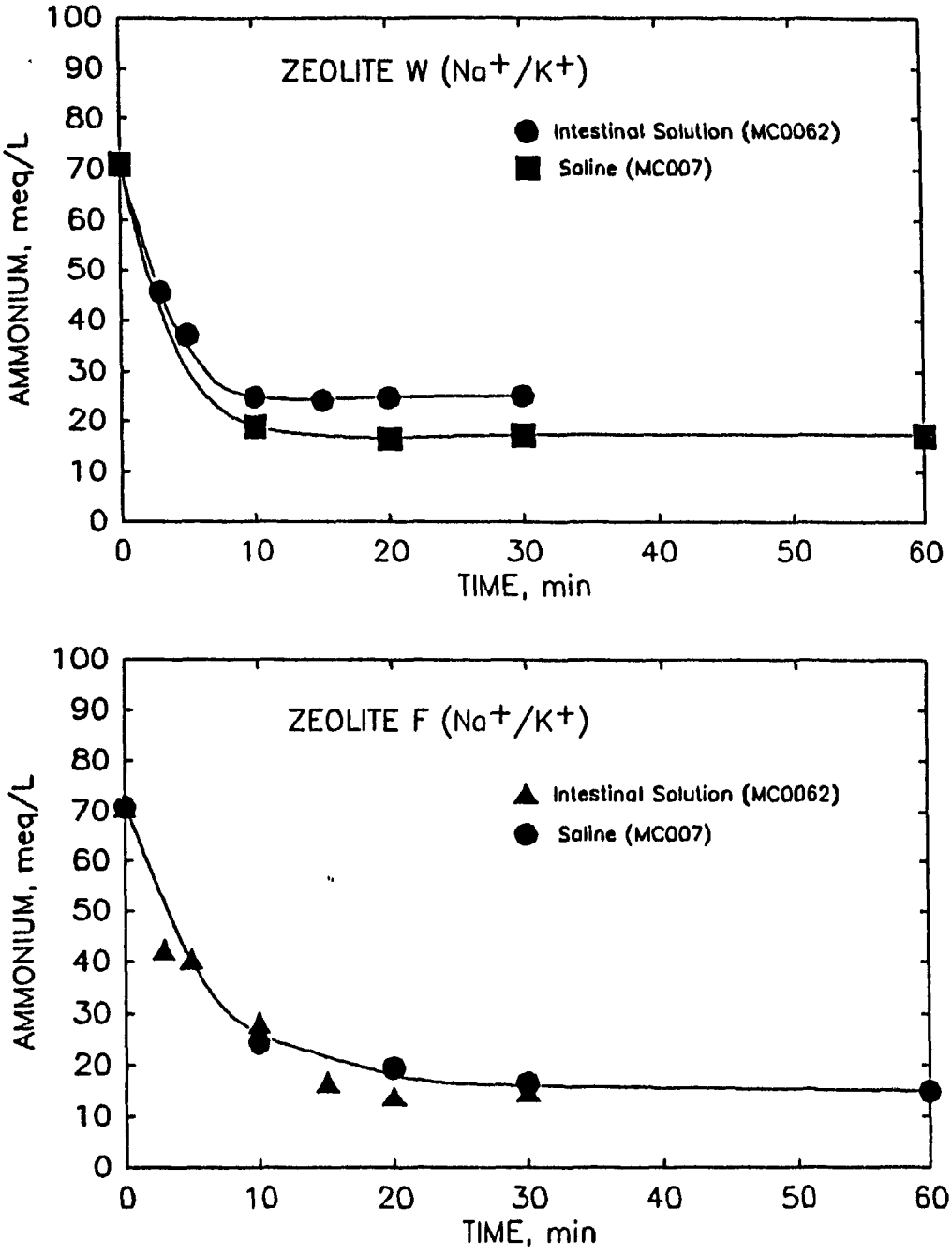



**FIGURE 3.4. SOLUTION COMPOSITION EFFECTS. Equilibrate 0.5 grams of zeolite W (figure 3.4a) and zeolite F (figure 3.4b) with intestinal and saline solutions at 37 °C and 120 RPM.**

In figure 3.5a the ammonium selective zeolites F and W are compared against zirconium phosphate (ZP) for ammonium removal. Evidently, ZP takes much longer than the zeolites to arrive at the same equilibrium value. Figure 3.5b is a magnification of figure 3.5a to show relative rates of uptake. Zeolites reach equilibrium in minutes versus hours for ZP.

The ammonium kinetics of both zeolites F and W in the calcium form are shown in figure 3.6. The break in the curve around 50 meq/l $NH_4^+$ shows that ammonium has difficulty exchanging and that calcium is held tightly within zeolite F (figure 3.6a). On the other hand, zeolite W's higher ammonium selectivity favours ion exchange with ammonium regardless of calcium. The ammonium uptake curve follows a smooth downward path (figure 3.6b). Calcium, potassium and sodium were measured as well as ammonium concentrations to provide a check on a total mass balance. This is validated since the total ion concentration equals the initial ammonium concentration of 71 meq/l.

Ammonium capacity curves (isotherms) for zeolite W are shown in figure 3.7. The plots in these figures differ in the severity of ion exchange with NaCl, prior to ammonium exchange. Zeolite W is available in a 50/50 $Na^+/K^+$ powder form. In run MC144 the zeolite was tested "as is", without prior $Na^+$ exchange, and the zeolite displayed a maximum ammonium capacity of 2.6 meq/g dry zeolite.

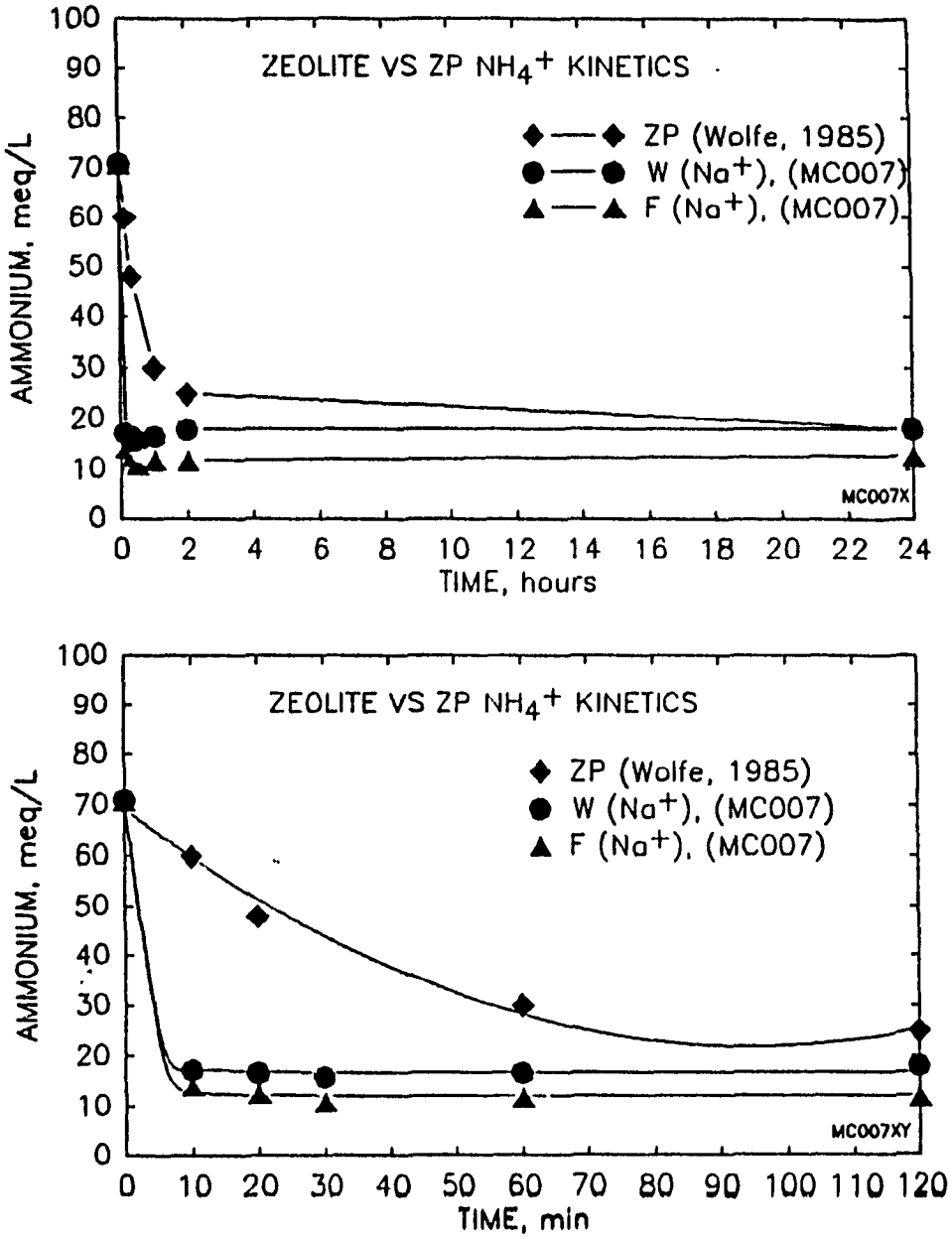



**FIGURE 3.5. ADSORPTION RATES FOR ZEOLITES F, W AND RESIN ZIRCONIUM PHOSPHATE. Add 0.5 grams of adsorbent to 15 ml ammonium chloride solution ($NH_4Cl$, 71 meq/L initial ammonium concentration) in saline (NaCl, 0.9 g/gL). Incubate at 37 °C and 120 RPM.**

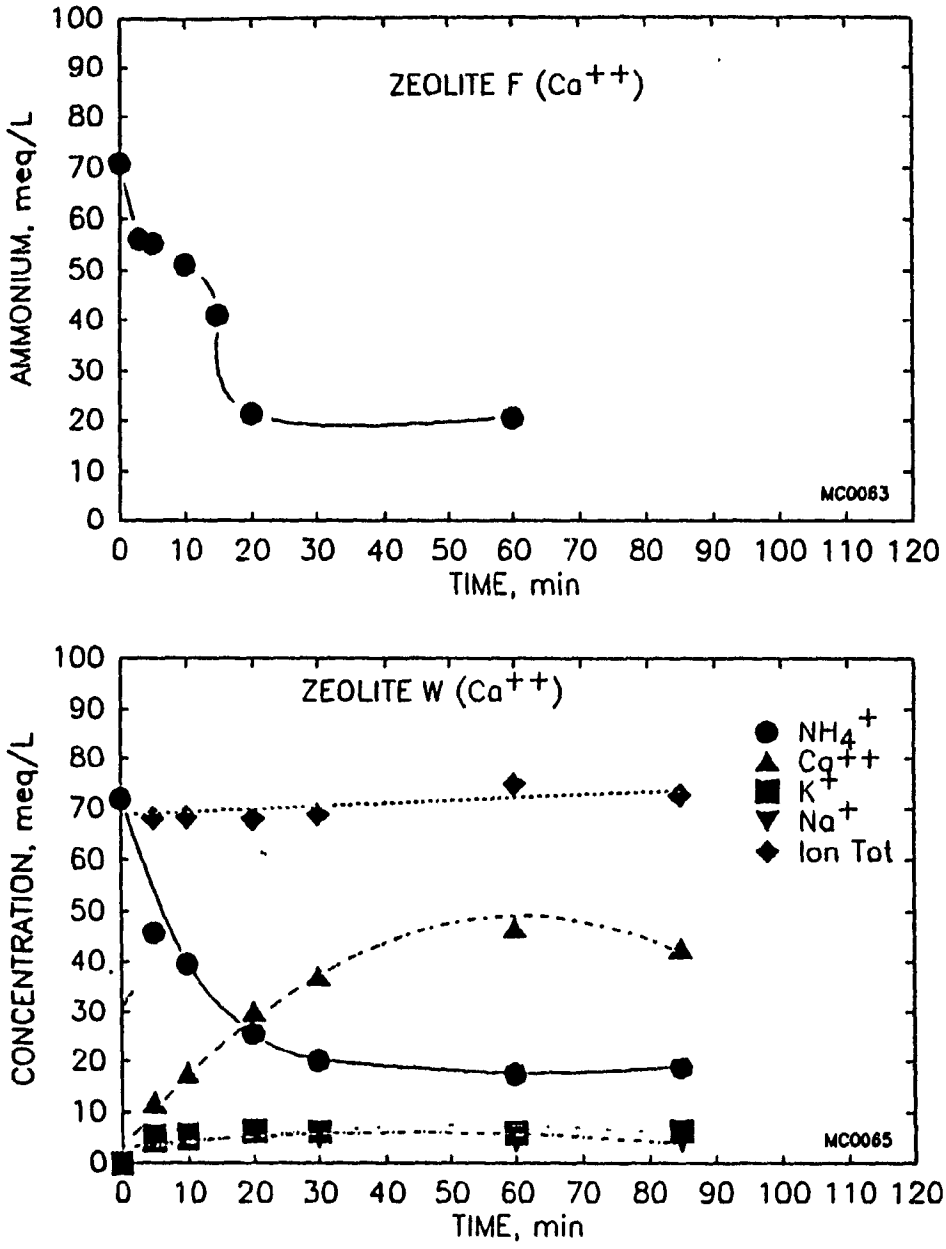



**FIGURE 3.6. CALCIUM ION EXCHANGE EFFECTS ON ADSORPTION RATES.** Add 0.5 grams of zeolite F (figure 3.6a, top) or zeolite W (figure 3.6b, bottom) both in the calcium form to 15 ml ammonium chloride solution ($NH_4Cl$, 71 meq/L initial concentration) in deionized water. Incubate at 37 °C and 120 RPM.

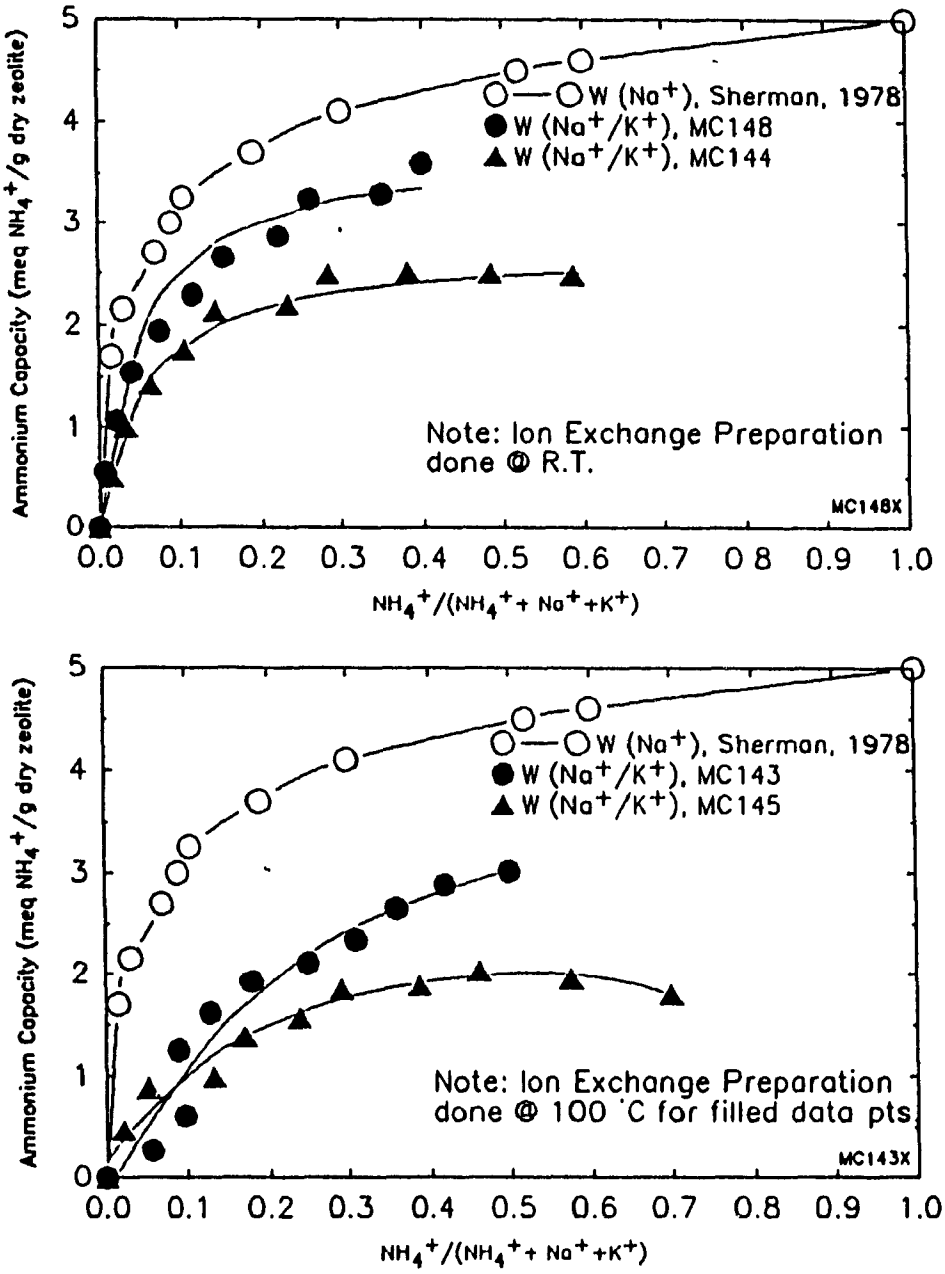



FIGURE 3.7. AMMONIUM ISOTHERMS FOR ZEOLITE W. Equilibrate 0.5 g of zeolite in 0.2 N solutions. The zeolite exchange with $Na^+$ was done at R.T. (figure 3.7a) and 100 °C (figure 3.7b).

The selectivity series for zeolite W is as follows:

$$Mg^{++} < Ca^{++} \approx Na^{+} << NH_4^{+} < K^{+}$$ (Sherman, 1978)

Ion exchange of potassium with sodium prior to ammonium exchange should improve the ammonium capacity of zeolite W since the zeolite is less selective for sodium. J.D. Sherman of Union Carbide converted zeolite W to the $Na^{+}$ form after ion exchange with 60 litres of 0.1 N NaCl down a column packed with zeolite-additive pellets (top curve -empty circles, Sherman, 1978, figure 3.7). In run MC148 10.4 g of zeolite W were equilibrated with 100 ml of 2N NaCl and stirred for half hour at room temperature, then filtered. This was repeated ten times. As shown in figure 3.7a the ammonium capacity for the MC148 sample is not as high as Sherman's. We obtained a maximum capacity of 3.6 meq/g whereas Sherman's value approaches 5.0. Evidently, sodium ion exchange in a column is more efficient than batch. Unfortunately, we were dealing with fine zeolite powder (1-20 $\mu$m) which is difficult to ion exchange in a column and we did not want to contaminate the zeolite with additive to form pellets. Pellets or bigger zeolite particles are necessary to minimize pressure drops in an ion exchange column.

We can obtain a simple relationship for the ion exchange reaction:

$$A_s + B_z \text{ ---> } A_z + B_s$$

where A and B are the cations, and s and z refer to the solution and zeolite phase respectively.

Let K be the separation factor, C and y the maximum and normal capacity of cation A in milliequivalents per gram of zeolite, and x the mole fraction of A in solution. Values of x and y are obtained experimentally as explained in the "Methods" section. For monovalent ions the following equilibrium condition applies under ideal conditions (Breck, 1974):

$$K = \frac{A_z * B_s}{B_z * A_s}$$

Substituting:

$$K = \frac{(y) * (1-x)}{(C-y) * (x)}$$

Rearranging:

$$1/y = (1/Cx) * (1/x) + (K-1)/KC$$

Both the maximum capacity C and the separation factor K are obtained by taking the slope and Y-intercept of a plot of 1/y

versus 1/x. The isotherm equation then becomes:

$$y = \frac{KC * x}{1 + (K - 1) * x}$$

The experimental data points in figure 3.7a tend to follow such a "saturation" pattern (solid line of runs MC144 and MC148).

The effect of temperature on ion exchange capacity is shown in figure 3.7b. Test run MC145 corresponds to sample MC143 further exchanged with 2 N NaCl @ 100 °C for 2 hours. The lowering of the $NH_4^+$ capacity curve for both samples may be the result of deterioration of the zeolite crystal structure due to a high temperature exposure.

In oral ingestion, the zeolites have to pass through the acidic environment of the stomach before entering the intestine. The effect of acid was therefore tested. The zeolites were acid treated (HCl, pH 2.1 for 2 hours) to simulate the gastric environment, as shown in figure 3.8. There is a small increase in the ammonium equilibrium value, indicating a potential loss of zeolite structure. This may lead to a release of aluminum in the stomach. Zeolites are more stable at the higher pH's found in the intestinal tract,

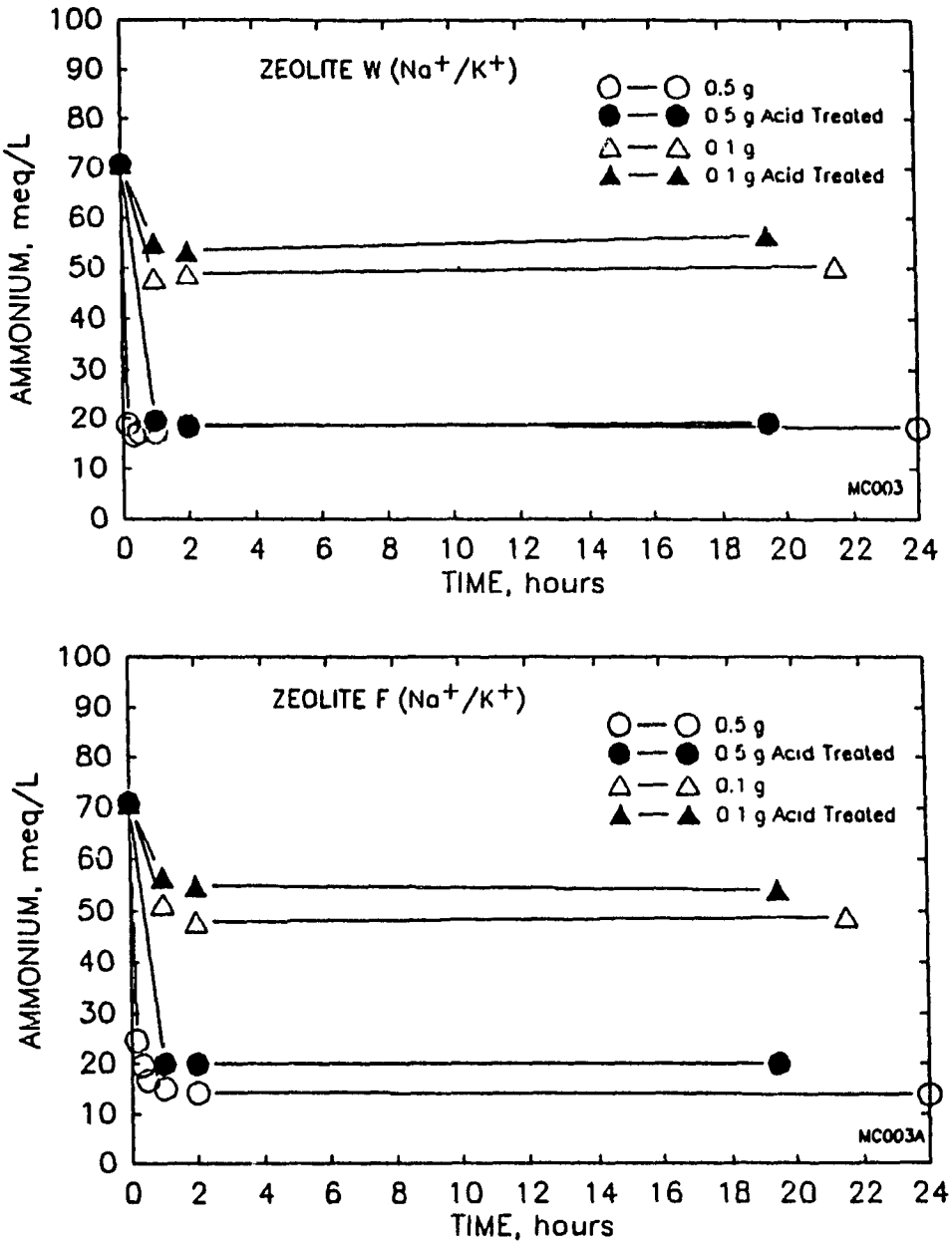



**FIGURE 3.8. ACID TREATMENT. Pretreat 0.5 grams of zeolite W (figure 3.8a, top) or zeolite F (figure 3.8b, bottom) in hydrochloric acid (HCl, pH 2.1) for 2 hours prior to ammonium adsorption.**

but not below 4-5 (Sherman, 1978). Their exposure to lower pH's should be minimized. Any buffering by proteins present in the stomach may reduce the severity of acid exposure. Microencapsulation and the presence of proteins and polymers inside the microcapsule may further enhance the zeolite's stability. If necessary, enteric coatings can be used to envelope and protect the zeolite during its passage through the stomach and conveniently rupture in the intestine.

Figure 3.9 shows the effect of solvent exposure prior to ammonium uptake. Methylene chloride, toluene and diethyl ether were chosen because of their use in microencapsulation. The zeolites were wetted in the solvents for 10 minutes prior to ammonium exchange. The solvents do not affect ammonium ion exchange.

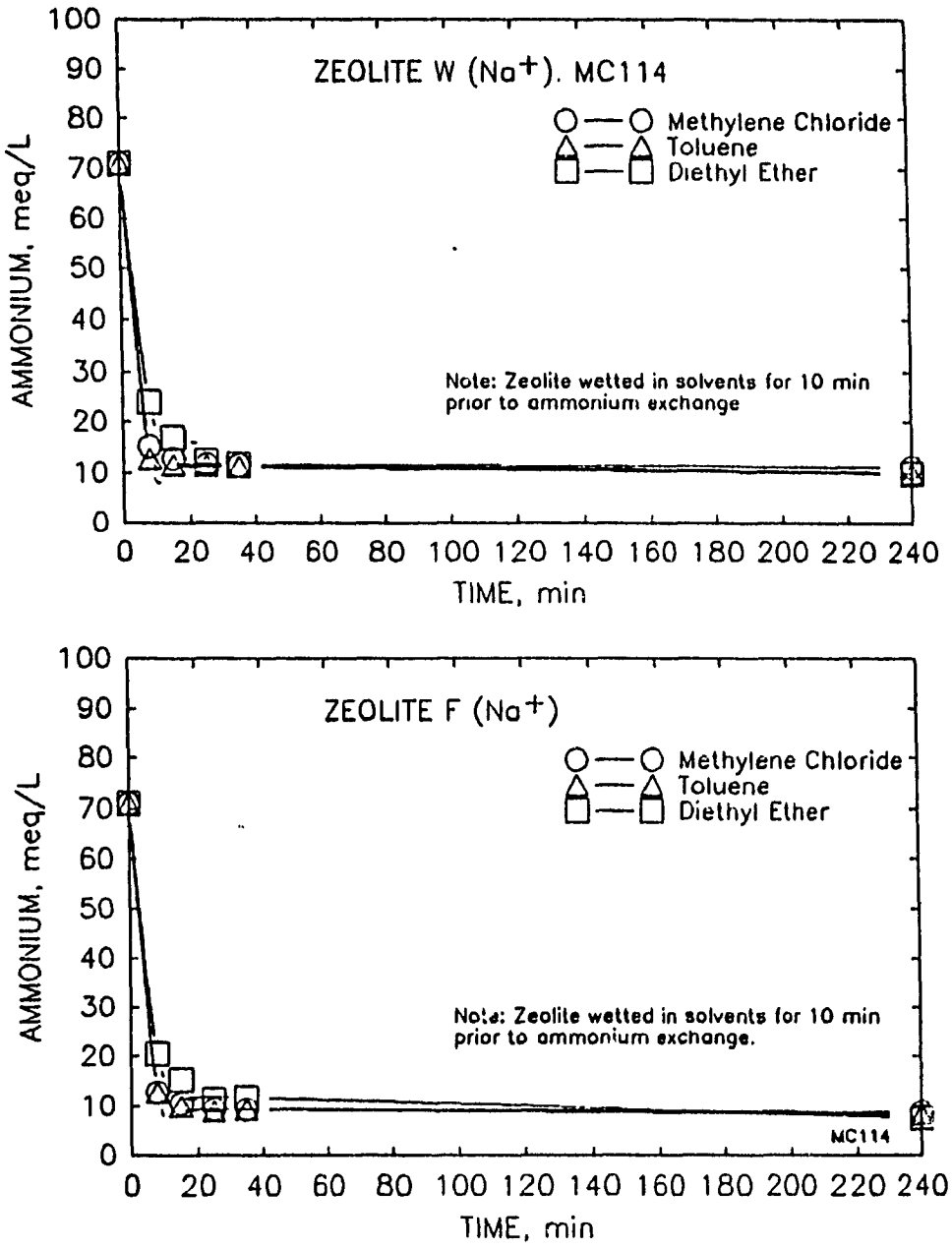



FIGURE 3.9. SOLVENTS' EFFECTS PRIOR TO AMMONIUM UPTAKE. Pretreat 0.5 grams of zeolite W (figure 3.9a, top) and zeolite F (figure 3.9b, bottom) with microencapsulation solvents for 10 min at room temperature prior to ammonium exchange.

## 3.2 Enzyme Envelopes

For immobilizing urease we adopted the enzyme envelope method referred to in the "Literature" section (Haynes, 1969). The effect of urease loading on silica is shown in figure 3.10. The amount of urease was varied from 12 to 100 mg per 250 mg of silica. One experiment at 500 mg silica was performed as well. Urea does not disappear as readily as urease loading approaches 12 mg, which indicates there may be insufficient enzyme on the support.

Urea and ammonium reaction kinetics are shown in figure 3.11 for urease immobilized on zeolite F and on silica. Urea concentrations decrease rapidly to zero from 100 mg/dl in the first few minutes of the reaction. For silica, the ammonium concentration is seen to rise toward the equilibrium value of 71 meq/l, whereas the zeolite lowers it to 20 meq/l (figure 3.11b). The zeolite-urease adduct performs like a true urea sorbent in removing more than 70% of the urea present in the reaction vessel.

The loading of urease on zeolite W (ZWU) is shown in figures 3.12a to 3.12f. The amounts vary from 1.5 to 100 mg urease per 500 mg of zeolite. Urea reaction velocity shows no further improvement for loadings beyond 12 mg/0.5 g of zeolite. The rise in ammonium concentration is limited to a maximum of 30 meq/l. Ammonium uptake curves in figure 3.12b are representative of this type of immobilization. An initial burst of ammonium, as shown by a peak in the first 10 minutes

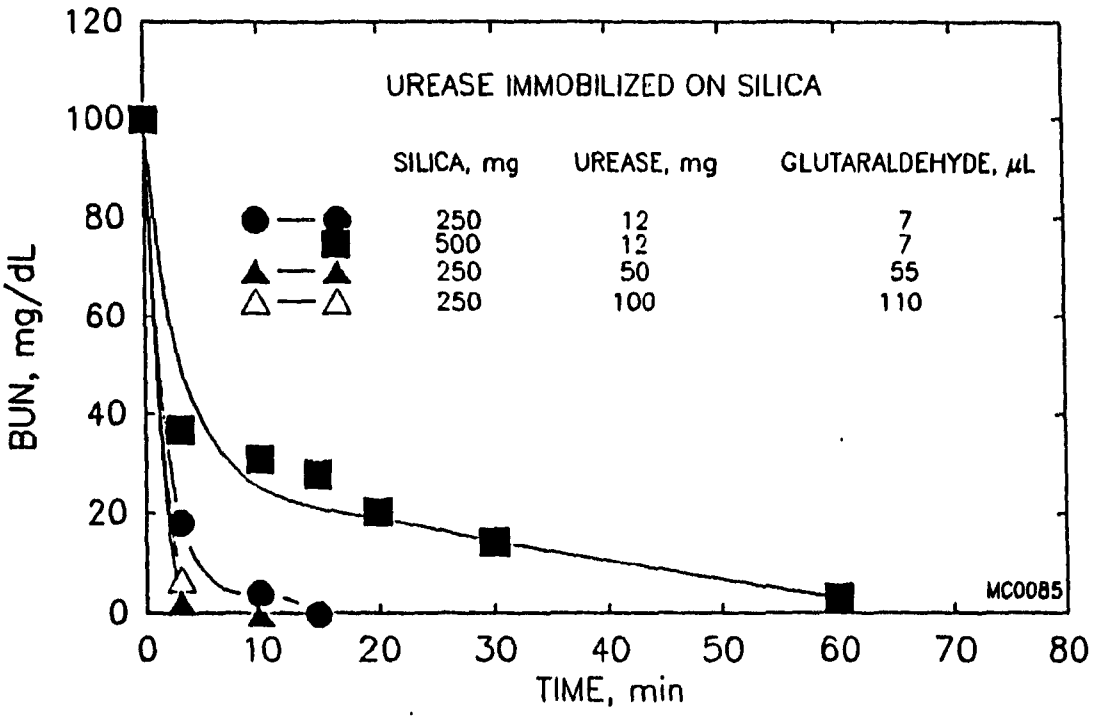



**FIGURE 3.10. UREASE IMMOBILIZED ON SILICA. The urease enzyme is immobilized at R.T. by the envelope method. The amount of urease enzyme varies from 12 to 100 mg/250 mg of silica colloid.**

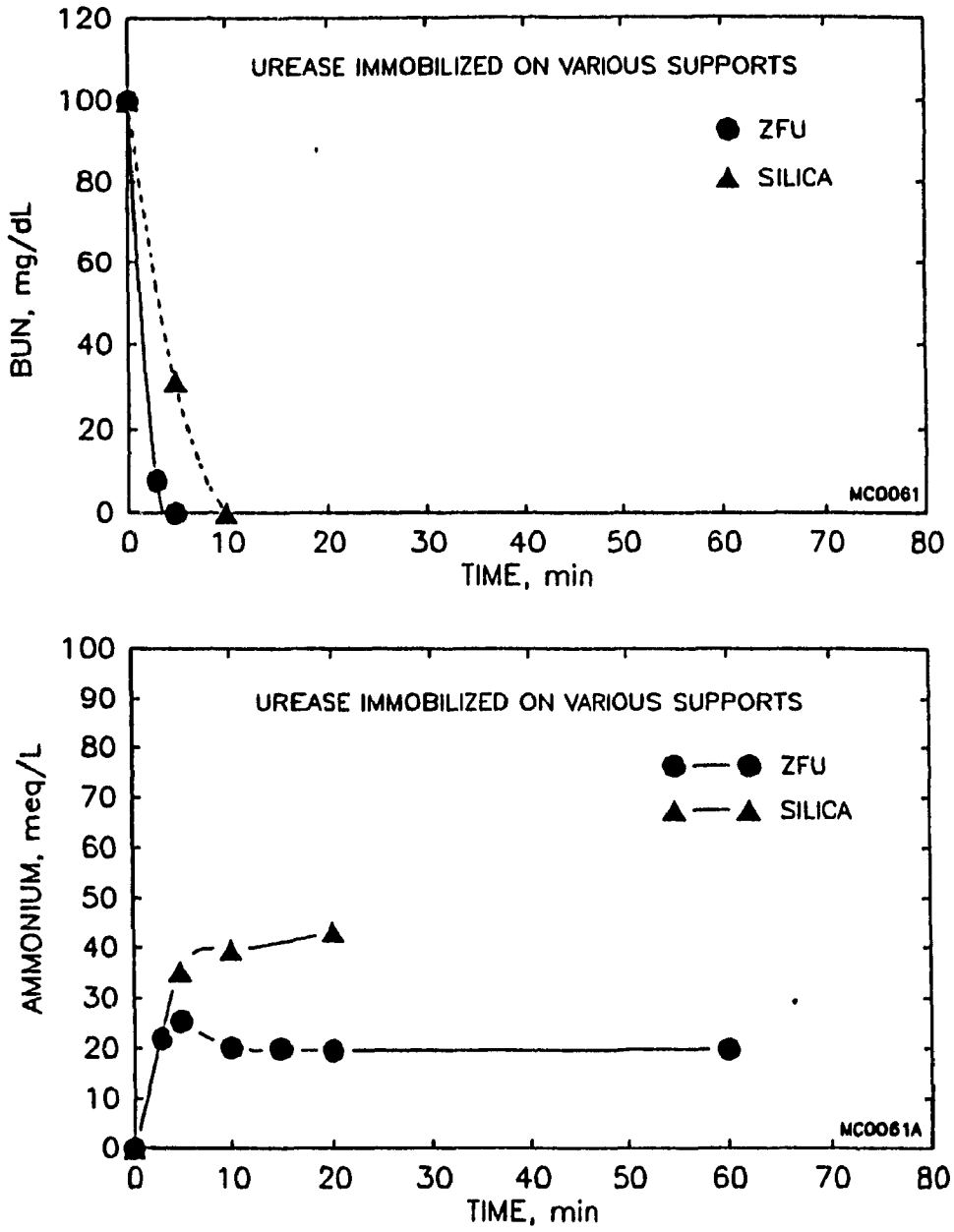



**FIGURE 3.11. UREASE IMMOBILIZED ON VARIOUS SUPPORTS. Both silica and zeolite F were loaded with 50 mg of urease per 250 mg of support material. The immobilization was carried out at room temperature. The BUN reaction is shown in figure 3.11a (top) while the ammonium uptake is in figure 3.11b (bottom graph).**

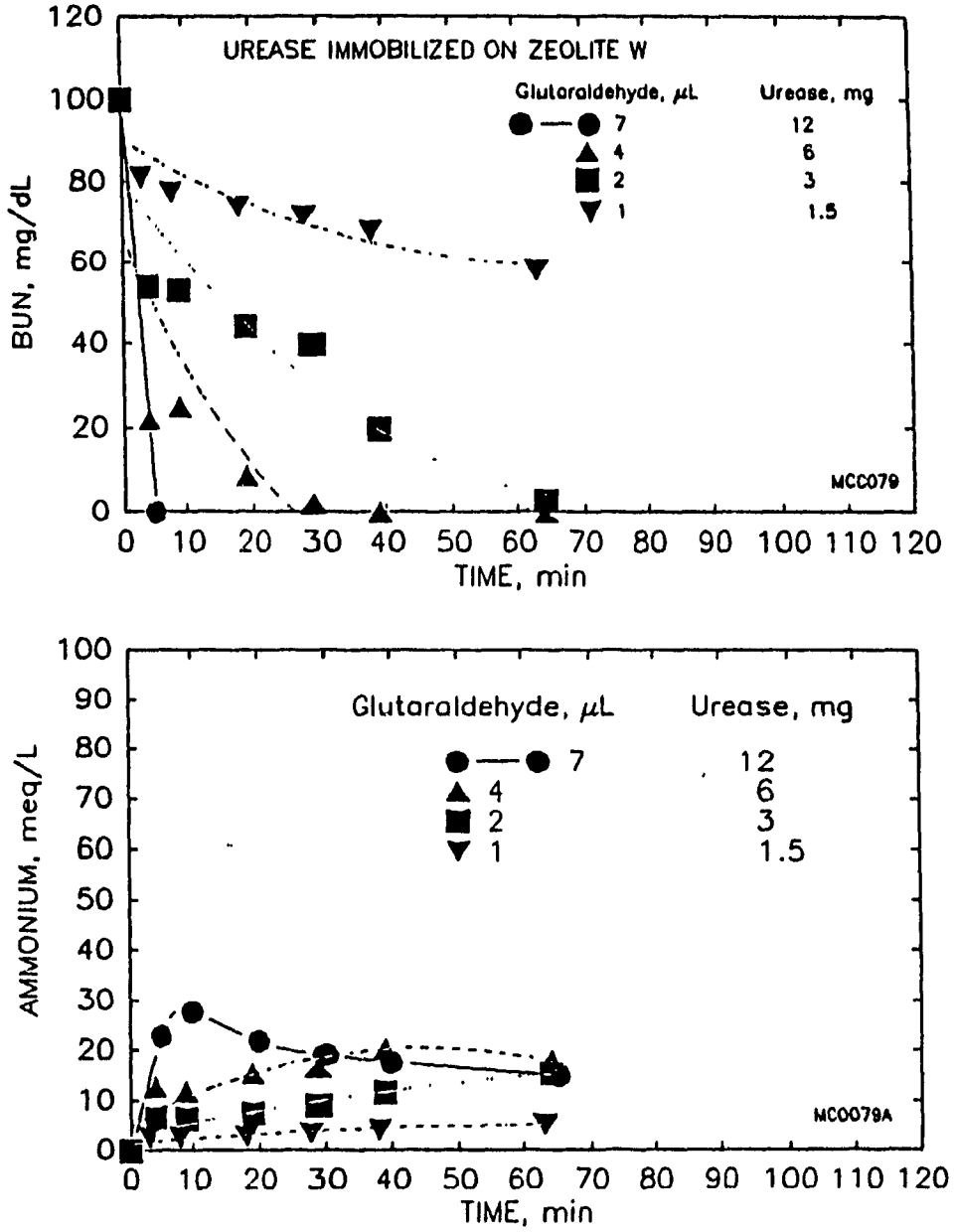



**FIGURE 3.12. UREASE IMMOBILIZED ON ZEOLITE W. The amounts of urease immobilized are as shown. The immobilization was performed at room temperature. Urease varies from 1.5 mg to 12 mg/0.5 grams of zeolite. BUN values are shown in figure 3.12a (top) while ammonium adsorption rates are in figure 3.12b (bottom graph).**

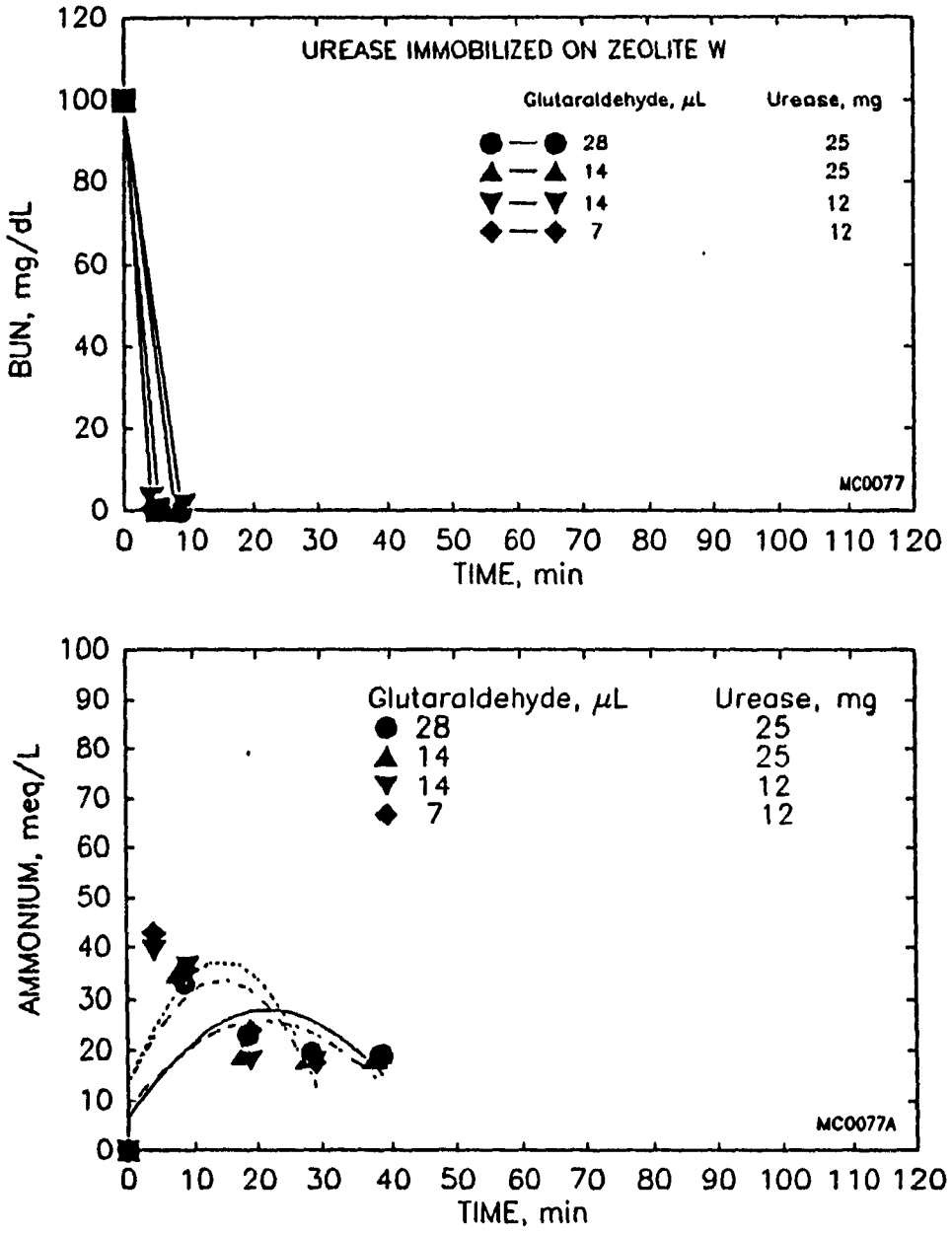



**FIGURE 3.12. UREASE IMMOBILIZED ON ZEOLITE W. The amounts of urease immobilized are as shown. The immobilization was performed at room temperature. Urease varies from 12 mg to 25 mg/0.5 grams of zeolite. BUN values are shown in figure 3.12c (top) while ammonium adsorption rates are in figure 3.12d (bottom graph).**

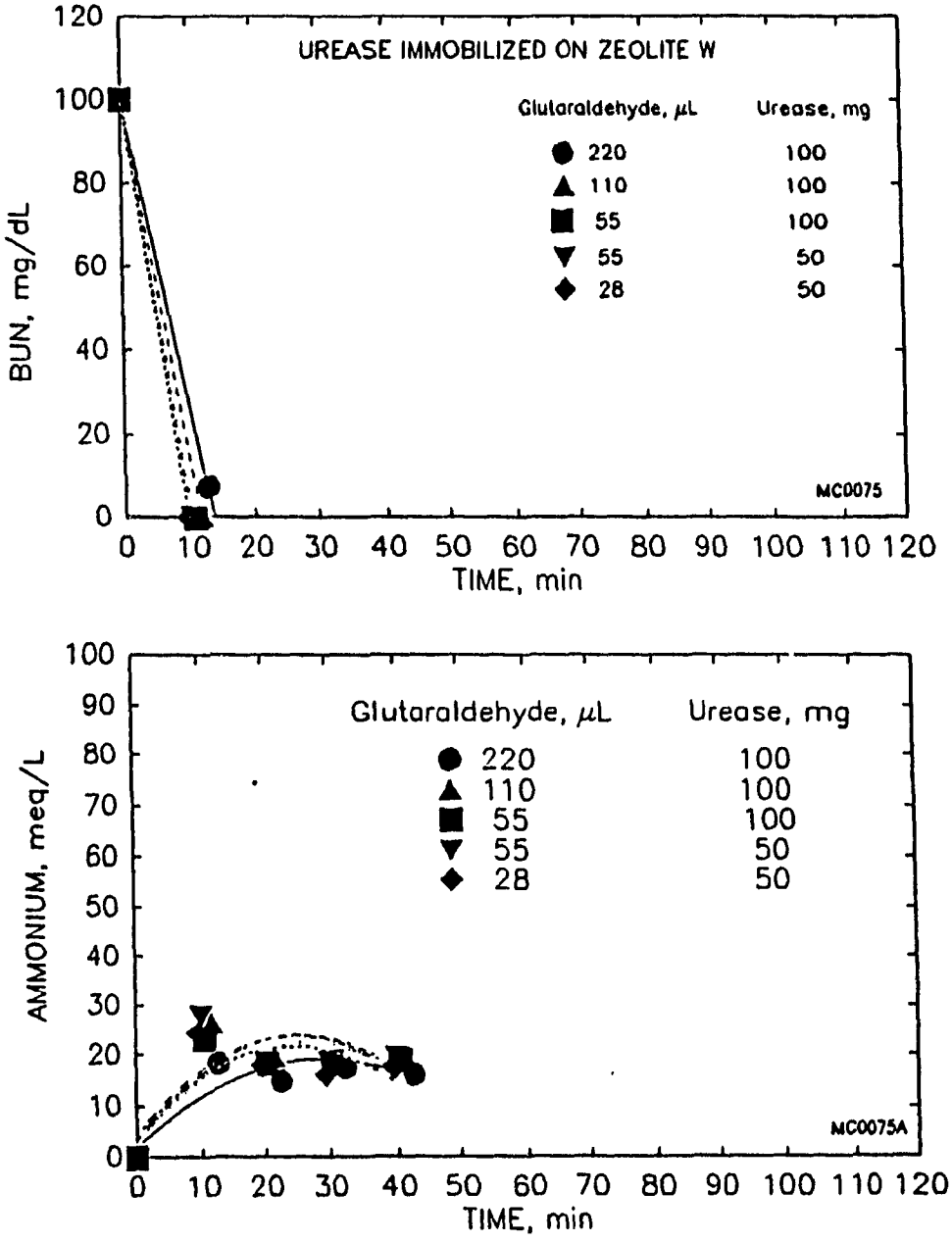



**FIGURE 3.12. UREASE IMMOBILIZED ON ZEOLITE W. The amounts of urease immobilized are as shown. The immobilization was performed at room temperature. Urease varies from 50 mg to 100 mg/0.5 grams of zeolite. BUN values are shown in figure 3.12e (top) while ammonium adsorption rates are in figure 3.12f (bottom graph).**

of measurement (12 mg urease plot), is a result of a urea reaction greater than the rate of ammonium uptake. As the rate of urea breakdown decreases so does the initial ammonium peak (6 mg urease or lower). Ammonium peaks are observed for all urease loadings greater than 12 mg per 0.5 g of zeolite.

A plot of absorbance versus NADH concentration is shown in figure 3.13a. The absorbance values can be converted into NADH concentrations, and to urea values as a result of the coupling between urea and NADH:

$$\text{Urea} + H_2O + 2H^+ \xrightarrow{\text{urease}} 2NH_4^+ + CO_2$$

$$2NH_4^+ + 2\alpha\text{-Ketoglutarate} + 2NADH \xrightarrow{\text{GLDH}} 2 \text{ Glutamate} + 2NAD^+ + 2H_2O$$

This coupling allows the determination of the urea initial reaction rates. Michaelis-Menten kinetics for native urease are shown in figure 3.13b. This is a plot of S/V (substrate concentration over initial reaction velocity) versus substrate concentration.

The Michaelis form of the equation:

$$V = \frac{Vmax * S}{Km + Vmax}$$

can be converted into a "Haynes plot" form:

$$S/V = S/Vmax + Km/Vmax$$

The maximum velocity (Vmax) is obtained from the reciprocal of the slope, and the Km value from the Y-intercept. The main advantage of the Haynes over the more common Lineweaver-Burk plot is that it gives a better indication of the quality of each observation and a smaller error deviation over the entire range (A. Cornish Bowden, Enzyme Kinetics). A Michaelis constant of 7 mM for native urease is of comparable magnitude to the literature value (Km= 3.4 mM, Chibata, 1978).

Figure 3.14a shows Michaelis plots for urease immobilized on zeolites F, Y and colloidal silica. Michaelis constants of 10, 5 and 4 mM were found. The envelope method does not affect the enzyme's active centre since these values are not significantly different from native urease. On the other hand, urease entrapped in liquid membrane microcapsules has a Km of 180 mM or two orders of magnitude greater than native urease (May and Li, 1972). The effect of enzyme loading on zeolite F is shown in figure 3.14b. Vmax values are 3 E-3 and 6 E-4 for 100 mg and 30 mg urease/0.5 g zeolite respectively indicate that different enzyme loadings may affect the concentration of urease on the support.

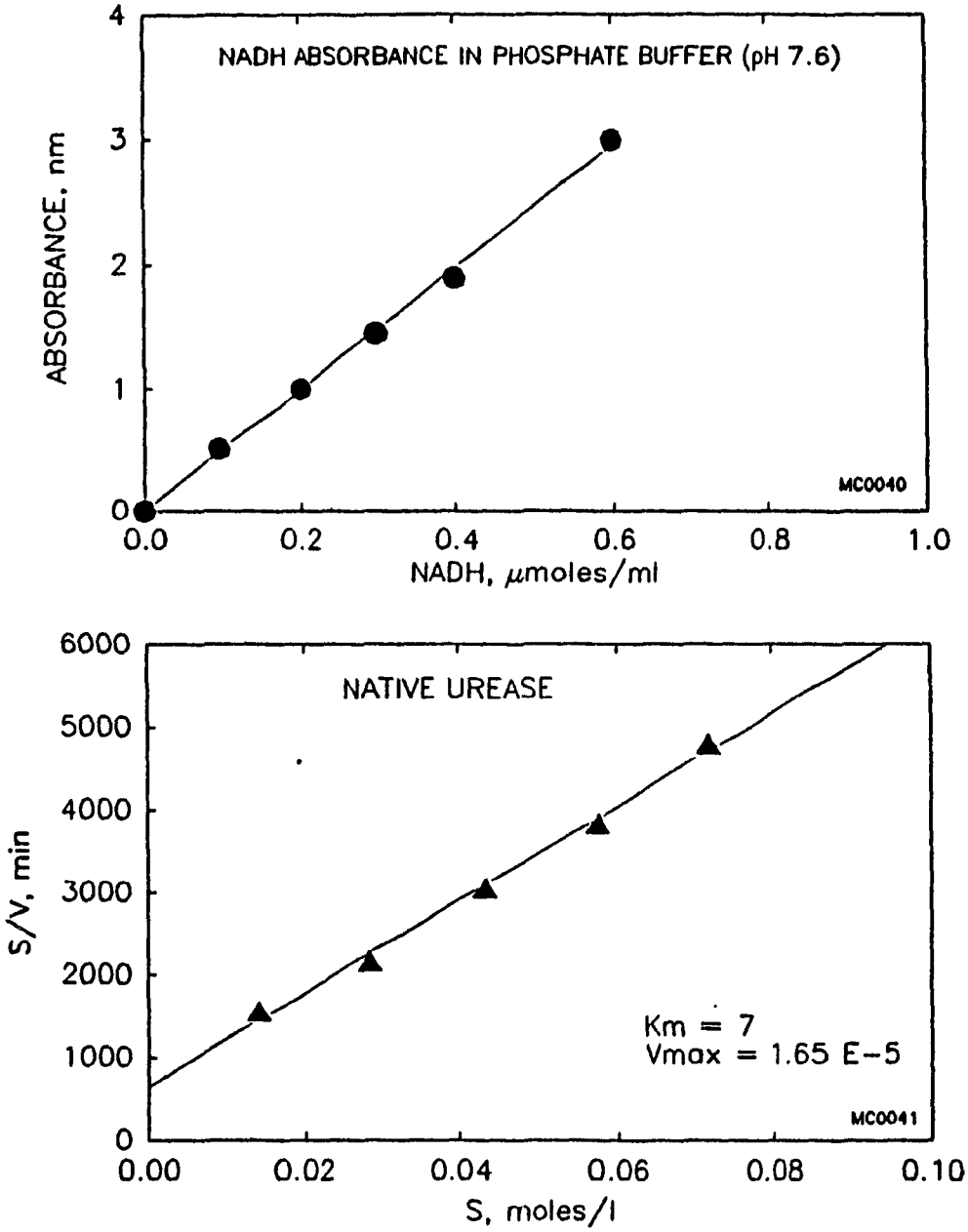



**FIGURE 3.13. A plot of NADH Absorbance in phosphate buffer is shown in figure 3.13a (top graph). In figure 3.13b (bottom graph) are shown Michaelis-Menten kinetics for native urease.**

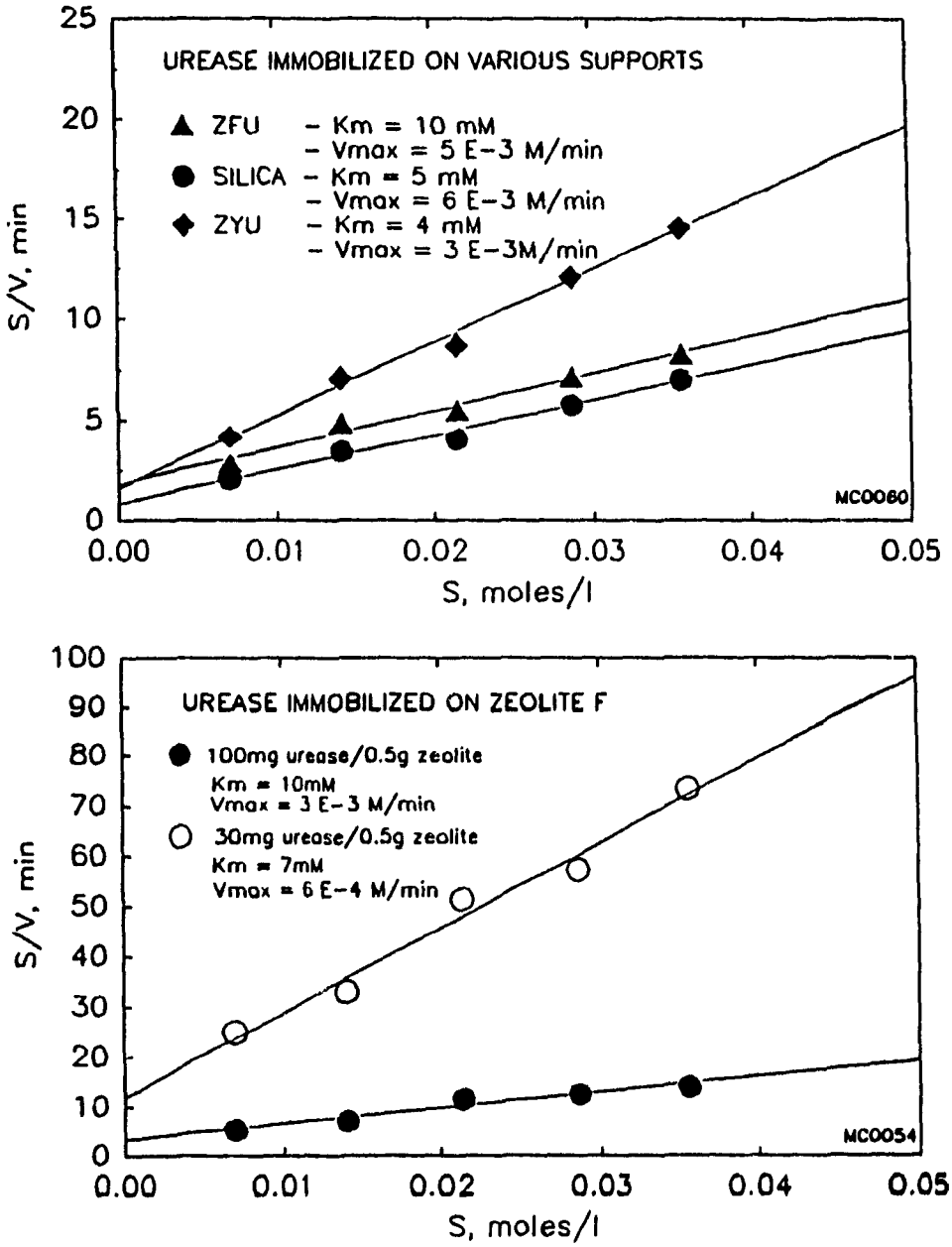



FIGURE 3.14. UREASE IMMOBILIZED ON VARIOUS SUPPORTS. Urease was immobilized on silica and zeolites F and Y (figure 3.14a, top graph). In figure 3.14b is shown the effect of enzyme loading on zeolite F.

Stability data in figure 3.15 compare a freshly prepared urease-zeolite sample with one stored for one month sealed in a jar at 4 °C. Km values of 10 and 8 mM for new and old samples do not differ significantly. On the other hand, Vmax decreased to 20% of its original value, from 5 E-3 down to 1.2 E-3 M/min, most likely as a result of enzyme deterioration.

This preparation in oral ingestion has to pass through the acidic environment of the stomach before reaching the intestine. A sample of urease immobilized on zeolite W was treated with acid to simulate gastric conditions. The acid inactivates the enzyme (figure 3.16). Studies were therefore carried out to see how to prevent this problem. One approach is by incorporating this in an oxycellulose matrix. Another approach is to use microencapsulation.

The effect of incorporating ZWU in an oxycellulose matrix is shown in figure 3.17. The matrix does not hinder the urea reaction rate and it seems to have a beneficial effect in reducing initial outbursts of ammonium. However, due to the large volumes of oxycellulose needed to entrap 0.5 g of the adduct, this method was discontinued.

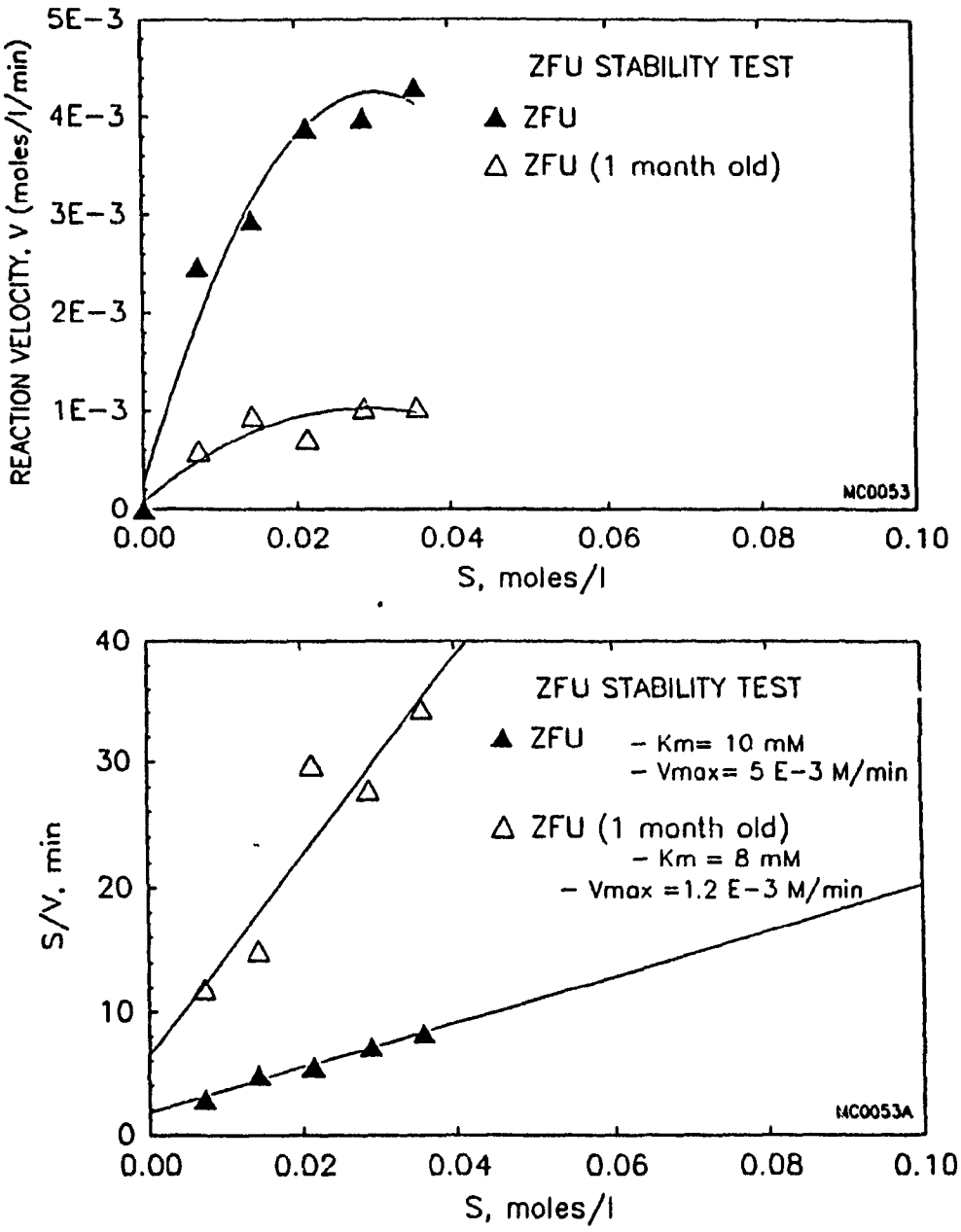



**FIGURE 3.15. STABILITY TEST FOR UREASE IMMOBILIZED ON ZEOLITE F. Reaction velocity data as a function of urea concentration is shown in figure 3.15a. In figure 3.15b Vmax values are obtained from a Haynes kinetic plot.**

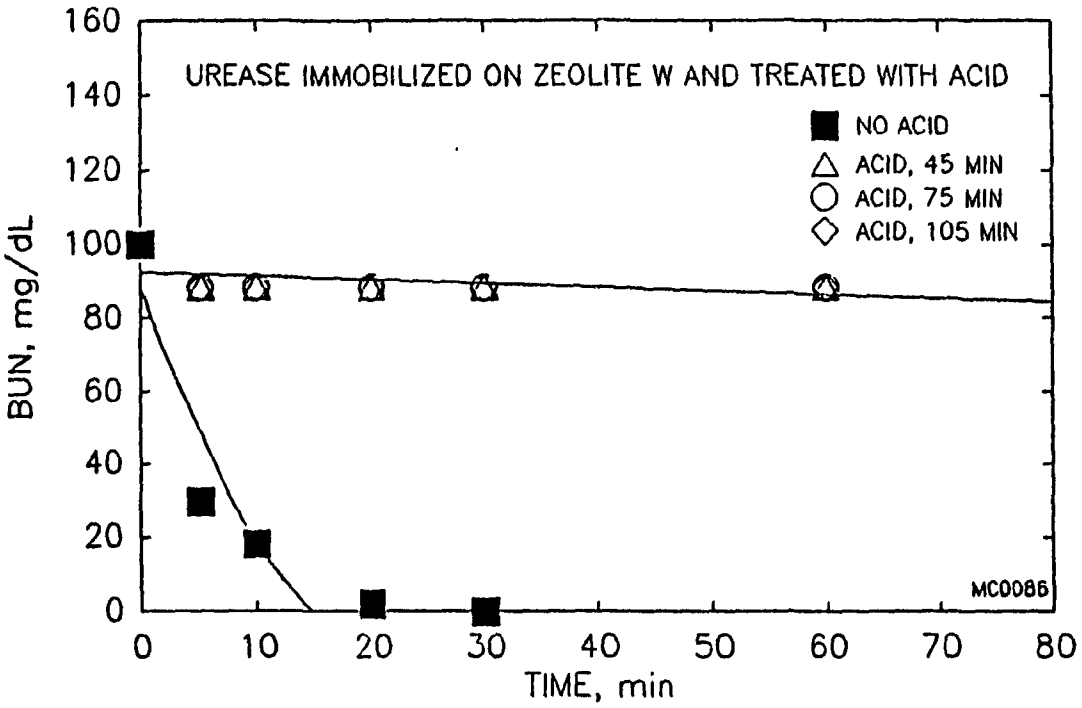



**FIGURE 3.16. UREASE IMMOBILIZED ON ZEOLITE W AND TREATED WITH ACID. The acid treatment was performed for a 45, 75, 105 min time interval at 37 °C and 120 RPM.**

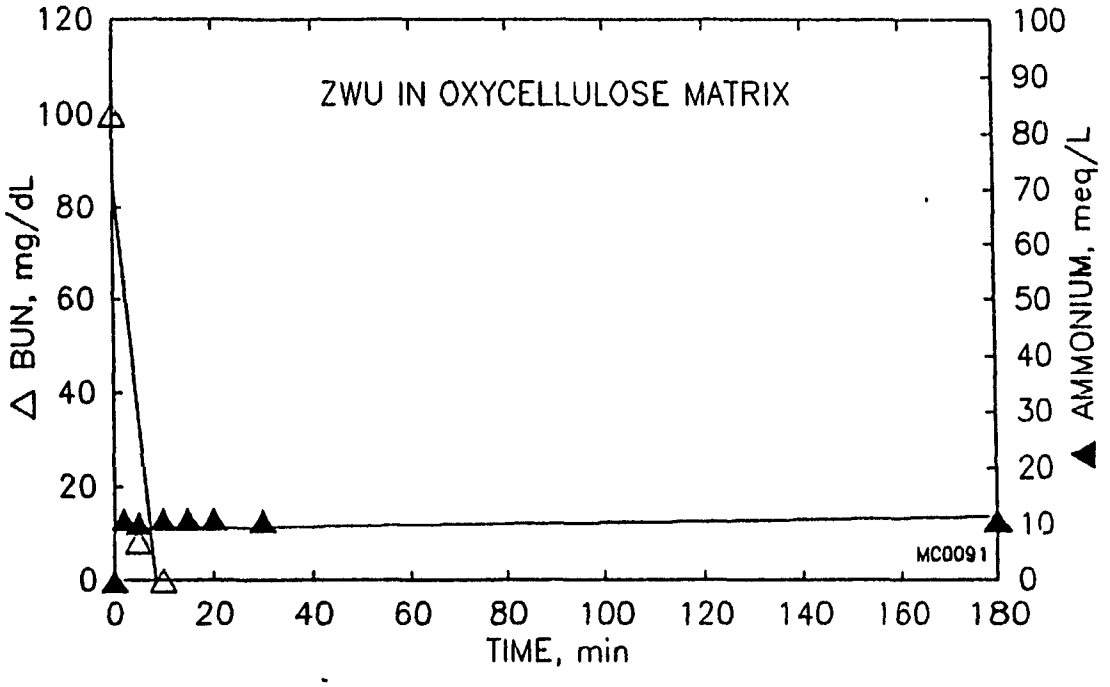



**FIGURE 3.17. ZWU IN OXYCELLULOSE MATRIX.**

## 3.3 Microencapsulation

Michaelis-Menten kinetics of urease collodion microcapsules is shown in figure 3.18a. The Km value of 7 mM does not differ from native urease (figure 3.13b). Evidently, the microenvironment within the microcapsule does not influence enzyme activity. The urea reaction rate as a function of urease concentration is shown in figure 3.18b. The rate does not increase significantly beyond 1.0 mg of urease per 0.5 ml of collodion microcapsules. This concentration of urease was used in all collodion microcapsule preparations.

In figure 3.19 both BUN and ammonium are measured as a function of time. Collodion microcapsules (0.5 ml) are placed in a 15 ml solution of 100 mg/dl BUN. As urea reacts, the ammonium concentration is seen to rise to its equilibrium value of 71 meq/l (figure 3.19a). On the other hand, the presence of zeolite F in the reaction vessel limits the ammonium concentration to 20 meq/l (figure 3.19b). When Zirconium Phosphate (ZP) is added the ammonium concentration rises to a maximum before falling (figure 3.20).

Urease immobilized on zeolite W (ZWU) was incorporated within alginate microcapsules to see whether its inclusion in a matrix-like environment would trap ammonium ions (figure 3.21). The problem with this method is the excessive volume of microencapsulated material due to alginate. This is a serious limitation in oral ingestion therapy and the use of alginate had to be discontinued.

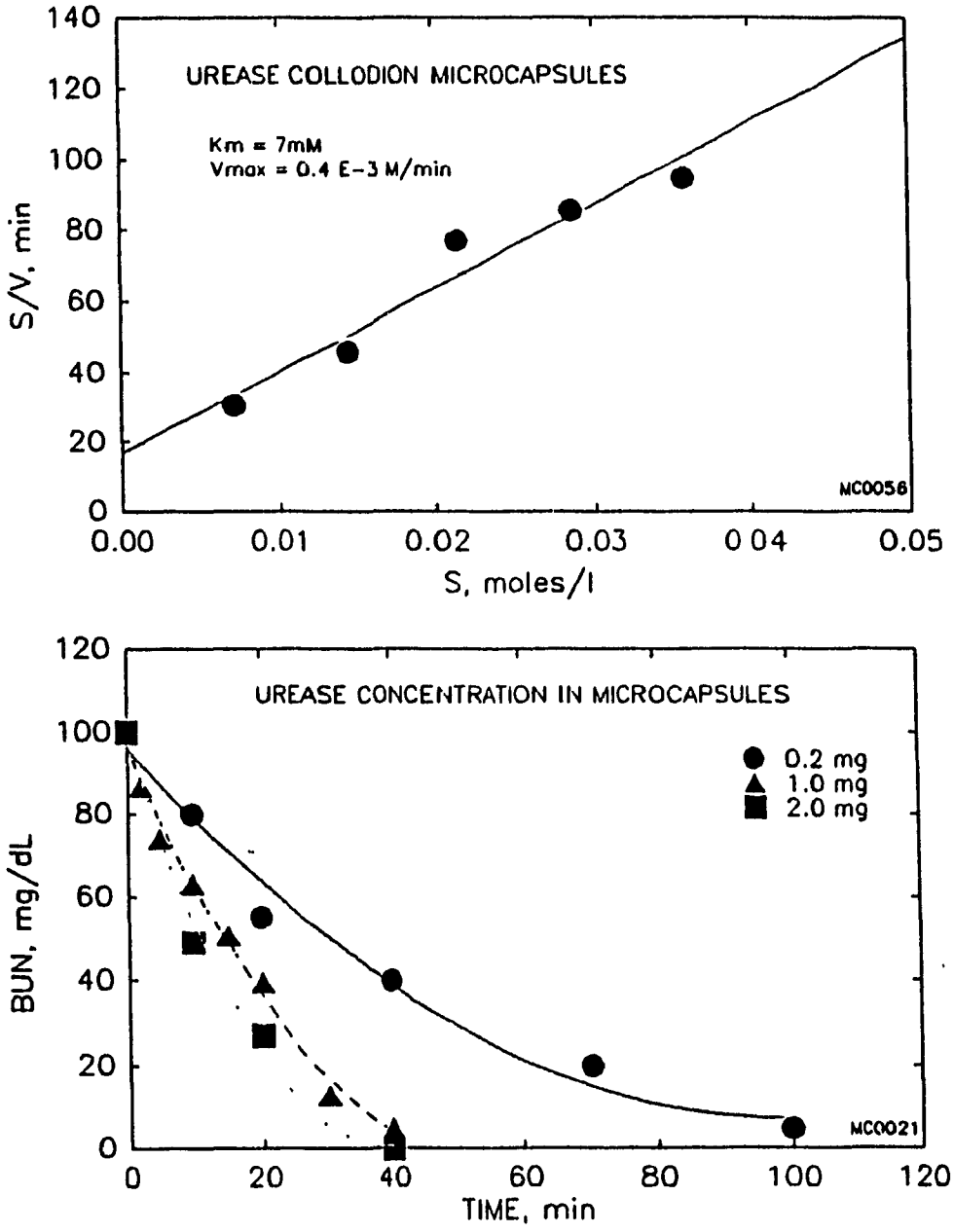



**FIGURE 3.18. Michaelis-Menten kinetics for collodion microcapsules containing urease enzyme are shown in figure 3.18a (top graph). In figure 3.18b (bottom graph) are shown urease concentration effects in collodion microcapsules. The starting 15 ml solution contains 100 mg/dl urea.N in 0.9g/dl NaCl.**

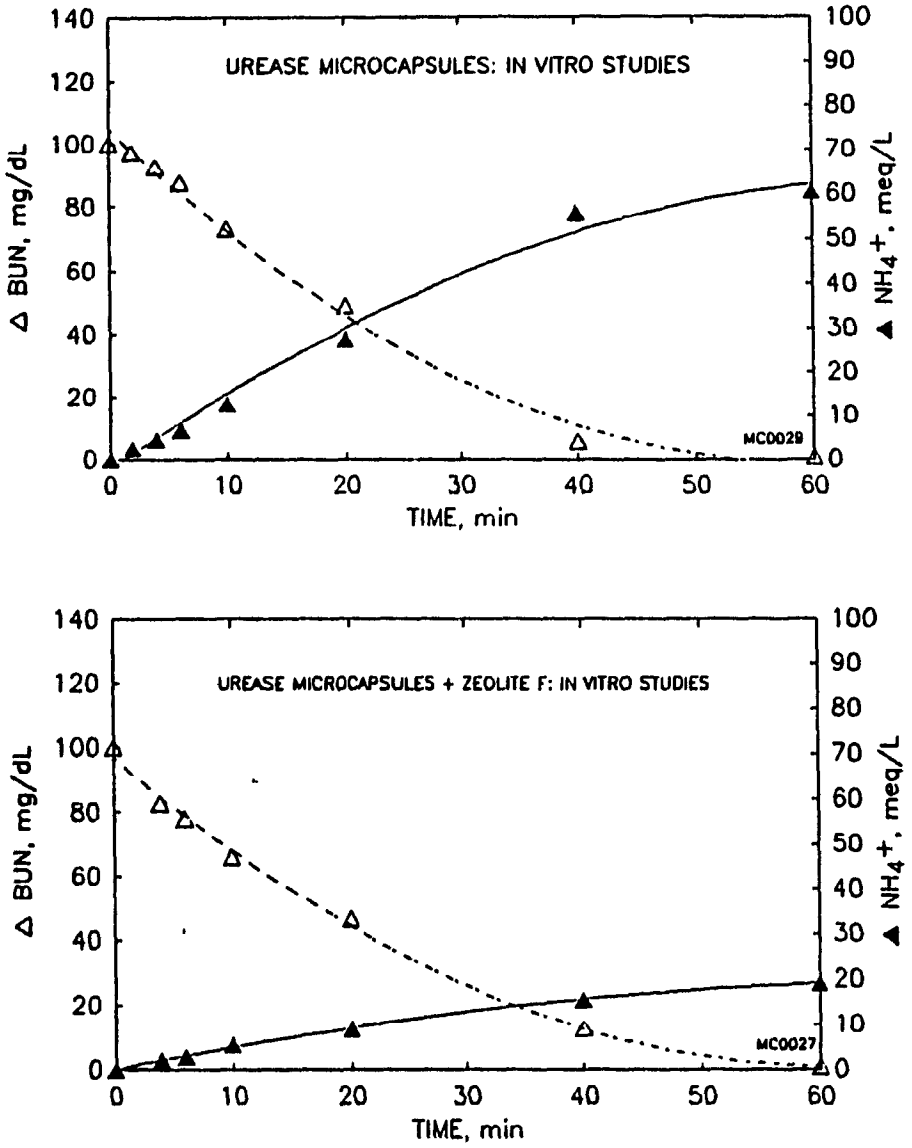



**FIGURE 3.19. IN VITRO STUDIES/UREASE MICROCAPSULES ONLY (FIG.3.19A, TOP) AND WITH ZEOLITE F (BOTTOM GRAPH). Add 1.0 mg of urease enzyme in 0.5 ml of collodion microcapsules to 15 ml solution of 100 mg/dl BUN in 0.9 g/dl NaCl (fig. 3.19a). Add 0.5 g of zeolite F to the microcapsules (fig. 3.19b).**

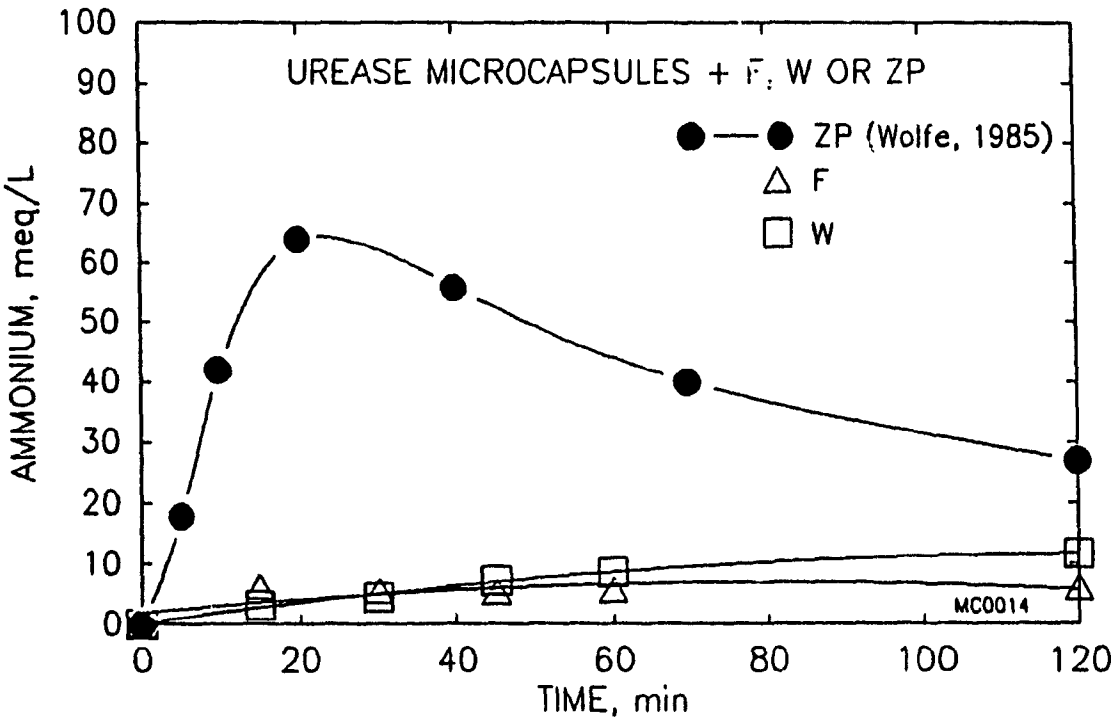



**FIGURE 3.20. IN VITRO STUDIES/MICROENCAPSULATED UREASE + LINDE F & W. Incubate 0.5 ml urease collodion microcapsules + 0.5 g of zeolite anhydrous or zirconium phosphate in 100 mg/dl urea.N solution in saline @ 37 °C and 120 RPM.**

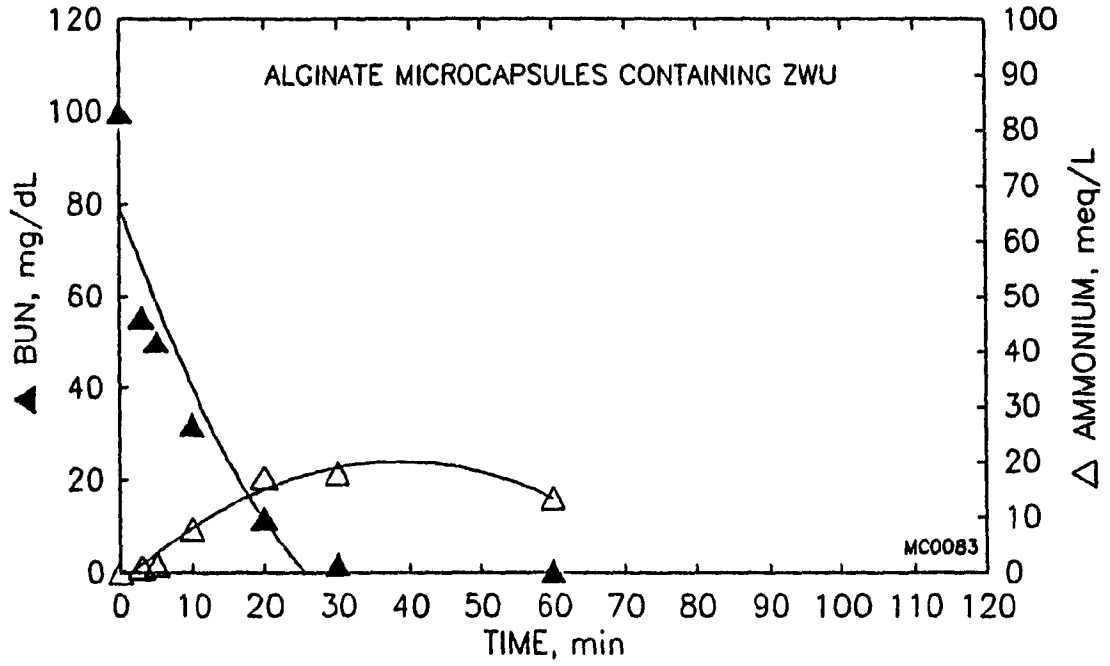



**FIGURE 3.21. ALGINATE MICROCAPSULES CONTAINING ZWU.**

On the other hand, microencapsulation of ZWU within Cellulose Acetate Butyrate (CAB) microcapsules was more successful. CAB microcapsules containing the adduct can be dried (crenated capsules) to reduce the bulk volume. The sphericity of the microcapsules returns with subsequent wetting (see figure 3.22 for crenated capsules immersed in distilled water). An additional benefit of these microcapsules is that they can be used to remove excess water as well as urea.

The amount of urease loaded on ZWU prior to microencapsulation was varied from 12 to 100 mg/0.5 g of zeolite W (figure 3.23). Unfortunately, microencapsulation of ZWU alone produces large releases of ammonium ions. We needed to stop the ammonium release out of the microcapsules. Perhaps, the use of coatings and/or additional core material could limit the ammonium's outward diffusion.

## 3.4 Coating Cellulose Acetate Butyrate microcapsules

The effect of coating on ammonium release is shown in figures 3.24 through 3.28. The lecithin and oxycellulose coated samples in figure 3.24 behave very much like the uncoated sample of figure 3.23. The presence of the ammonium peak shows the coating does not limit ammonium release. The microcapsules were then impregnated with a positive electrolyte 2-polyvinyl methylpyridinium iodide (2-PVMP) solution which would fix on the membrane after drying. The positive electrolyte would allow urea, a non charged molecule, to pass freely,

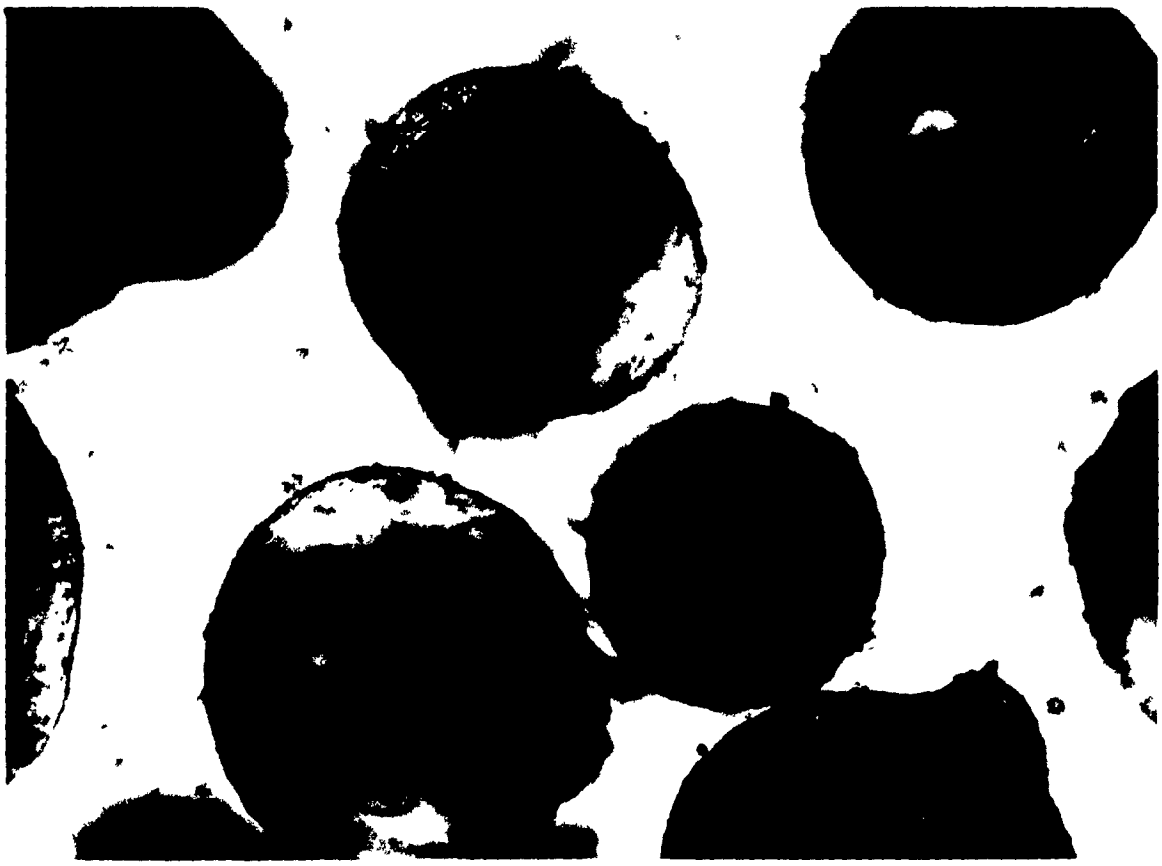


FIGURE 3.22. CELLULOSE ACETATE BUTYRATE MICROCAPSULES

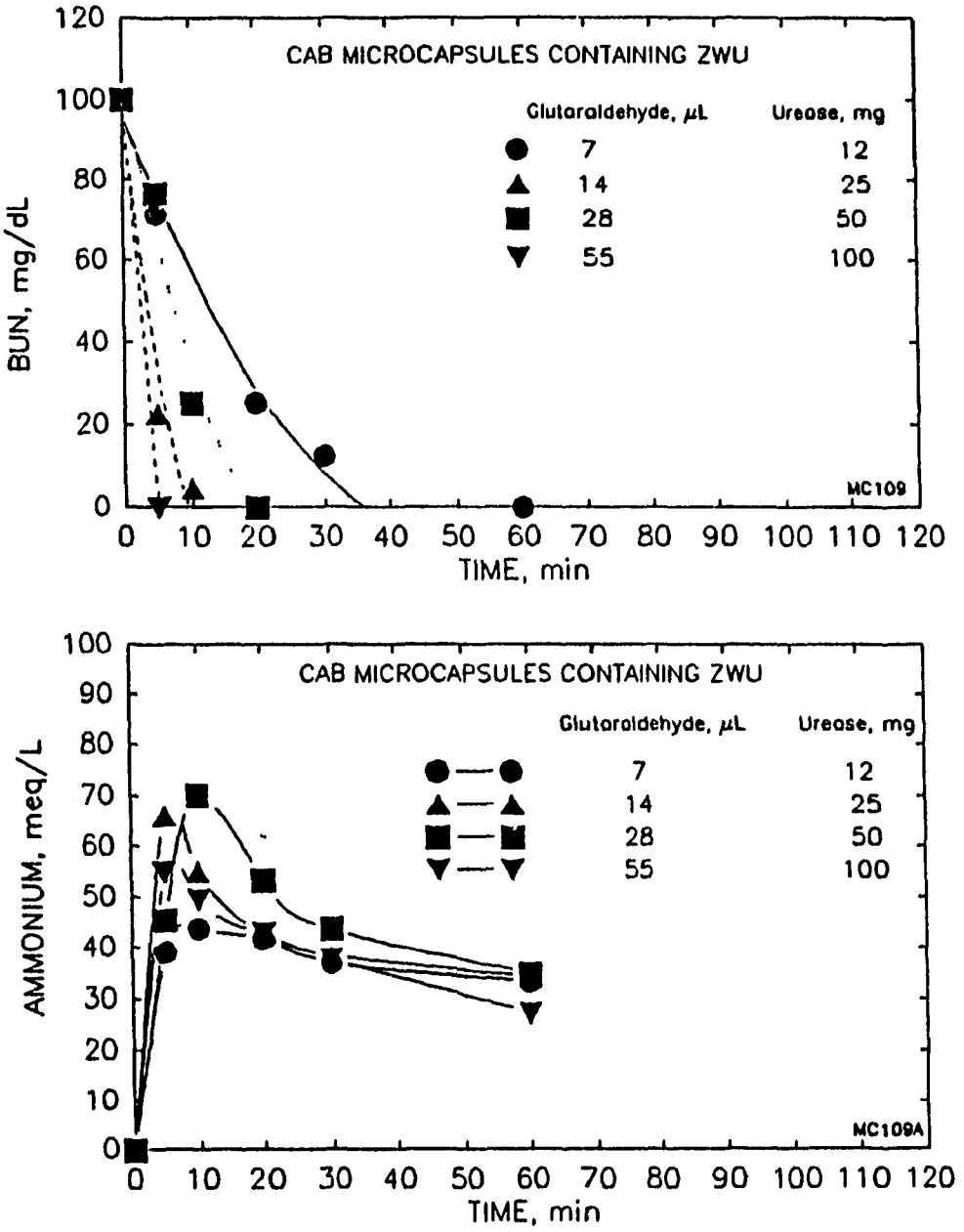



**FIGURE 3.23. CELLULOSE ACETATE BUTYRATE MICROCAPSULES CONTAINING THE UREASE-ZEOLITE W PREPARATION.** Urea reaction rates are shown in figure 3.23a (top graph). In figure 3.23b (bottom graph) ammonium release curves are obtained at different urease concentrations on the zeolite W support.

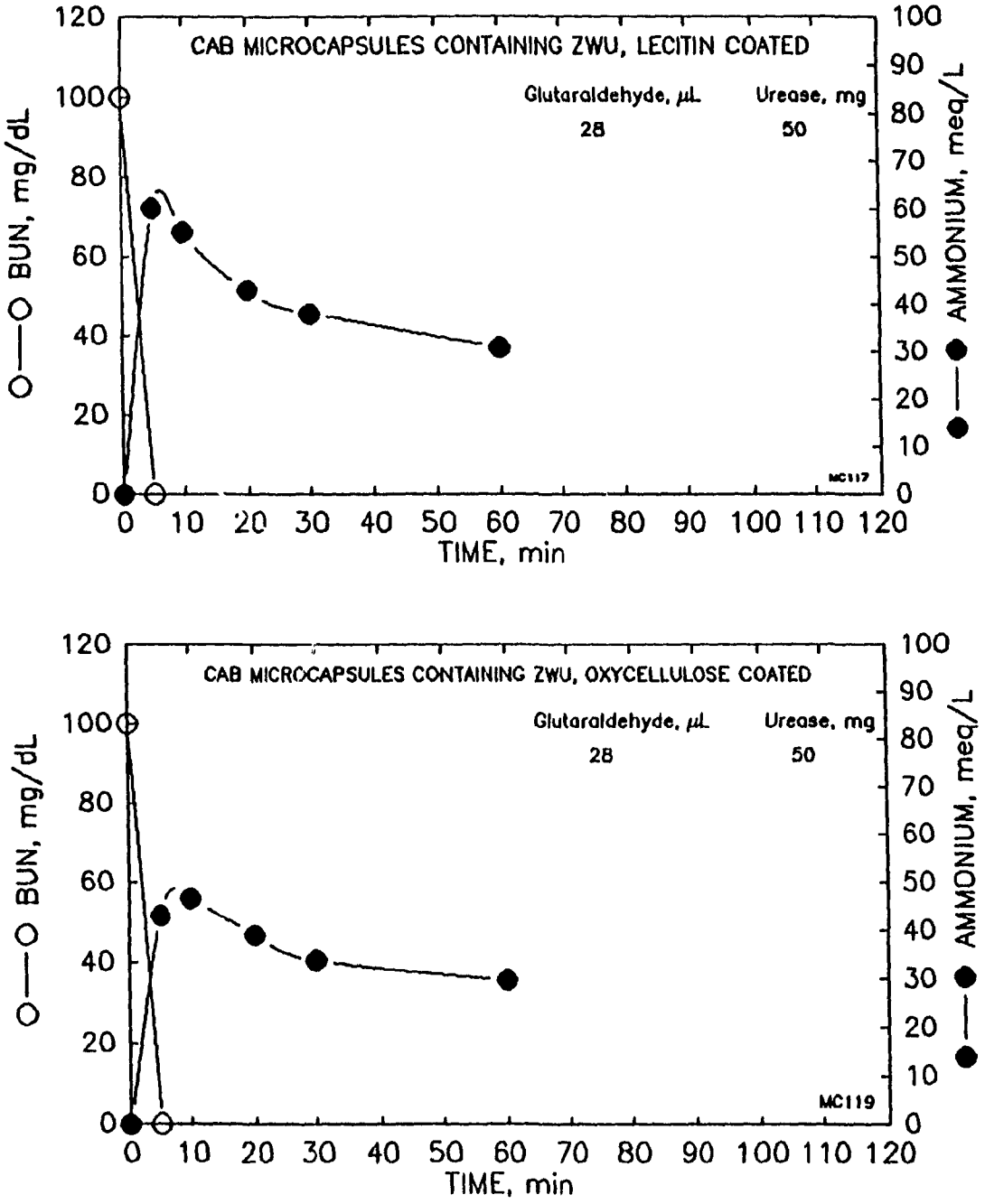



**FIGURE 3.24. CAB MICROCAPSULES COATED WITH LECITHIN (FIG. 3.24A, TOP GRAPH) AND OXYCELLULOSE (FIG. 3.24B, BOTTOM GRAPH).**

while entrapping ammonium. The disappearance of the IR band at 1473 cm-1 in figure 3.25 shows complete quaternization of the vinyl pyridine units:

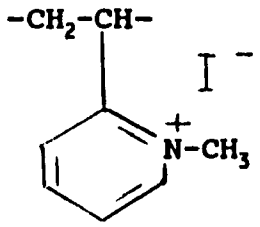



Figure 3.26 shows the reaction of urea with native urease before and after exposure to 2-PVMP. Unfortunately, 2-PVMP was found to affect enzyme's activity and its use as a coating had to be discontinued. Ethylene oxide, alginate, polyethylene imine-epichlorohydrin and polyethylene imine act as ammonium barriers but slow down urea diffusion as well (figure 3.25 and 3.26).

The activity of CAB microcapsules containing ZWU at different urease concentrations is shown in figure 3.29. The ammonium peak is not as pronounced since urea reactions are also low. In further experiments we had to compromise between faster urea reaction rates and lower ammonium peaks.

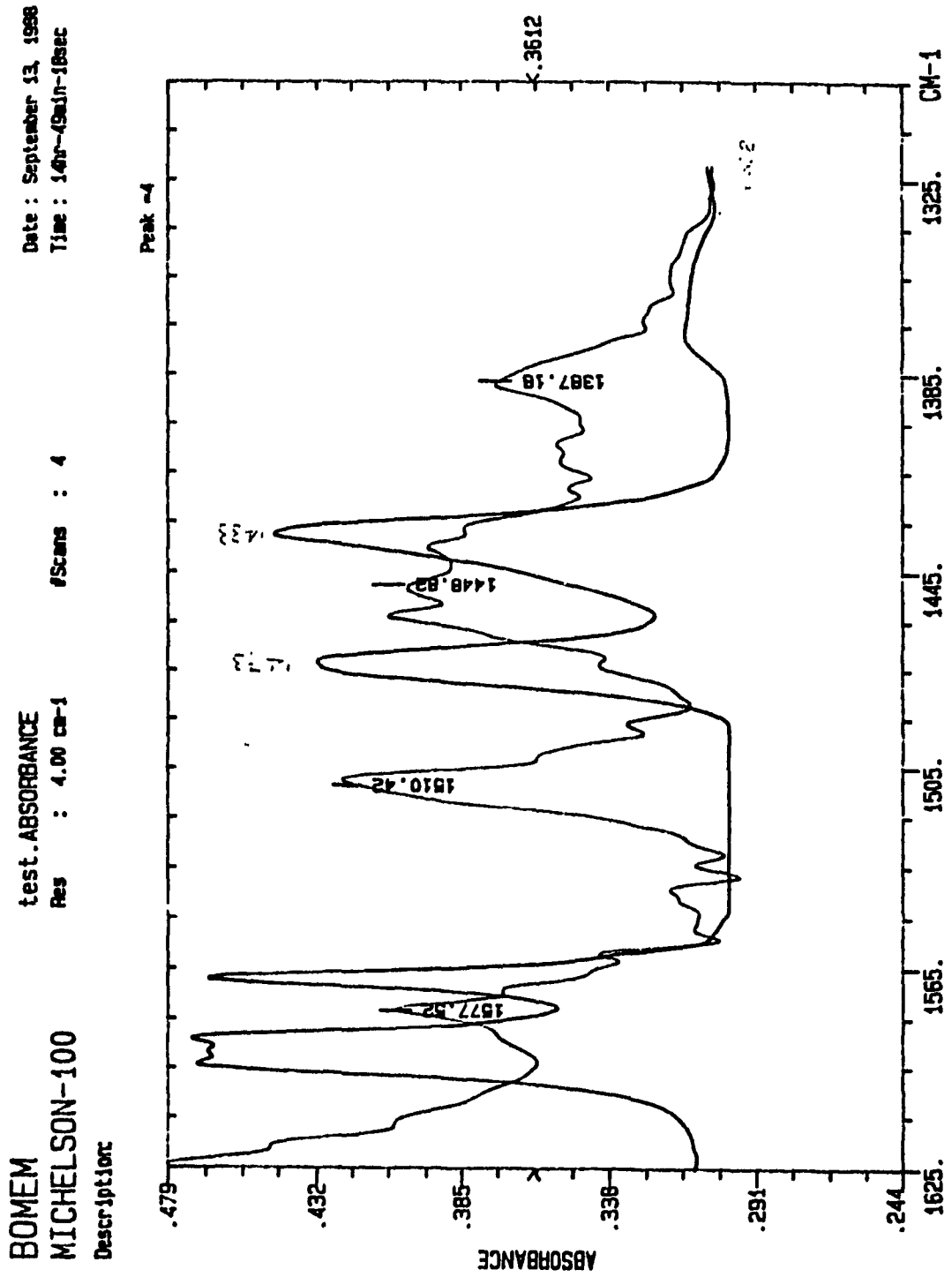



FIGURE 3.25. INFRARED DATA ON QUATERNIZED AND UNQUATERNIZED 2-PVMP.

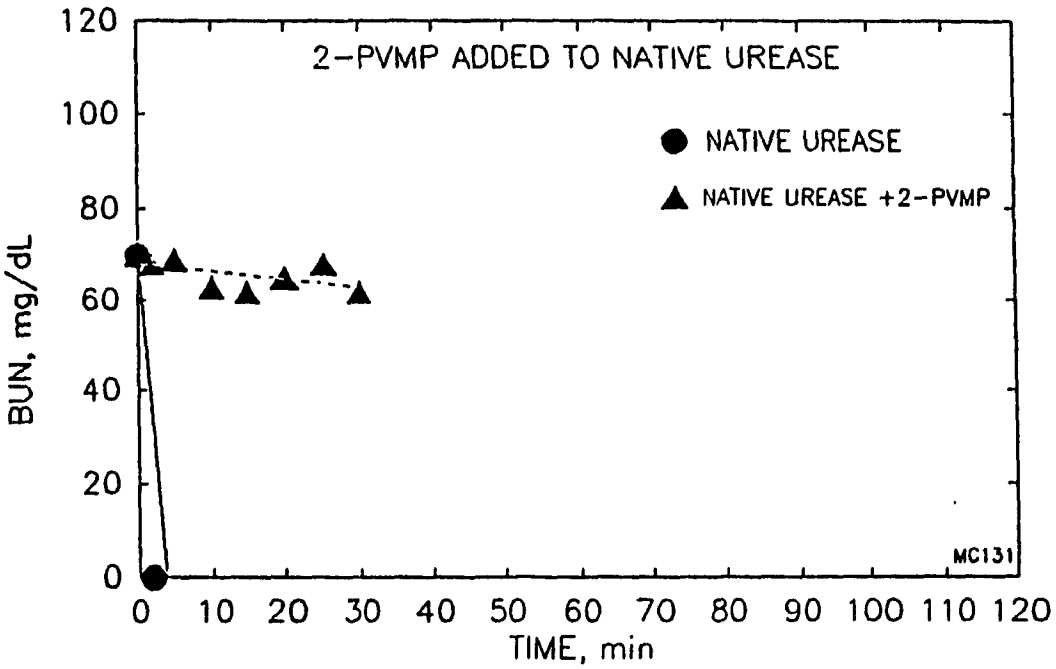



**FIGURE 3.26. 2-PVMP ADDED TO NATIVE UREASE. The BUN value remains at 70 mg/dl after exposure of native urease to the electrolyte 2-PVMP.**

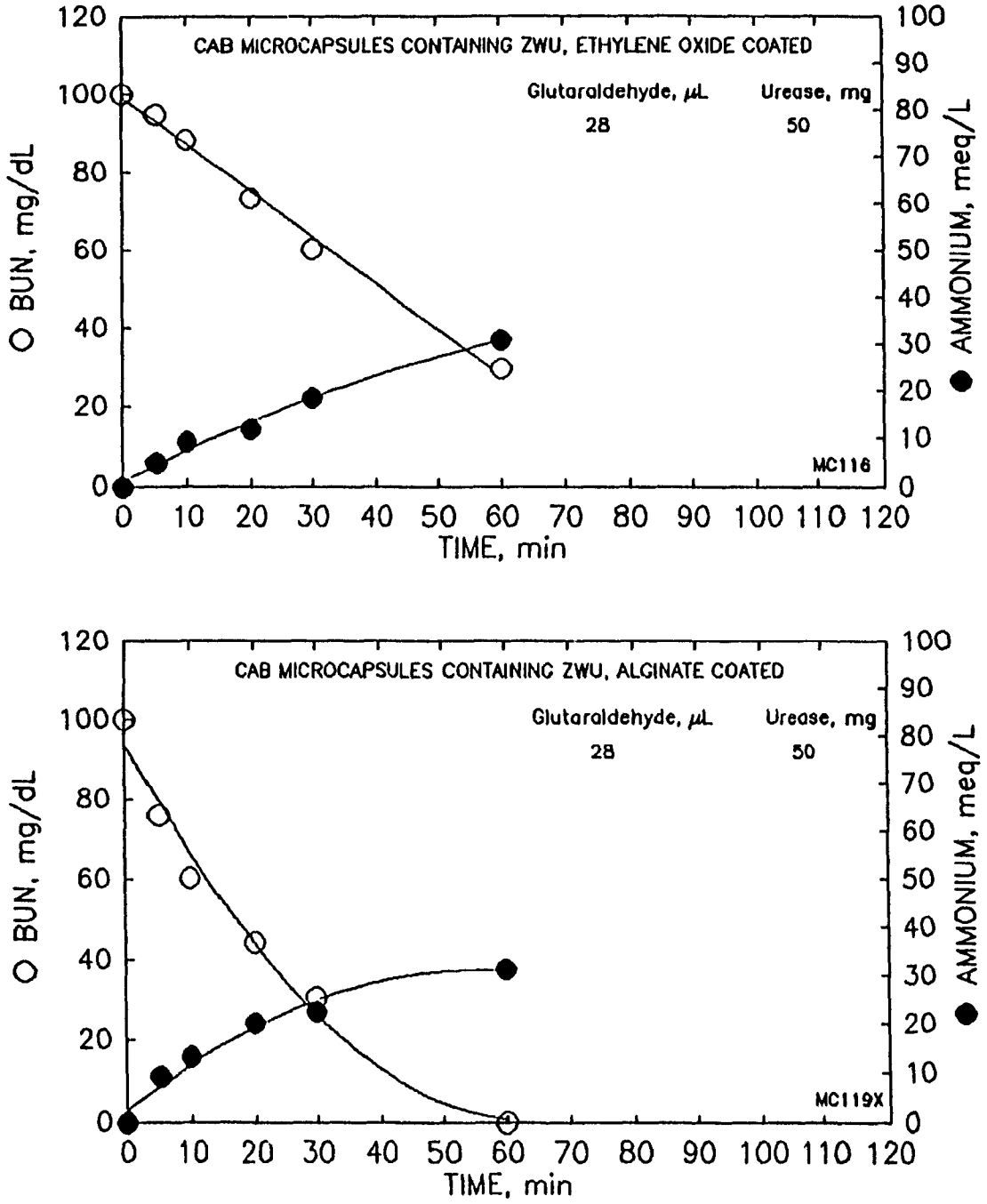



FIGURE 3.27. COATING OF CAB MICROCAPSULES. Ethylene oxide (figure 3.27a, top graph) and alginate (figure 3.27b, bottom graph) are applied as coatings. BUN and ammonium concentration are measured simultaneously.

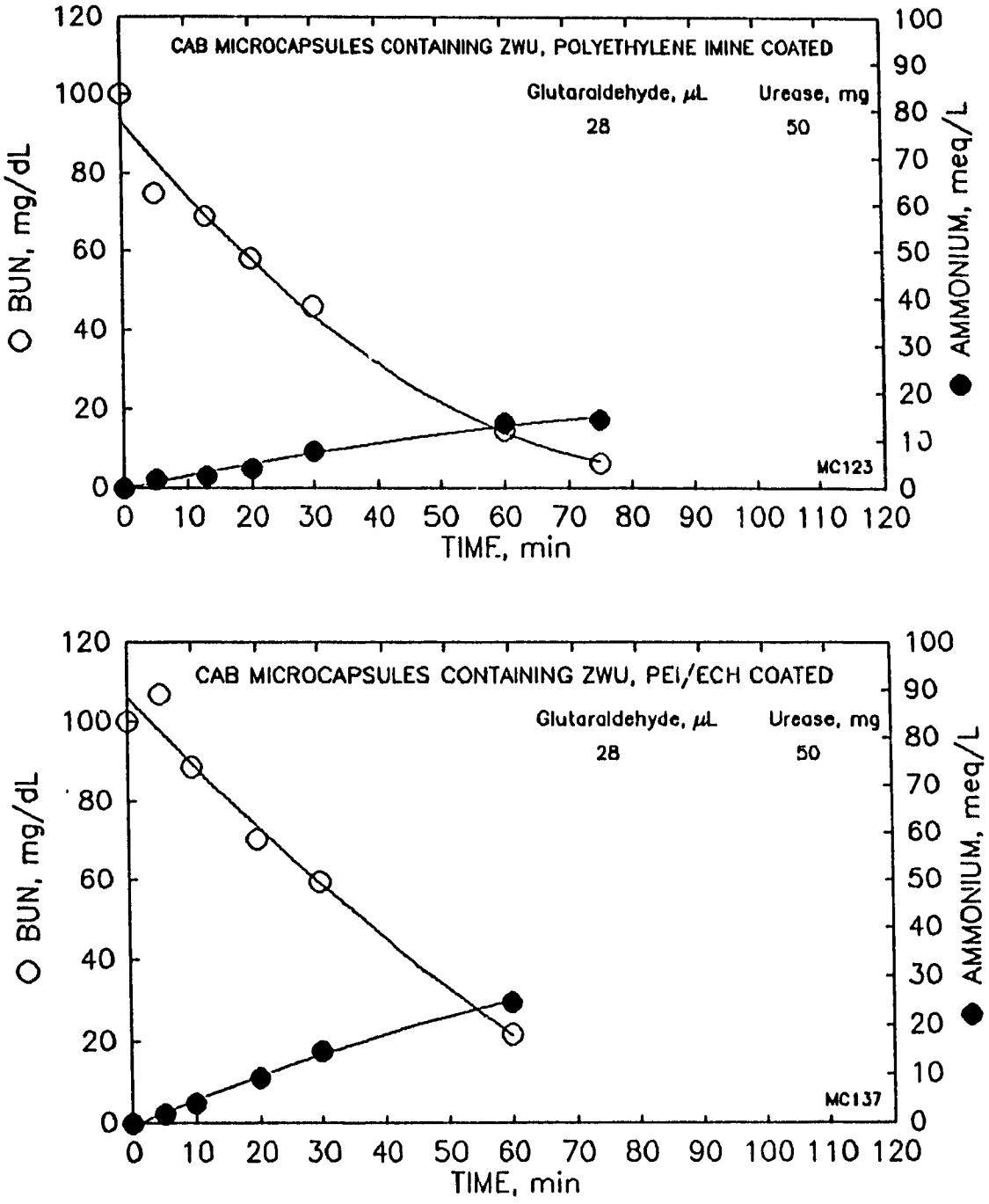



**FIGURE 3.28. COATING OF CAB MICROCAPSULES.** Polyethylene Imine (figure 3.28a, top graph) and polyethylene imine/epichlorohydrin (figure 3.28b, bottom graph) are applied as coatings. BUN and ammonium concentration are measured simultaneously.

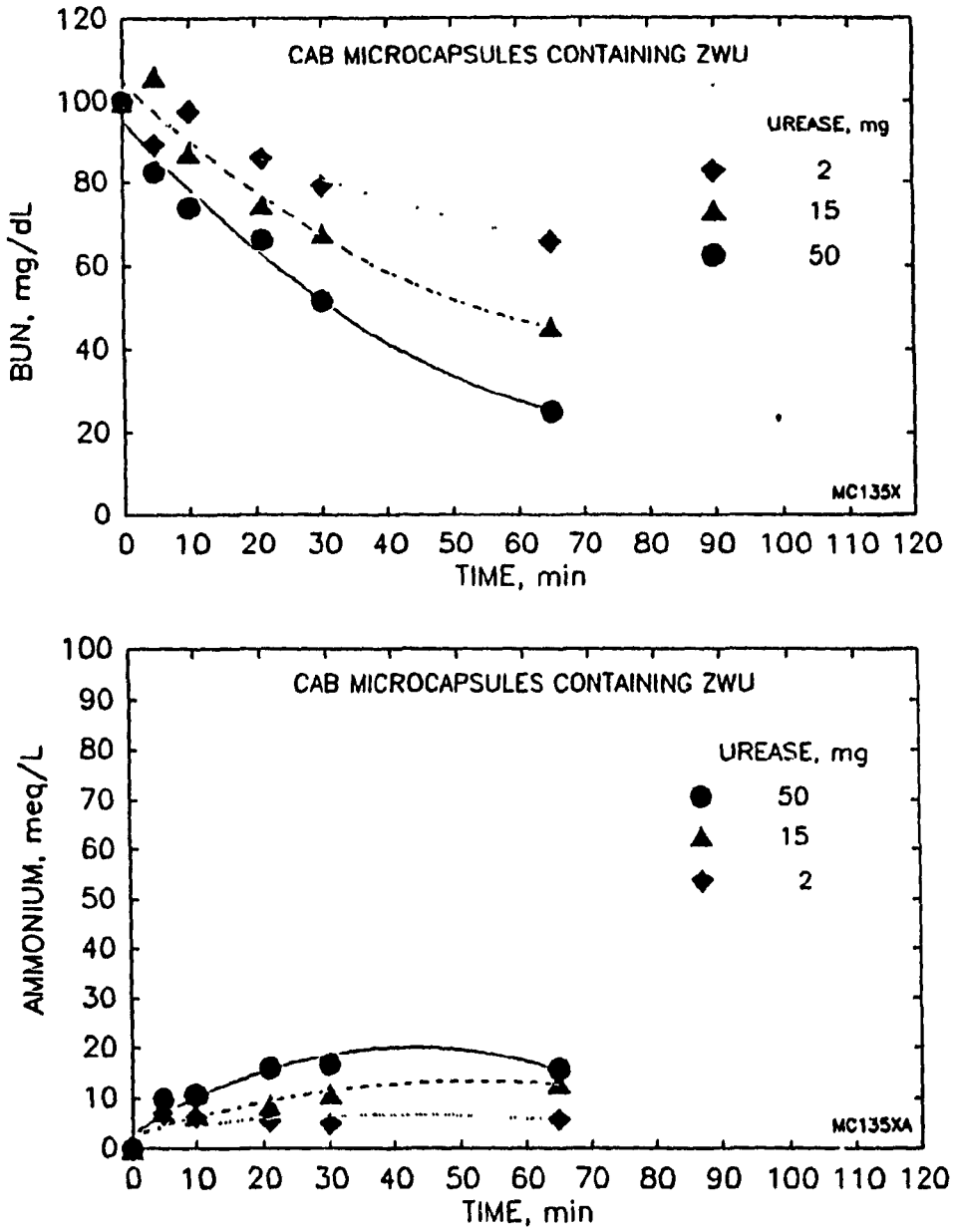



**FIGURE 3.29. CELLULOSE ACETATE BUTYRATE MICROCAPSULES CONTAINING THE UREASE-ZEOLITE PREPARATION. Urea.N (figure 3.29a, top graph) and ammonium (fig. 3.29b) are measured against time for different urease loadings.**

## 3.5 Cellulose Acetate Butyrate microcapsules with Polyethylene imine (PEI)

In figure 3.30 CAB microcapsules with equivalent amounts of urease are tested for PEI addition. A flaky suspension forms when PEI is added to ZWU suspended in a 3 ml phosphate buffer solution. With the addition of PEI, the suspension changes from colloidal to a gel-like substance. The rate difference between samples with and without PEI is significant. Obviously, PEI has changed the microenvironment in a favourable way. The urea concentration decreases rapidly to zero within the first hour of measurement (figure 3.30a). The ammonium concentration is limited to less than 20 meq/L. The CAB microcapsules are removing 80% of urea in one hour or less.

## 3.6. The effect of particle size

Figure 3.31 shows the effect of particle size on the ammonium rate. The 233 and 118 μm curves apply to CAB microcapsules. The 1-20 μm curve is for ZWU envelopes prior to microencapsulation. Evidently, the size of the particles is of primary importance. It affects diffusion, hence the reaction rate. When the initial ammonium flux derived from figure 3.31 is plotted against particle size we obtain an inverse relationship, as shown in figure 3.32a. A semilog plot of these data produces a straight line (figure 3.32b).

Lower enzyme loadings and PEI addition counteract the

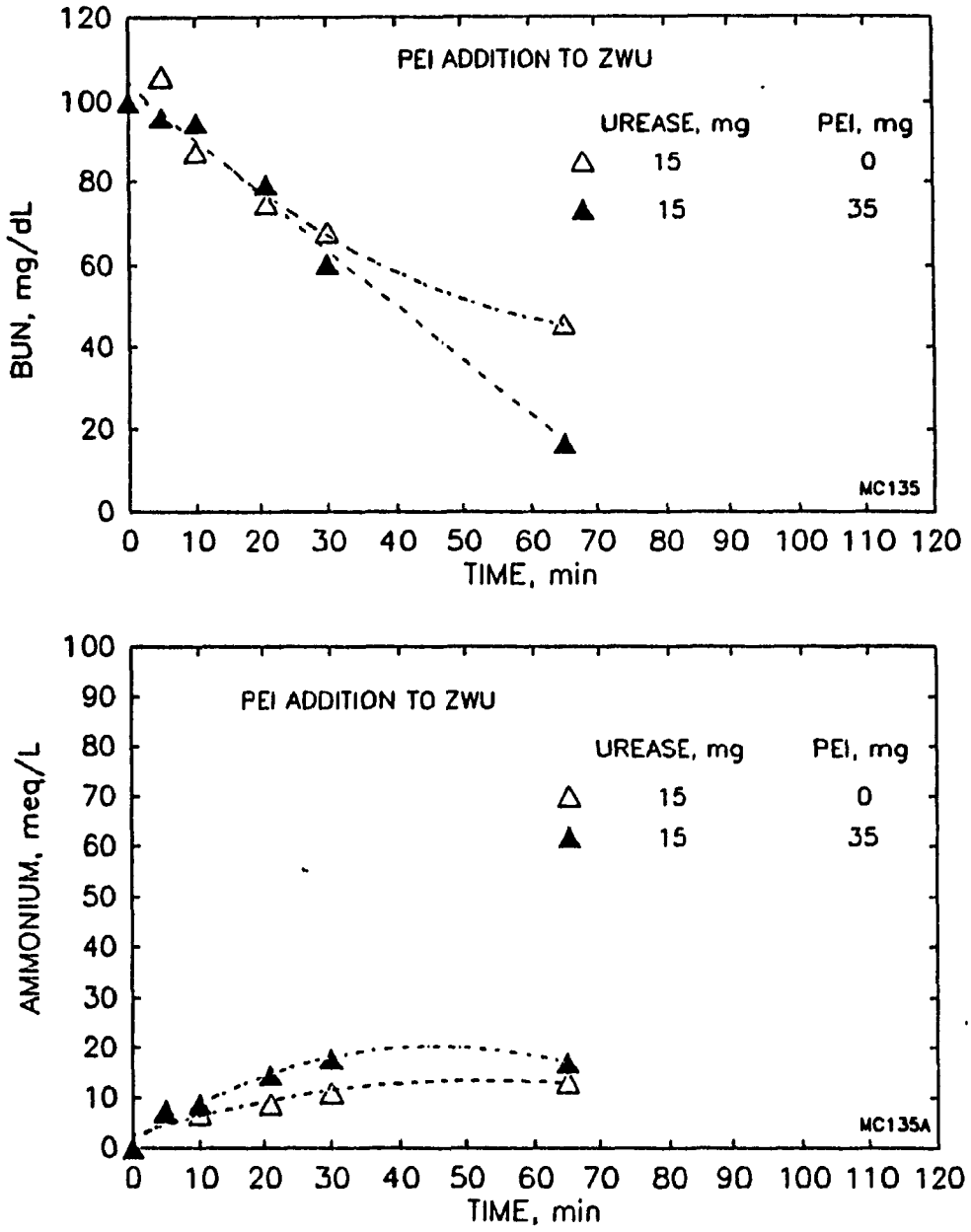



**FIGURE 3.30. POLYETHYLENE IMINE ADDITION TO UREASE-ZEOLITE PREPARATION PRIOR TO MICROENCAPSULATION.** Urea.N (figure 3.30a, top graph) and ammonium (fig. 3.30b) are measured against time for CAB microcapsules with and without PEI.

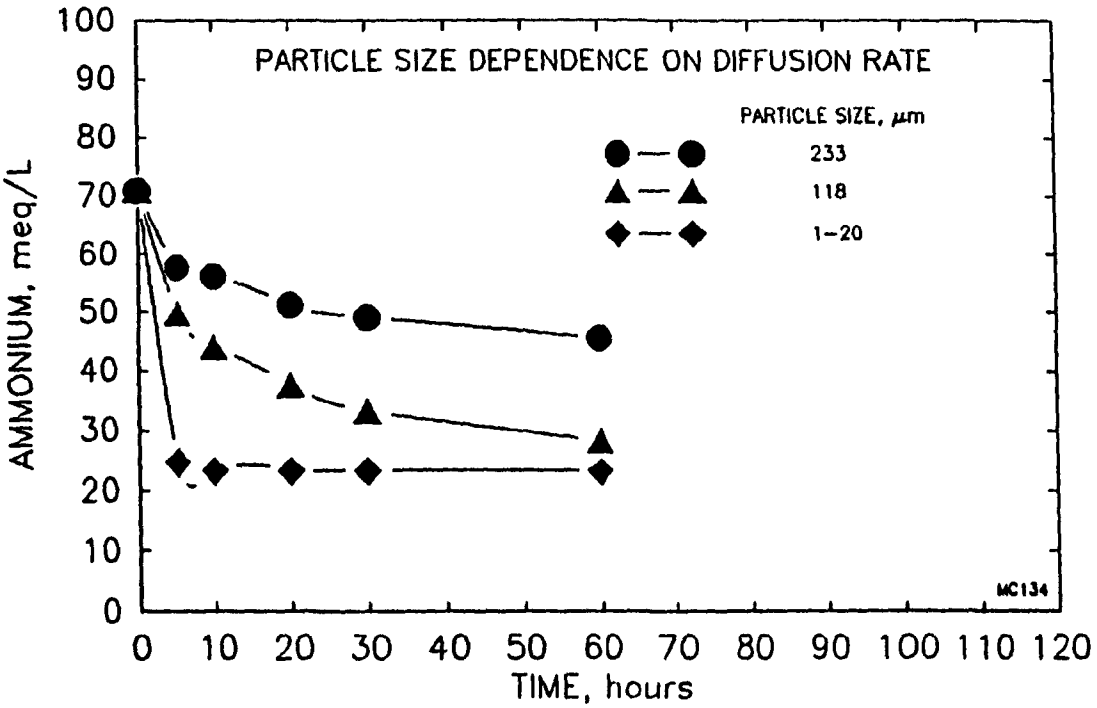



**FIGURE 3.31. PARTICLE SIZE DEPENDENCE ON THE DIFFUSION RATE.**

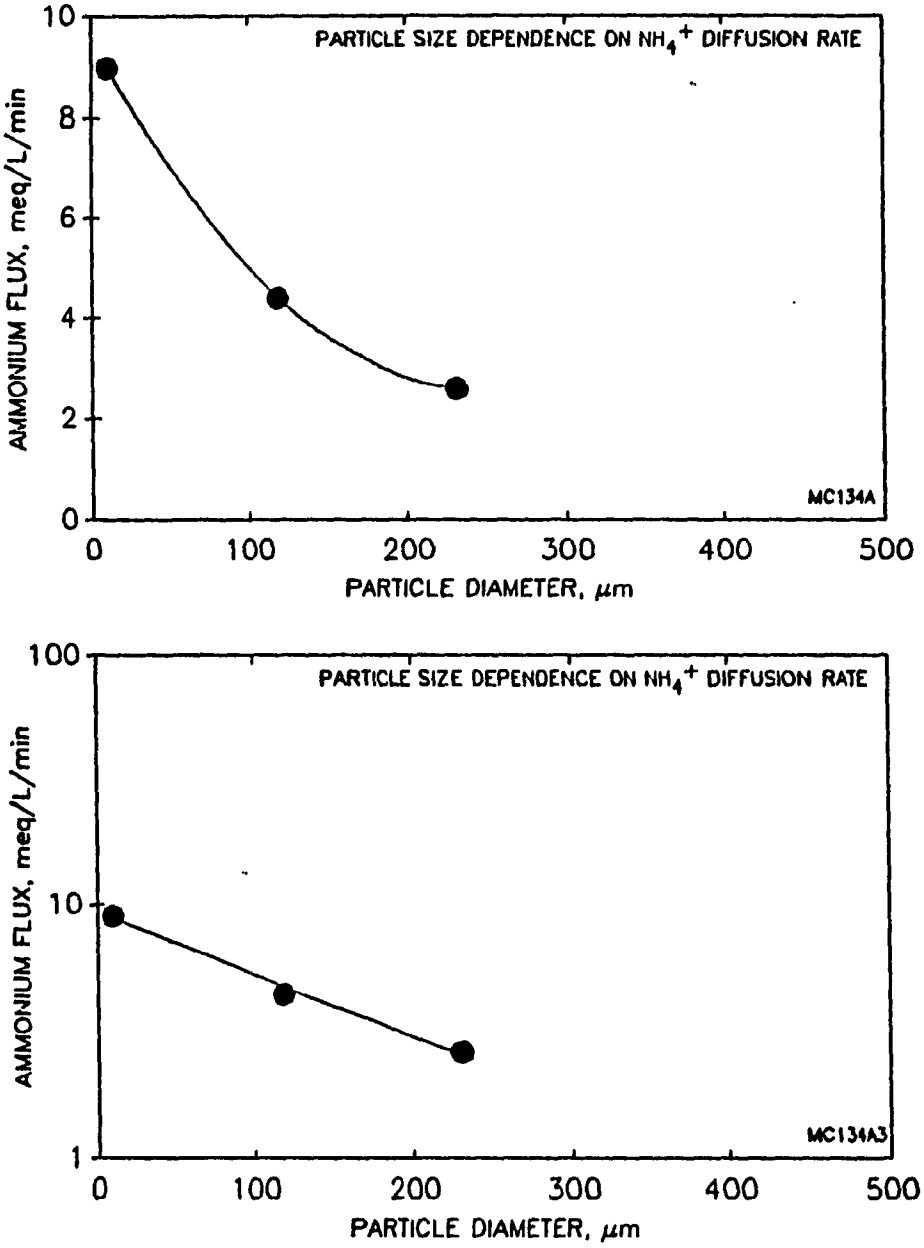



**FIGURE 3.32. PARTICLE SIZE DEPENDENCE ON THE DIFFUSION RATE. A normal (fig.3.32a, top) and semilog plot (fig. 3.32b, bottom graph) relate the ammonium flux into CAB microcapsules with the particle diameter.**

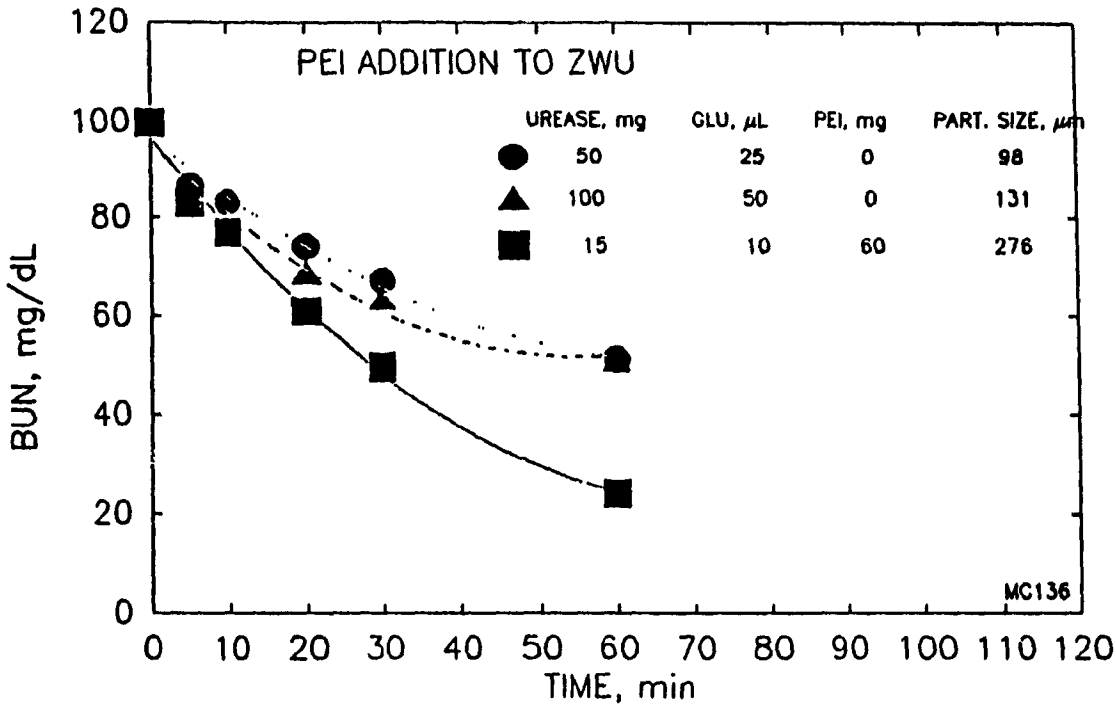



**FIGURE 3.33. POLYETHYLENE IMINE ADDITION TO CELLULOSE ACETATE BUTYRATE MICROCAPSULES. Particle size was the additional variable included in these studies.**

effect of particle size. In figure 3.33 microcapsules containing Polyethylene imine (PEI) show a marked improvement in urea reaction rate. This improvement is the more significant since these microcapsules contain less urease and have larger particle sizes, two factors that tend to reduce urea reactions.

### 3.7. CAB microcapsules in a haemoperfusion device

CAB microcapsules packed in a haemoperfusion shunt reduce urea levels to 30% of the original value in 16 hours of operation (figure 3.34). Unfortunately, the ammonium build-up is not within physiologically acceptable levels. More work on how to limit ammonium release is required.

### 3.8. Preliminary in vivo experiments

Preliminary in vivo experiments were conducted on 5/6 nephrectomized rats having urea levels up to 100 mg/dL BUN to simulate kidney diseased humans. The high BUN levels were obtained by constricting arterial blood flow to their kidneys, as detailed in the experimental section. The rats were given orally, by gastric tube, a mixture of zeolite F and Urease microcapsules (ZFU) once, sometimes twice per day. A 2 days administration of ZFU shows a 25% reduction in urea levels, down to 42 from 58 mg/dL BUN for rat 21 (figure 3.35, day 10-

12). This is a good indication of the efficacy of collodion urease microcapsules and zeolite F as a urea oral sorbent. When taken off the mixture of zeolite and microencapsulated urease the rat resumes the higher 70 mg/dL BUN levels. Again, feeding ZFU at day 20 drops urea levels by 15% down to 85 from 100 mg/dL BUN. A similar response is obtained for rat 31 (figure 3.36).

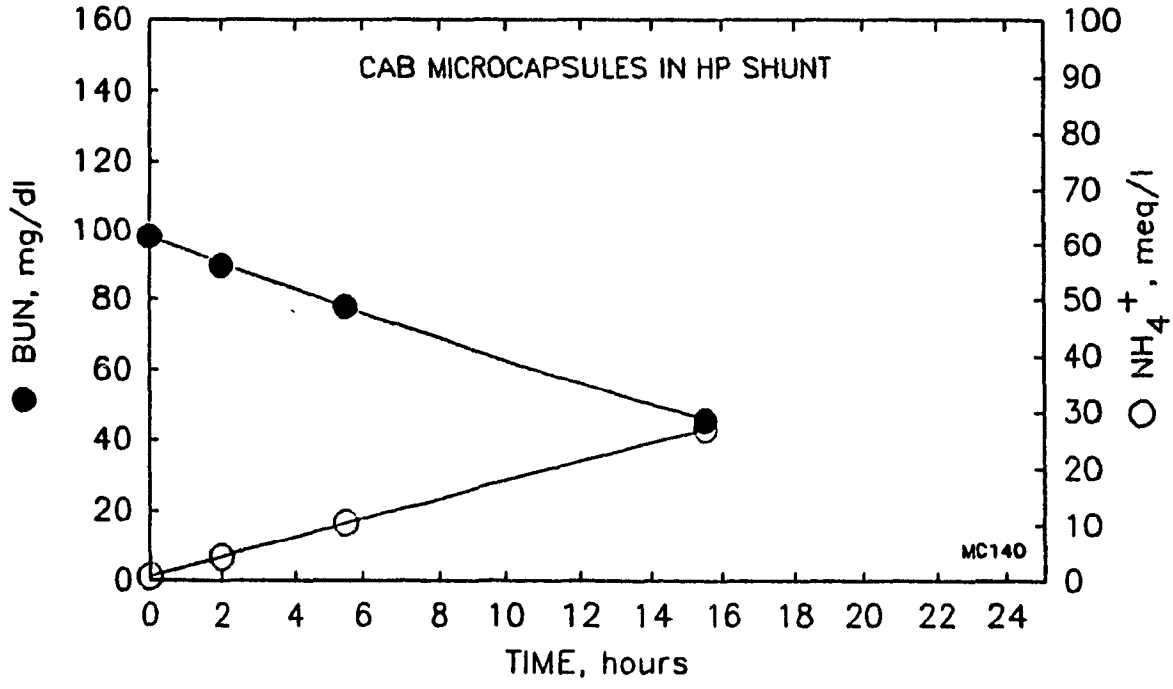



**FIGURE 3.34. CAB MICROCAPSULES INCORPORATED IN A HAEMOPERFUSION SHUNT.**

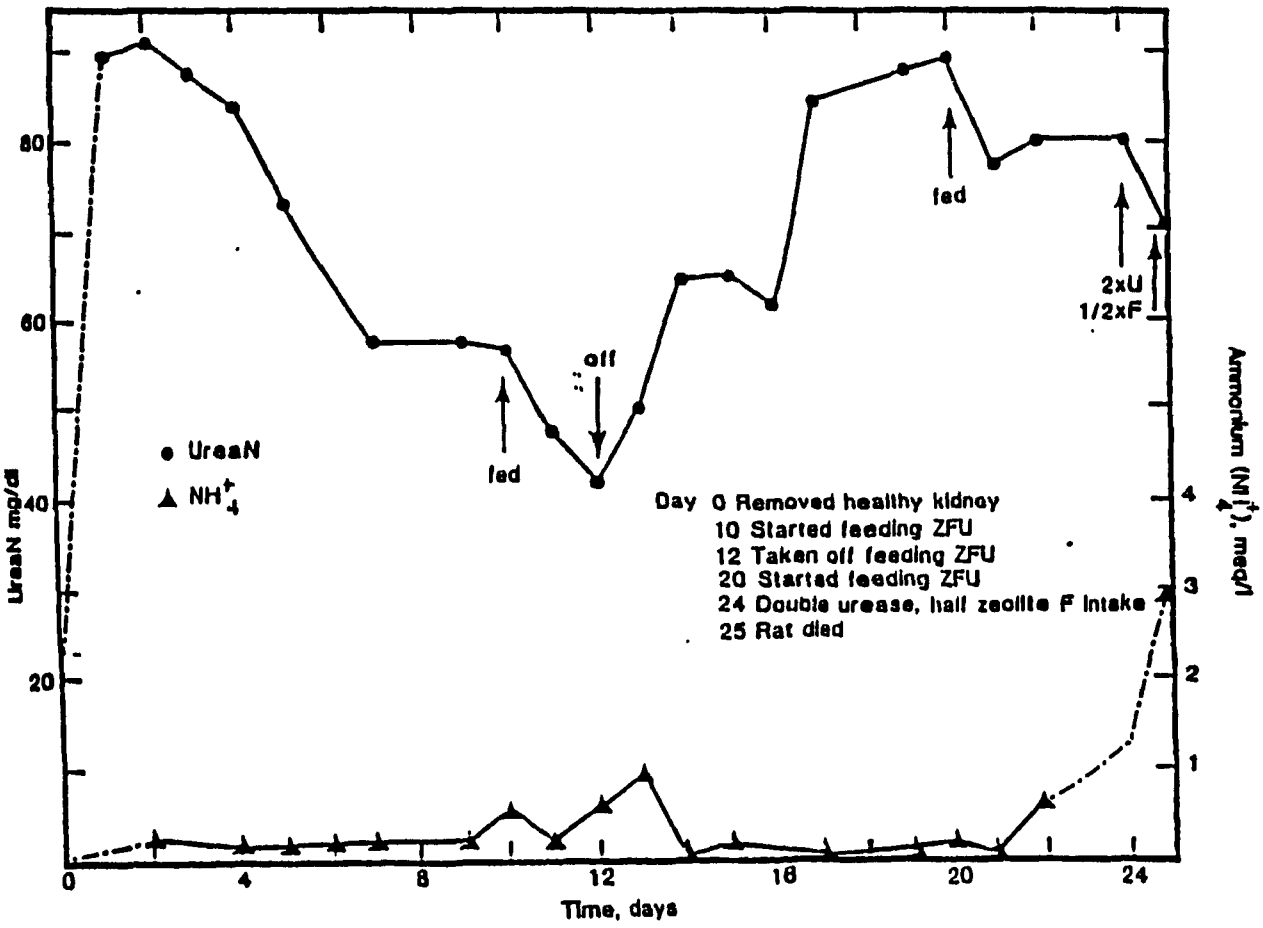



FIGURE 3.35. BLOOD UREA NITROGEN LEVELS AS A FUNCTION OF TIME IN URAEMIC RAT 21 (Technicon MC0022-0026).

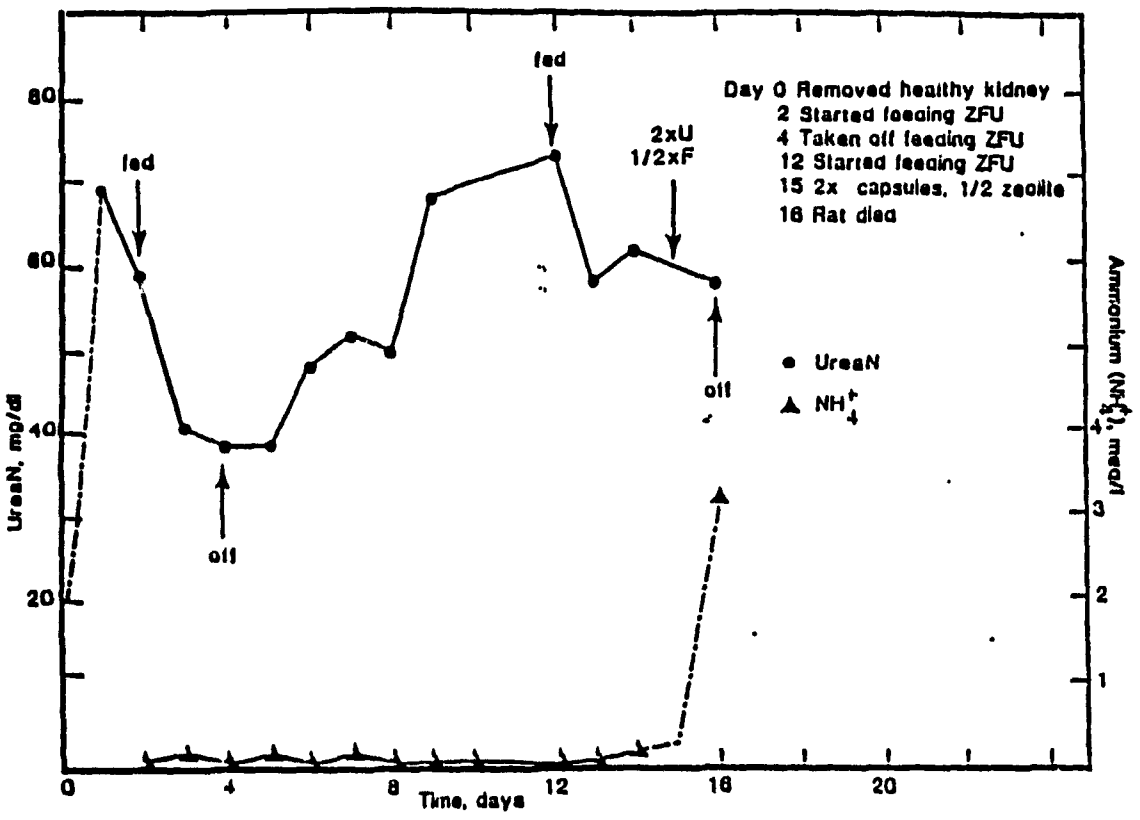



FIGURE 3.36. BLOOD UREA NITROGEN LEVELS AS A FUNCTION OF TIME IN URAEMIC RAT 31 (Technicon MC0022-0026).

# DISCUSSION

## 4. DISCUSSION OF RESULTS

### 4.1 Feasibility of sorbent therapy in uraemia

The two main questions that have to be answered before undertaking serious studies on oral sorbent therapy are: (1) Does the gut have sufficiently elevated urea concentrations? and (2) Is the clearance of urea across the intestinal membrane sufficiently high? The first question deals with equilibrium and sorbent capacity considerations, the second is important from a rate or kinetics standpoint.

Gut aspiration studies and triple lumen studies indicated that urea was indeed a compound which could be removed via an intestinal sorbent. To answer the first question, Gardner had shown that the concentration of urea in the small intestine was 70% of the serum urea concentration (Gardner, 1984):

TABLE 4.1

Comparison of serum and gut urea concentration in two segments of the intestinal tract (Gardner, 1984).

| | Duodenum | | Jejunum | |
|---|---|---|---|---|
| | Control | Uraemic | Control | Uraemic |
| Serum urea-N, mg% | 20 | 100 | 20 | 100 |
| Gut urea-N , mg% | 14 | 66 | 19 | 86 |

The extremely low value of urea clearance across the intestinal wall reported by Gotch (3.5 ml/min; Gotch, 1976), raised many doubts on the future of oral sorbent therapy for the removal of urea.

It is true that the urea clearance into the lumen of the large intestine is negligible and therefore the large intestine cannot be used for binding urea (Wolpert, 1971). Fortunately, the low urea clearance quoted by Gotch and reported by Wolpert was found to refer to the large intestine and not the small one.

Indeed, Sparks reviewed the literature and found a much higher urea clearance of 31.4 ml/min, which clearly indicates that a significant amount of urea can be removed from the small intestine, providing the availability of an effective urea-binder (Sparks, 1979). In addition, Gardner had confirmed, in further experiments on uraemic dogs, that large quantities of urea do move into the small intestinal tract (Gardner, 1984). His value of 33 g urea/d excreted into a 10 ft length of small intestine is nearly identical to the value obtained by Sparks (70 g urea/d for 21 ft), who derived it from reference data (Sparks, 1979).

## 4.2 Microencapsulated urease and zirconium phosphate oral sorbent

In 1970 Chang and Lo administered microencapsulated urease and a Dowex ion exchanger zirconium phosphate to reduce urea levels in rats. One year later Gardner experimented on a urease-zirconium phosphate microcapsule "cocktail" to act as an oral sorbent to remove urea (Gardner et al 1971, 1975). He used a combination of urease immobilized on silica and ion exchanger zirconium phosphate. Urea in the intestinal tract would react in the presence of microencapsulated urease enzyme to form ammonium:

$$\text{urea} + H_2O \xrightarrow[\text{urease}]{} 2NH_4^+ + CO_3^{=} \qquad (4.1)$$

The ammonium would exchange for sodium inside the microcapsules containing zirconium phosphate:

$$NH_4^+ + ZP.(Na^+) \longrightarrow Na^+ + ZP.(NH_4^+) \qquad (4.2)$$

Gardner succeeded in testing urea sorbent microcapsules in patients in 1981 (Kjellstrand et al, 1981). He showed a reduction in urea values in patients from 75 down to 60 mg/dL. Unfortunately, the patients started complaining of nausea, hard stools, heart burns and hypertension. One serious problem was the depletion of bodily calcium. Calcium depletion is

especially deleterious in kidney failure patients. We wanted to avoid some of the problems associated with his experiments. Some of these complications may be due to the ion exchanger zirconium phosphate. Zirconium phosphate has a high affinity for calcium ions and is not very stable at the high pH found in the intestinal tract.

We were determined to find a replacement for zirconium phosphate, something that would not bind calcium, would not hydrolyse at intestinal pH, and be of higher capacity per unit volume. The result reported in this thesis is to use a different ammonium adsorbent, a zeolite. Different approaches to immobilizing urease were also studied.

## 4.3 Zeolite Selection

The ammonium capacity of a zeolite depends on many factors, namely the surrounding ammonium concentration, other competing ions such as sodium, potassium, calcium, etc. present in solution, and the temperature. The two main contributors to the ion exchange capacity are ion sieving and ion selectivity effects.

### Ion Sieving

When the ion to be exchanged is larger than the pore openings, the ion is totally or partially excluded from the zeolite interior. In the case of clinoptilolite the ions shown ($Ca^{2+}$, $NH_4^+$, $Na^+$, $K^+$) all have diameters less than the pore openings and the full capacity of the exchangers is utilized by $NH_4^+$, ie. there is full exchange between the ionic species (see table 4.2 and 4.3 and figure 4.1).

Table 4.2 Typical properties of some zeolites (Sherman,1978a)

| Zeolite Type | Pore Openings, Å | Si/Al | Max. Theor. Ion Exch. Capacity meq/g |
|---|---|---|---|
| Clinoptilolite | 4.0x5.5, and 4.4x7.2, and 4.1x4.7 | 5 | 2.6 |
| Phillipsite | 4.2x4.4, and 2.8x4.8, and 3.3 | 2.2 | 4.7 |
| Linde A | 4.2, $\alpha$-cage 2.2, $\beta$-cage | 1 | 7 |
| Linde F | 3.7 | 1 | 7 |
| Linde W | 4.2x4.4 | 1.8 | 5.3 |
| Linde X | 7.4, $\alpha$-cage 2.2, $\beta$-cage | 1.25 | 6.4 |
| Linde Y | 7.4, $\alpha$-cage 2.2, $\beta$-cage | 2.4 | 4.4 |

Table 4.3 Some data on selected ions

| Ion | Dia., Å(i) | -ΔH, Kcal/g ion (ii) |
|---|---|---|
| $Na^+$ | 1.94 | 203.294 |
| $K^+$ | 2.66 | 183.11 |
| $NH_4^+$ | 2.86 | - |
| $Cs^+$ | 3.38 | 169.281 |
| $Ca^{2+}$ | 1.98 | 593.41 |

(i) Pauling's crystal diameters (Sherry, 1969)

(ii) Enthalpy of hydration (Sherry, 1969)

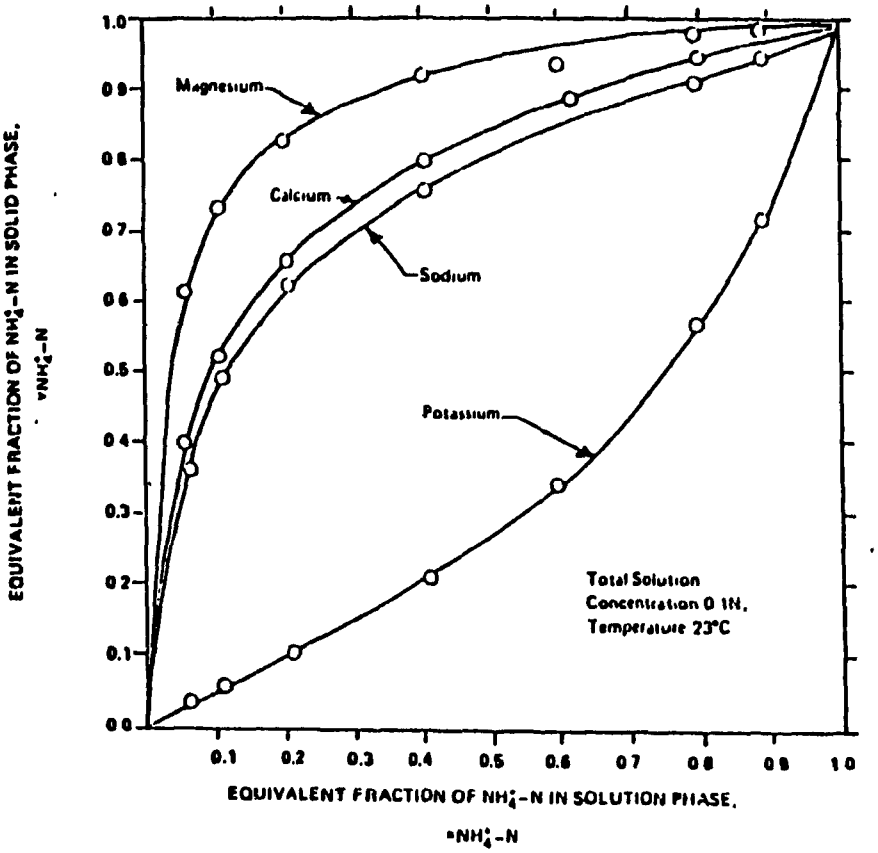



Fig.4.1 Isotherm for exchange of $NH_4^+$ for $K^+$, $Na^+$, $Ca^{2+}$ (Sherman, 1978) on clinoptilolite.

Zeolite Y, on the other hand, has two three-dimensional channels, one with pore openings of 7.4 Å into the $\alpha$-cages and the other with pore openings of 2.2 Å into the $\beta$-cages. If the ion to be exchanged has a diameter greater than 2.2 Å it will not be able to penetrate the $\beta$-cage channel, and the ion exchange capacity for this ion will be reduced accordingly.

For example, cesium ion (diameter 3.38 Å, table 4.3) can easily penetrate the $\alpha$-channel but is totally excluded from the $\beta$-channel. As a result ion exchange with cesium terminates at 68% capacity:

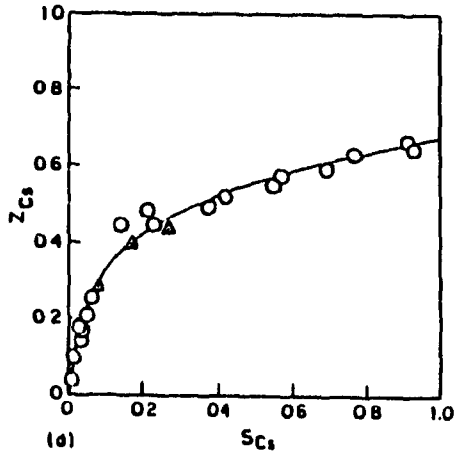



Fig.4.2 Isotherm for exchange of $Cs^+$ for $Na^+$ (Sherry, 1969)

The ions involved in our studies that are present in significant quantities in "simulated-plasma" solutions such as $Na^+$, $K^+$, $Ca^{2+}$ and $NH_4^+$ are easily accessible to the ammonium selective zeolites Clinoptilolite, Phillipsite, Linde W and F. As a result, the full theoretical ion exchange capacity is available. Sherman has indeed shown such a behaviour (Sherman, 1978a, 1978b, 1980, 1981, 1983).

Now that we have established selected ions such as ammonium, sodium and potassium can easily penetrate the zeolite interior, further differences in capacity between different zeolites are due to selectivity effects.

## Ion Selectivity

The first comprehensive treatise on ion selectivities for glasses and extended to zeolites has been given by Eisenman (Eisenman, 1962).

Eisenman divided the free energy of the ion exchange reaction in two components, an electrostatic component involving the ions and the fixed charged sites on the solid and an hydration component:

$$G = (G_A^{el} - G_B^{el}) - (G_A^{hyd} - G_B^{hyd}) \qquad (1)$$

for the reaction:

$$A^+ + B^+_{ex} = A^+_{ex} + B^+$$

He then assigned a value for the electrostatic energy based upon Coulomb's Law:

$$F = K*q^2/(r+ + r-) \qquad (2)$$

where:

K= 1 for widely spaced exchange sites

q= electron charge, Kcal/mole

r+= radius of cation, Å

r-= radius of anion, Å

Knowing the free energy of hydration from both enthalpy (see table 4.3) and entropy terms, Eisenman was able to calculate values of the free energy exchange and summarize them as shown below for the ion exchange between alkali and sodium ions (Sherry, 1969) (figure 4.3).

Zeolites with low values of $r^-$ (high ionic field strength and high ion exchange capacity are those with a higher proportion of aluminum (lower Si/Al ratio in table 4.2). As we move from left to right we move in the direction of decreasing field strength (higher Si/Al ratio) and consequently lower exchange capacity. Linde A and X exhibit the selectivity pattern of group XI, the high field strength exchangers (Si/Al=1), while Phillipsite and W exhibit the weak field pattern I (Si/Al=2). In the weak field pattern the

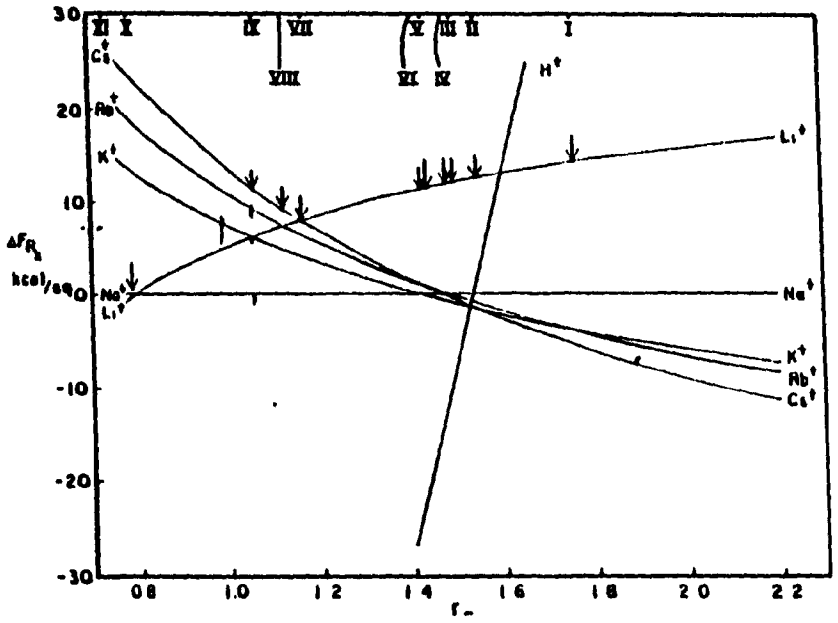



Fig.4.3 Selectivity for alkali other than sodium.

The 11 selectivity series that are resolved are listed in table 4.4.

Table 4.4. Selectivity series predicted for closely spaced sites by varying anionic field strength from figure 4.3.

| Groups | |
|---|---|
| I. | $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$ |
| II. | $Cs^+ > K^+ > Rb^+ > Na^+ > Li^+$ |
| III. | $K^+ > Cs^+ > Rb^+ > Na^+ > Li^+$ |
| IV. | $K^+ > Cs^+ > Na^+ > Rb^+ > Li^+$ |
| V. | $K^+ > Na^+ > Cs^+ > Rb^+ > Li^+$ |
| VI. | $K^+ > Na^+ > Rb^+ > Cs^+ > Li^+$ |
| VII. | $Na^+ > K^+ > Rb^+ > Cs^+ > Li^+$ |
| VIII. | $Na^+ > K^+ > Rb^+ > Li^+ > Cs^+$ |
| IX. | $Na^+ > K^+ > Li^+ > Rb^+ > Cs^+$ |
| X. | $Na^+ > Li^+ > K^+ > Rb^+ > Cs^+$ |
| XI. | $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$ |

electrostatic term of equation 1 can be neglected and the energy of hydration determines the selectivity. In this case the fixed ion exchange sites compete with water in attracting cations. Large cations such as ammonium, of lower energy of hydration than sodium, will be preferentially attracted to the zeolite.

The difference in selectivity can be readily observed in figure 3.1b. Zeolites X, Y, A and W were tested for ammonium uptake. When the solution contains sodium as a competing cation only zeolite W maintains high ammonium uptakes. Clearly, zeolite W outperforms the other zeolites in ammonium selectivity.

In figure 3.5 the ammonium selective zeolites F and W are compared against zirconium phosphate (ZP) for ammonium removal. This is a graph of ammonium concentration in milliequivalents per litre versus time over a 24 hour period. Zeolites reach equilibrium in minutes versus hours for ZP. The rapid kinetics may be advantageous in the small intestine where the sorbent's residence time is of the order of a few hours. The urea clearance across the intestinal membrane is highest in the small intestine (Gardner, 1984; Sparks, 1979). After the sorbent has passed the small intestine the urea clearance drops precipitously from about 30 ml/min down to 3 ml/min in the large intestine (Walser, 1974).

The field strength distinguishes ammonium selective zeolites as well. The ammonium kinetics of both zeolites F and

W in the calcium form are shown in figure 3.6. Electrostatic forces will have a greater effect on zeolite F than on W since F has a higher charge density (Si/Al=1 for F versus Si/Al=1.8 for W, table 4.1). The break in the curve around 50 meq/l shows that ammonium has difficulty exchanging and that calcium is held tight within zeolite F (figure 3.6a). The presence of calcium greatly affects zeolite F's ammonium performance since calcium wants to occupy potential ammonium sites. Column experiments performed by Union Carbide also indicate a loss of ammonium capacity for zeolite F after repeated exposure to calcium (Sherman, 1980). On the other hand, zeolite W's higher ammonium selectivity and weak field strength permits ion exchange with ammonium regardless of calcium. The ammonium uptake curve follows a smooth downward path (figure 3.6b). Zeolite W's selectivity pattern is attractive, especially for oral sorbent therapy in kidney failure patients.

Ion selectivity is particularly important in maximizing the available ion exchange capacity. The selectivity series for zeolite W is as follows:

$$K^+ > NH_4^+ > Na^+ \qquad \text{(group I)}$$

If the zeolite is loaded with a preferred ion, say potassium, it will be very difficult to displace it with ammonium, and

the ammonium capacity will suffer. On the other hand, if sodium is present as counter ion, its exchange for ammonium will be favourable. A zeolite should be loaded with the least preferred ion prior to ammonium exchange. Potassium is highly preferred and should be replaced by sodium prior to ammonium exchange to fully exploit the zeolite's selectivity pattern.

Ammonium capacity curves for zeolite W are shown in figure 3.7. The plots in these figures differ in the severity of ion exchange with NaCl, prior to ammonium exchange. Zeolite W is available in a 50/50 $Na^+/K^+$ powder form. Since the maximum theoretical capacity is 5.3 meq/gm (table 4.2) and half the sites are occupied by $K^+$ ions, the maximum ammonium capacity expected was half the theoretical or 2.7 meq $NH_4^+/g$. In run MC144 the zeolite was tested "as is", without prior $Na^+$ exchange, and the zeolite displayed a maximum ammonium capacity of 2.6 meq/g dry zeolite.

Ion exchange of potassium with sodium should improve the ammonium exchange capacity. J.D. Sherman of Union Carbide converted zeolite W to the $Na^+$ form after ion exchange with 60 litres of 0.1 N NaCl down a column packed with the zeolite (top curve -empty circles, Sherman, 1978, figure 3.7). This method was possible because Union Carbide developed an experimental 20x50 mesh (297-841 μm) particle. The particle contained zeolite as well as support material, something undesirable for our purposes. Unfortunately, column ion exchange is difficult with zeolite powder (1-20 μm) because

of the high flow resistance through a packed bed. With only zeolite powder available we had to settle for a batch operation. As shown in figure 3.7a the ammonium capacity for the MC148 sample is not as high as Sherman's. We obtained a maximum capacity of 3.6 meq/g whereas Sherman's value approaches 5.0.

### 4.4 Enzyme Envelopes on Zeolites

Gardner immobilized urease on silica to form enzyme envelopes, according to the procedure by Haynes (Haynes, 1969), to obtain stable enzymes which could be trapped inside microcapsules. Haynes found that optimum performance was obtained when a monolayer of enzyme was formed around a particle. Additional layers had little effect on the rate of reaction. Experimentally, in the case of trypsin Haynes found a monolayer to form at approximately 150 mg of enzyme per 250 mg of silica particles. Theoretically, given a surface area of 220 $m^2/g$ (I.E. Dupont, technical bulletin), a molecular diameter of trypsin of 40 Å and trypsin molecules packed as rigid spheres, the number of molecules per gram of silica is: $220/(3.14 * 20\ E\text{-}10)^2$. Multiplying by the molecular weight over Avogadro's number (23000/6.02 E+23) gives 150 mg of protein per 250 mg of silica, which confirms Haynes's experimental value.

For urease the effect of enzyme loading on silica is shown in figure 3.10. Note the molecular weight difference between

urease and trypsin (Trypsin MW = 23000, Urease MW = 482,700). The amount of urease was varied from 12 to 100 mg per 250 mg to 500 mg of silica. The urea reaction rate is slow for urease loadings of 12 mg/500 mg silica. In this case there might be insufficient enzyme to form a monolayer on the support. Gardner's experiments with urease-silica adduct were done with 100 mg of urease per 250 mg of silica. According to the data in figure 3.10 this enzyme loading value would ensure fast urea reactions.

In taking the idea of enzyme envelopes further than previous workers, we thought of replacing the "useless" silica gel core with "useful" catalyst zeolite particles. These would not only serve as enzyme support but would also represent the ion exchanger component of the system. The zeolite and the enzyme combine to reduce the distance between enzyme and ion exchanger active sites, thus minimizing diffusional resistances. Enzyme envelopes can now be called "bio-catalyst" particles, particles that utilize both the enzyme and the core material.

Urea and ammonium reaction kinetics are shown in figure 3.11 for urease immobilized on zeolite F and on silica. For silica, the ammonium concentration is seen to rise toward the equilibrium value of 71 meq/l. All the ammonium produced by the urea conversion reaction is released into solution. On the other hand, with zeolite the ammonium concentration is limited to 20 meq/l (figure 3.11b). The urease-zeolite adduct

accomplishes both the task of removing urea and the task of eliminating ammonium, a definite plus over the urease-silica method. The urease-zeolite combination performs like a true urea sorbent in removing more than 70% of the urea present in the reaction vessel. In addition, the enzyme-zeolite adduct eliminates one component: silica. The amount of silica used by Gardner is estimated at around 25% of the urease-silica microcapsule preparation (Gardner, 1975). Without silica the amount to be ingested as oral sorbent decreases substantially. This is important when large amounts of oral sorbent may have to be ingested on a daily basis.

Larger zeolite particle sizes (10 μm for zeolite versus 0.01 μm for silica) produce a smaller surface area. Thus, enzyme loadings to form enzyme envelopes will be different. Urea and ammonium kinetics of urease immobilized on zeolite W (ZWU) are shown in figures 3.12a to 3.12f. Urease amounts vary from 1.5 to 100 mg per 500 mg of zeolite. For all urease concentrations beyond 12 mg/0.5 g of zeolite all urea was converted to ammonia within 10 minutes. On the basis of surface area, as we go from silica to zeolite we predict a decrease in enzyme loading (zeolite surface area = 2 $m^2/g$, derived from Union Carbide data on Linde W, particle size 1-20 μm, average 10 μm). With urease (approximate molecular diameter = 100 Å) we calculate an optimum loading of 20 mg per gram of zeolite or 10 mg per 0.5 g of zeolite, a result which is in fair agreement with experiment (12 mg per 0.5 g of zeolite, figure

3.12a).

These results show that the carrier crosslinking procedure with glutaraldehyde is valid for any particulate in addition to the colloidal sols of Haynes and Walsh. Ion exchangers are well suited for this type of immobilization since they readily fix the enzyme by physical adsorption. This makes it easier to allow the step of glutaraldehyde cross-linkage of the enzyme while it is already adsorbed to the particles.

Michaelis Constants for native and immobilized urease shown in figures 3.13b, 3.14a, 3.18a and summarised in table 4.5 are of the same order of magnitude.

Drastic changes in Km after immobilization (Chibata, 1978) are clearly not the case here. On the other hand, urease entrapped in liquid membrane microcapsules has a Km of 180 mM or two orders of magnitude greater than native urease (May and Li, 1972).

Table 4.5. Michaelis Constants for native and immobilized urease.

| Enzyme preparation | Km (mmol/l) |
|---|---|
| Native urease | 7 |
| Entrapping (Collodion microcapsules) | 7 |
| Carrier crosslinking silica gel + glutaraldehyde | 5 |
| Carrier crosslinking zeolite F + glutaraldehyde | 10 |
| Carrier crosslinking zeolite Y + glutaraldehyde | 4 |

## 4.5 Microencapsulation

Urease enzyme in collodion microcapsules convert urea to form ammonium which is subsequently ion exchanged by zeolite present in the external solution. Michaelis-Menten kinetics of urease collodion microcapsules is shown in figure 3.18. The Km value of 7 mM is in the same order of magnitude as native urease (figure 3.13b). In figure 3.19 both BUN and ammonium are measured as functions of time. Collodion microcapsules (0.5 ml) are placed in a 15 ml solution of 100 mg/dl BUN. As urea reacts, the ammonium concentration is seen to rise to its equilibrium value of 71 meq/l. On the other hand, the presence of zeolite F in the reaction vessel maintains the ammonium concentration low (20 meq/l, figure 3.19b). When Zirconium Phosphate (ZP) is added the ammonium concentration rises to a maximum before falling (figure 3.20). The rise in ammonium levels is not desirable in oral sorbent therapy because ammonium ions could be readsorbed. The ammonium concentration should be as low as possible, especially when there is rapid exchange with the circulation, as in the small intestine (Gardner, 1984). In vivo, ammonium has to travel a "long distance" to the zeolite interface outside the urease microcapsules before reaching the intestinal wall where it would return to the blood circulation. Chang (1969) and Sparks (1971) suggested the incorporation of the urea reaction and the ammonium exchange into a single system within a microcapsule. Gardner in 1975 incorporated both ammonium binder

zirconium phosphate and urease-silica adduct within the same microcapsule. Microencapsulation of the enzyme-zeolite preparation would provide a barrier between enzyme and the external environment, protect the enzyme and improve its stability, especially when exposed to the harsh conditions present within the gastro-intestinal tract.

The incorporation of both ammonium ion exchanger and urease within the same microcapsules has definite advantages in addition to the above: 1) the high ammonium concentration within the microcapsule -high concentrations of this ion are locally available for ion exchange with the zeolite since urease is continuously producing ammonium and 2) the potential of further transforming the membrane into an ion exchanger membrane which retains ammonium within the capsule.

To see whether the inclusion of ZWU in a matrix-like environment would trap ammonium ions, ZWU was incorporated within alginate microcapsules (figure 3.21). Both urea and ammonium react normally. Unfortunately, microencapsulation of the adduct by alginate was found to produce too much extra matrix material for ingestion. The experiment showed no particular advantage in using alginate. Perhaps the only benefit in using alginate is that the microcapsules are hard and can easily be packed in a column without significant pressure drop.

ZWU was then microencapsulated within a cellulose acetate butyrate membrane. Urea easily crosses the microcapsule

membrane and the reaction is virtually immediate which indicates minimal diffusion resistance (100 mg urease plot). A fast rate of reaction is necessary in light of the short transit time available in the small intestine where urea concentrations are high. As explained earlier, high urea fluxes across the intestinal wall do not extend into the large intestine where equilibrium is much longer. Unfortunately, these microcapsules display a prominent ammonium peak (figure 3.23b). A large ammonium release is to be avoided since ammonium can be reabsorbed by the intestine. Only minimal quantities of ammonium should be allowed out of the microcapsule and into the portal circulation. Several attempts were carried out toward a microcapsule membrane that would block the escape of ammonium ions. The use of coatings and/or additional core material could possibly limit this ammonium release into solution and increase nitrogen excretion out of the intestinal tract.

### 4.6 Coating CAB microcapsules

The effect of coating cn ammonium release is shown in figures 3.24 through 3.28. The lecithin and oxycellulose coated samples in figure 3.24 behave very much like the uncoated sample of figure 3.23. The presence of the ammonium peak shows the coating does not limit ammonium release. Coating the CAB microcapsules with these compounds was not encouraging.

We applied the method for producing positively charged

cellulose nitrate membranes (Gottlieb, 1959) that could block positive ions diffusion to the microcapsules. Our results seemed to be promising. The microcapsules were impregnated with a positive electrolyte 2-polyvinyl methylpyridinium iodide (2-PVMP) solution which would fix on the membrane after drying. The positive electrolyte would allow urea, a non charged molecule, to pass freely, while entrapping ammonium. Unfortunately, the activity of urease after exposure to 2-PVMP was reduced to zero (figure 3.26). Most likely there was interaction between 2-PVMP and the enzyme's active site. For this reason the use of 2-PVMP had to be discontinued.

Ethylene oxide, alginate, polyethylene imine epichlorohydrin and polyethylene imine act as ammonium barriers but slow down urea diffusion as well (figure 3.27 and 3.28).

At this point we decided to take a middle path by decreasing the urea reaction rate and allow more time for ammonium adsorption and therefore lower ammonium releases. The activity of uncoated CAB microcapsules containing ZWU at different urease concentrations is shown in figure 3.29. The ammonium peak is not as pronounced since urea reactions are lower. We now wanted to optimize this microcapsule preparation by further examining the microcapsule contents and the effect of microcapsule size. An orthogonal method was used to select among the many variables and streamline the optimization procedure (Kang Fu Gu, 1988).

## 4.7 CAB microcapsules containing PEI

In Figure 3.30 CAB microcapsules with equivalent amounts of urease are tested for PEI addition. The urea rate difference between the two samples is significant. Obviously, PEI has changed the microenvironment significantly. A flaky suspension forms when PEI is added to ZWU suspended in a 3 ml phosphate buffer solution. With the addition of PEI, the suspension changes from colloidal to a gel-like substance. This could imply some form of reaction between exposed glutaraldehyde groups and PEI. PEI is also known to act as an enzyme stabilizer and it may protect the enzyme against denaturation during microcapsule preparation. In the uncoated sample of figure 3.23 the necessity of using enzyme loadings greater than 15 mg per 500 mg of zeolite for the enzyme envelope preparation (which goes beyond the formation of the monolayer) may have been to protect the enzyme monolayer from denaturation during the microcapsule preparation. PEI may in fact take the place of these additional enzyme layers to further stabilize the monolayer.

The addition of polyethylene imine (PEI), as an ingredient in the formation of microcapsules, turned out to be a mixed blessing. For an equivalent amount of enzyme (15 mg) PEI promotes urea conversion (figure 3.30a). Ammonium peaks have virtually disappeared (figure 3.30b). By taking into account the residual ammonium concentration of 15 meq/L or 21 mg/dL BUN equivalents, the microcapsules are effectively removing 80%

of urea in one hour or less.

The addition of PEI to the microcapsules not only enhances the urea reaction rate but is also beneficial for the preparation of good microcapsules.

### 4.8 Preliminary in vivo experiments

Preliminary in vivo experiments on 5/6 nephrectomized rats show a 40% reduction in BUN upon feeding collodion microcapsules and zeolite F (figures 3.35 and 3.36, Cattaneo, 1986). More in vivo experiments need to be performed to substantiate this observation.

### 4.9 Potential of sorbents in uraemia

A simple mass balance on urea provides some insight on the potential of sorbents in uraemia:

$$IN - OUT = ACCUMULATION$$

$$G - (A + KrC) = V*dC/dt$$

where

G= generation rate of urea, mg/min

A= sorption rate of urea, mg/min

Kr= residual kidney clearance, ml/min

C= BUN concentration, mg/ml

V= total body water, ml

t= time, min

Solving the differential equation we obtain:

$$C = Co*EXP(-Kr/V)t + (G-A)/Kr*(1-EXP(-Kr/V)t)$$

At steady state this reduces to:

$Cs = G/Kr$ (control)

$Cs = (G-A)/Kr$ (treated)

These curves can be plotted, as shown in figure 4.4. We used a generation rate of urea (G) of 3 mg/min for an average patient of 65 kg (Gotch, 1976). For the sorbent rate of uptake we used the following values:

1. 100 grams of microcapsules administered per day.
2. A capacity for the CAB microcapsules of removal of 24 mg BUN/g of capsule, based on our data from figure 3.30.

Figure 4.4 shows the effect of this sorbent therapy on urea level. With the CAB microcapsules as oral sorbents the BUN level of 0.9 mg/ml is reached at a residual renal function of 1-2 ml/min. Without this oral sorbent therapy this BUN level of 0.9 mg/ml will be reached when the residual kidney function is 3-4 ml/min.

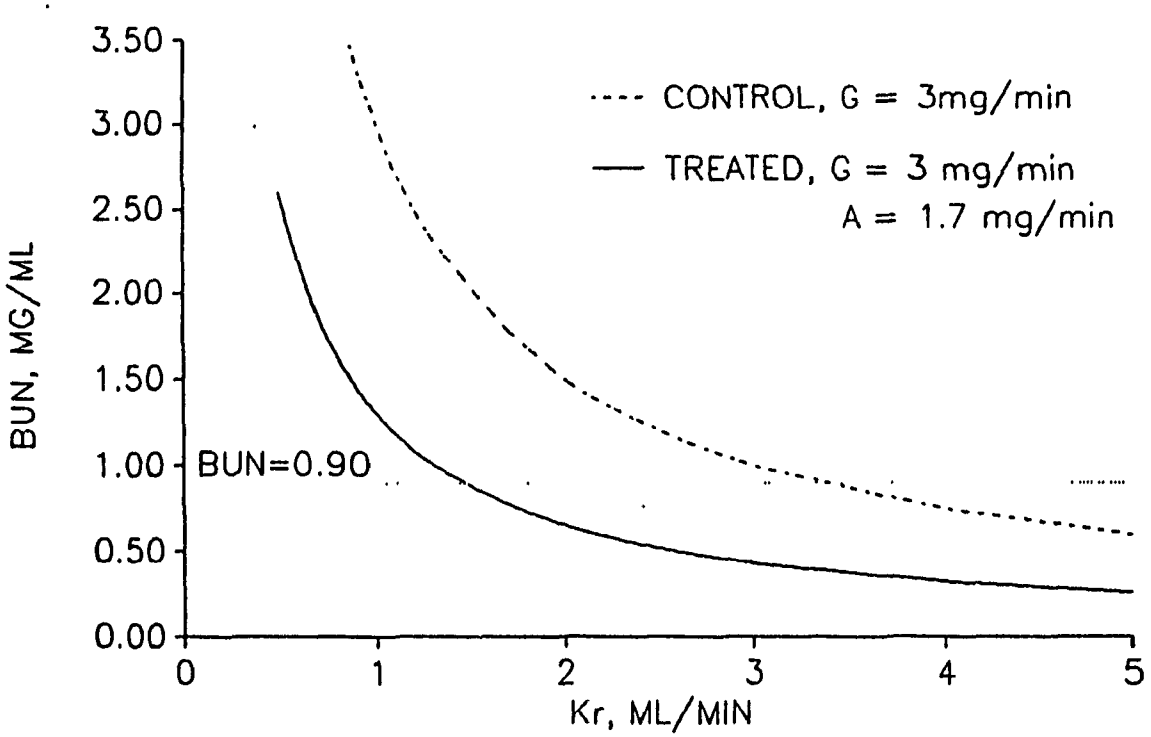



**Figure 4.4. Delayed Dialysis with Sorbent Therapy.**

# CONCLUSIONS

## 5. CONCLUSIONS AND RECOMMENDATIONS

The use of microcapsules containing a urease-silica adduct and ion exchanger zirconium phosphate, though successful in reducing urea levels, resulted in a number of problems including a negative calcium balance in patients. In this thesis it is demonstrated that the use of microcapsules containing a urease-zeolite preparation may be a potential route to urea removal. The use of zeolite ion exchangers, and zeolite W in particular, can alleviate the above mentioned problems of zirconium phosphate.

Zeolite W when present in the sodium form has a high ammonium capacity of 3.6 meq $NH_4^+$/g zeolite under simulated intestinal conditions. Unlike zirconium phosphate, zeolite W is non selective toward calcium ions and is stable at the high pH found in the intestinal tract. Its reactivity to ammonium is also higher.

The application of enzyme envelopes on zeolite particles is a novel immobilization procedure that does not involve the use of colloidal silica. The use of enzyme envelopes on zeolite support, which replaces silica, can reduce the amount of ingested material by at least 25%.

The present "in vitro" study shows that cellulose acetate butyrate microcapsules, containing a urease-zeolite preparation, remove up to 80% of urea in less than one hour. The microcapsules can be dried and retain activity when sealed in a jar at 4 °C.

Preliminary "in vivo" experiments on Sprague-Dawley uraemic rats treated with ingested microcapsules indicate reductions in urea levels and a lengthening of survival times compared to controls.

My experience with urease and zeolites is encouraging. Further experiments on a larger animal than the rat can be more relevant in establishing the feasibility of this system in man. Ultimately, patient acceptability and performance of this oral sorbent can only be determined in clinical trials.

# REFERENCES

## 6. REFERENCES


Abel, J.J., L.G. Rountree and B.B. Turner, J. Pharmacol.Exp. Therap., 5:275, 1913-4.

Alwall, N., Acta Med. Scand., 128:317, 1947.

Ames, L.L. Jr., "Zeolite removal of NH4+ ions from agricultural and other wastewaters", Proc. 13th Pacific NW Indus. Waste Conf., Wash. State Univ., Publ. by Tech. Ext. Serv., Wash. State Univ., Pullman, Wa., April 6-7, 1967, p.135.

Ash, S.R., R.G. Barile, J.A. Thornhill, J.D. Sherman and N-H.L. Wang, "In vivo evaluation of calcium-loaded zeolites and urease for urea removal in haemodialysis", Trans. Am. Soc. Artif. Intern. Organs, 26:111, 1980.

Asher, W.J., K.C. Bovee, J.W. Frankenfeld, R.W. Hamilton, L.W. Henderson, P.G. Holtzapple and N.N. Li, "Liquid membrane system directed toward chronic uraemia", Kidney Int., 7:S409, 1975.

Asher, W.J., T.C. Vogler, K.C. Bovee, P.G. Holtzapple and R.J. Hamilton, "Projections and measurements of in vivo performance of liquid membrane capsules", Kidney Int., 10:S254, 1976.

Asher, W.J., K.C. Bovee, T.C. Vogler, R.W. Hamilton and P.G. Holtzapple, "Liquid membrane capsules administered to the gastrointestinal tracts of dogs for removal of urea from the blood", Clin. Nephrol., 11:92, 1979.

Bourget, L. and T.M.S. Chang, "Artificial Cell-Microencapsulated Phenylalanine Ammonia-Lyase", Appl. Biochem. Biotech., 10:57, 1984.

Bourget, L. and T.M.S. Chang, "Phenylalanine ammonia-lyase immobilized in semipermeable microcapsules for enzyme replacement in phenylketonuria", FEBS, 180:5, 1985.

Breck, D.W., "Zeolite Molecular Sieves", Robert E. Krieger Publisher, 1984, p.530.

Brescia, M.J., J.E. Cimino, K. Appel and B.J. Hurwich, N. Eng. J. Med., 275:1089, 1960.

Bryan, F.A., "Final Report National Dialysis Registry, Artificial Kidney-Chronic Uraemia Program, National Institute of Health, Report No. AK-8-7-1387-F, PB259174/AS (August, 1976).

Campbell, J. and T.M.S. Chang, "The recycling of NAD+ (Free and Immobilized) within semipermeable aqueous microcapsules

containing a multi-enzyme system", Biochem. Biophys. Res. Comm., 69:562, 1976.

Campbell, J. and T.M.S. Chang, "Immobilized Multienzyme Systems and Coenzyme Requirements: Perspectives in Biomedical Applications", in Biomedical Applications of Immobilized Enzymes and Proteins, T.M.S. Chang, Editor, Plenum Press, 1977, p.281.

Campbell, J. and T.M.S. Chang, "Microencapsulated Multi-Enzyme Systems as Vehicles for the cyclic regeneration of free and immobilized coenzymes", Enzyme Engineer., 3:371, 1978.

Carr, P.W. and L.D. Bowers, "Immobilized Enzymes in Analytical and Clinical Chemistry", in Chemical Analysis, John Wiley and Sons Publishers, 56:45, 1980.

Cattaneo, M.V., " The potential applications of zeolites and microencapsulated urease for the removal of urea in uraemia", M.Eng. Thesis, McGill University, 1986.

Chang, T.M.S., "Haemoglobin Corpuscles", Report of a research project for the BSc Honours, McGill University, 1957.

Chang, T.M.S., "Semipermeable microcapsules", Science, 146:524, 1964a.

Chang, T.M.S. and F.C. MacIntosh, Pharmacologist, 6:198, 1964b.

Chang, T.M.S., "Semipermeable aqueous microcapsules (Artificial Cells): with emphasis on experiments in an extracorporeal shunt system", Trans. Amer. Soc. Artif. Int. Organs, 12:13, 1966a.

Chang, T.M.S., F.C. MacIntosh and S.G. Mason, "Semipermeable aqueous microcapsules, Ind.Preparation and Properties, Can. Jour. Physiol. Pharmacol., 44:115, 1966b.

Chang, T.M.S., L.J. Johnson and O.J. Ransome, "Semipermeable aqueous microcapsules: IV. Nonthrombogenic microcapsules with heparin-complexed membranes", Can. J. of Phys. and Pharm., 45:705, 1967.

Chang, T.M.S. and M.J. Poznansky, "Semipermeable microcapsules containing catalase for enzyme replacement in acatalasaemic mice", Nature, 218:243, 1968.

Chang, T.M.S. and M.J. Poznansky, "Semipermeable Aqueous Micro-capsules (Artificial Cells). V. Permeability Characteristics", J.Biomed. Mater. Res., 2:187, 1968b.

Chang, T.M.S., Proc. Can. Fed. Biol. Soc., 12:62, 1969.

Chang, T.M.S. and S.K. Lo, "Urea removal by urease and ammonia absorbents in the intestine", Physiologist 13:70, 1970.

Chang, T.M.S., "The in vivo Effects of Semipermeable microcapsules containing L-Asparaginase on 6C3HED Lymphosarcoma",Nature, 229:117, 1971.

Chang, T.M.S., Biochem. Biophys. Res. Commun., 44:1531, 1971.

Chang, T.M.S., "Effects of Local Applications of Microencapsulated Catalase on the Response of Oral Lesions to Hydrogen Peroxide In Acatalasemia", J. Dental Res., 51:319, 1972.

Chang, T.M.S., "L-Asparaginase Immobilized within Semipermeable Microcapsules: in vitro and in vivo Stability", Enzyme, 14:95, 1972/73.

Chang, T.M.S., "Haemoperfusion alone and in series with ultrafiltration or dialysis for uraemia, poisoning, and liver failure", Kidney Int., 10:S305, 1976.

Chang, T.M.S., "Protective Effects of Microencapsulation (Coating) on Platelet Depletion and Particulate Embolism in the Clinical Applications of Charcoal Haemoperfusion, in Artificial Organs," Proc. Seminar on the Clinical Applications

of Membrane Oxygenators and Sorbent-based Systems, R.M. Kenedi et al (ed.), pp.164-177, Macmillan, London, (1977).

Chang, T.M.S., "Encapsulation of Enzymes, Cell Contents, Cells, Vaccines, Antigens, Antiserum, Cofactors, Hormones and Proteins", in Biomedical Applications of Immobilized Enzymes and Proteins, T.M.S. Chang, Editor, Plenum Press, 1977, p.69.

Chang, T.M.S., "Rationale and Strategies for the Therapeutic Applications of Immobilized Enzymes", in Biomedical Applications of Immobilized Enzymes and Proteins, T.M.S. Chang, Editor, Plenum Press, 1977, p.93.

Chang, T.M.S., "Experimental Therapy Using Semipermeable Microcapsules Containing Enzymes and Other Biologically Active Materials", in Biomedical Applications of Immobilized Enzymes and Proteins, T.M.S. Chang, Editor, Plenum Press, 1977, p.147.

Chang, T.M.S. and C. Malouf, "Artificial cells microencapsulated multienzyme system for converting urea and ammonia to amino acid using a-Ketoglutarate and glucose as substrates", Trans. Am. Soc. Artif. Intern. Organs, 24:18, 1978a.

Chang, T.M.S. and N. Kuntarian, "Galactose conversion using a microcapsule immobilized multienzyme cofactor recycling

system", Enzyme Eng., 4:193, 1978b.

Chang, T.M.S. et al, "Haemoperfusion-Haemodialysis in a single unit: Composite artificial kidney", J. Artif. Support Systems, 1:63, 1982a.

Chang, T.M.S. et al, "Phase one clinical trial of a new composite artificial kidney: a single unit combining dialysis with haemoperfusion", Trans. Am. Soc. Art. Intern. Organs, 28:43, 1982b.

Chang, T.M.S., "Artificial Cells in Medicine and Biotechnology", Appl. Biochem. Biotech., 10:5, 1984.

Chang, T.M.S., "Artificial Cells Containing Multienzyme Systems", Methods in Enzymol., 112:195, 1985.

Chang, T.M.S., "Recycling of NAD(P) by Multienzyme Systems Immobilized by Microencapsulation in Artificial Cells", Meth. in Enzymol., 136:67, 1987.

Chang, T.M.S., "Applications of Artificial Cells in Medicine and Biotechnology", Biomat. Art. Cells Art. Organs, 15:1, 1987.

Chanutin, A. and E.B. Ferris, "Experimental Renal

Insufficiency produced by partial nephrectomy", Arch. Intern. Med., 49:767, 1932.

Chapman, G.V., R.A. Ward and P.C. Farrell, "Separation and quantification of the middle molecules in uraemia", Kid. Intern., 17:82, 1980.

Chibata, I., Immobilized Enzymes, Halsted Press Publishers, John Wiley & Sons Inc., p.5, 1978.

Chong, E.D.S. and T.M.S. Chang, "In vivo Effects of Intraperitoneally Injected L-Asparaginase Solution and L-Asparaginase Immobilized within Semipermeable Nylon Microcapsules with Emphasis on Blood L-Asparaginase, 'Body' L-Asparaginase, and Plasma L-Asparagine Levels", Enzyme, 18:218, 1974.

Chong, E.D.S. and T.M.S. Chang, "Asparaginase as a Model for Enzyme Therapy of Substrate-Dependent Tumours", in Biomed. Appl. of Imm. Enz. and Proteins, T.M.S. Chang, Editor, Plenum Press, 1977, p.105.

Cole, J.J., T.L. Pollard and J.S. Murray, Trans. Am. Soc. Art. Int. Organs, 9:67, 1963.

Colton, C.K. et al, "Permeability Studies with cellulosic

membranes", J.Biomed.Mater.Res., 5:459, 1971.

Colton, C.K., "The Interleukin hypothesis: A quantitative assessment", Kidney Int., 33:S27, 1988.

Cousineau, J., Chang, T.M.S., "Formation of amino acid from urea and ammonia by sequential enzyme reaction using a microencapsulated multienzyme system. Biochem. Biophys. Res. Commun.,79:24, 1977.

Daugirdas, J.T. and S.I. Todd, "First-use reactions during haemodialysis: A definition of subtypes", Kidney Int., 33:S37, 1988.

Dempsey, E.F., E.L. Carrol, F. Albright and P.H. Henneman, "A study of Factors Determining Fecal Electrolyte Excretion", Metabolism Clin. and Exp., 7:108, 1958.

Denti, E., M.P. Luboz, V.A. Tessore, F. Castino and P.F. Gaglia, Kidney Int., 7:S401, 1975.

Eisenman, G., Biophys. J., 2:259, 1962.

Fordtran, J.S., F.C. Rector Jr., M.F. Ewton, N. Soter and J. Kinney, "Permeability Characteristics of the Human Small Intestine", J. Clin. Invest., 44:1935, 1965.

Friedman, E.A., J. Fastook, M.M. Beyer, T. Rattazzi and A.S. Josephson, Trans. Am. Soc. Artif. Int. Organs, 20:161, 1974.

Friedman, E.A., G.B. Laungani and M.M. Beyer, Kidney Int., 7:377, 1975.

Friedman, E.A., M.J. Saltsman, M.M. Beyer and A.S. Josephson, "Combined oxystarch-charcoal trial in uraemia: Sorbent-induced reduction in serum cholesterol", Kid. Intern., 10:S273, 1976.

Friedman, E.A., "Sorbent Therapy in Uremia", in Clinical Aspects of Uremia and Dialysis, S.G. Massry and H.L. Sellers (ed.), p.671, Thomas, Springfield (1976).

Furst, P., J. Bergstrom, A. Gordon, E. Johnson and L. Zimmerman, "Separation of peptides of 'middle molecular weight from biological fluids of patients with uremia", Kidney Int., 7:S272, 1975.

Gardner, D.L., Falb, R.D., Kim, B.C. and D.C. Emmerling, Trans. Amer. Soc. Artif. Int. Organs, 17:239,1971a.

Gardner, D.L. and D.C. Emmerling, Chem.Eng.News, 49:31, 1971b.

Gardner, D.L., D.C. Emmerling, K.D. Williamson, W.C. Baytos and C.R. Hassler, "Encapsulated adsorbents for removal of

nitrogerous metabolites via oral ingestion", Kid. Intern., 7:S393, 1975.

Gardner, D.L., C.M. Kjellstrand, C.R. Hassler, D.J. Fink and D.C. Emmerling, "An orally administered microcapsule system for treating chronic renal failure patients", Appl. Biochem. Biotech., 10:27, 1984.

Gauthier, S., "Synthesis and physical properties of styrene-vinylpyridinium ionomers of various architectures", Ph.D. Thesis, McGill University, Montreal, Canada, 1985.

Giordano, C., Proc. Eur. Dial. Transplant Assoc., 9:419, 1972.

Giordano, C., R. Esposito and M. Pluvio, "Further studies with oxystarch", Kid. Inter., 10:S266, 1976.

Giordano, C.,R. Esposito, M. Pluvio and F. Gonzalez, "Oxycelluloses: A group of sorbents to remove extracorporeal urea", Kid. Int., 10:S348, 1976.

Giordano, C., R. Esposito and P. Bello, "A cold charcoal depurator for the adsorption of high quantities of urea", Kid. Int., 10:S284, 1976.

Giordano, C. and R. Esposito, "Oxystarch and other

polyaldehydes: the present status in the treatment of uremia", in Art. Kidney, Art. Liver and Detoxification, Chang T.M.S. editor, New York Plenum Press, p.47, 1978.

Giordano, C., "Polyaldehydes", in Sorbents and Their Clinical Applications, C. Giordano Editor, Academic Press, 1980, p.148.

Goldenhersh, K.K., W-D. Huang, N.S. Mason and R.E. Sparks, "Effect of microencapsulation on competitive adsorption in intestinal fluids", Kid. Intern., 10:S251, 1976.

Goosen, M.F.A., G.M. O'Shea, H.M. Gharapetian, S. Chou and A.M. Sun, "Optimization of Microencapsulation Parameters: Semipermeable Microcapsules as a Bioartificial Pancreas", Biotech. and Bioeng., 27:146, 1985.

Gordon, A., Greenbaum, M.A., Marantz, C.B., Gral, T. and M.H. Maxwell, "A sorbent based low volume recirculating dialysate system", Trans. Am. Soc. Art. Int. Organs, 15:347, 1969.

Gordon, A., A. Lewin, L.B. Marantz and M.H. Maxwell, "Sorbent regeneration of dialysate", Kid. Int., 10:S277, 1976.

Gotch, F.A., P.C. Farrell and J. Sargent, "Theoretical considerations on molecular transport in dialysis and sorbent therapy for uremia", J. Dialysis, 1:105, 1976.

Gotch, F.A., J.A. Sargent, M. Keen, M. Lam, M. Prowitt and M. Grady, "Clinical results of intermittent dialysis therapy (IDT) guided by ongoing kinetic analysis of urea metabolism", Trans. Am. Soc. Artif. Int. Organs, 22:175, 1976.

Gotch, F.A., "Dialysis of the future", Kidney Int., 33:S100, 1988.

Gottlieb, M.H., R. Neihof and K. Sollner, "Preparation and properties of strong base type collodion matrix membranes", J. Phys. Chem., 61, 154, 1959.

Gregor H.P. and K. Sollner, "Improved methods of preparation of electropositive permselective protamine collodion membranes", J. Phys. Chem., 50:88, 1946.

Grunwald, J. and T.M.S. Chang, "Nylon polyethyleneimine microcapsules for immobilizing multienzymes with soluble dextran-NAD+ for the continuous recycling of the microencapsulated dextran-NAD+", Biochem. Biophys. Res. Comm., 81:565, 1978.

Grunwald, J. and T.M.S. Chang, "Continuous recycling of NAD+ using an immobilized system of collodion microcapsules containing dextran-NAD+, alcohol dehydrogenase, and malic dehydrogenase", J. Appl. Biochem., 1:104, 1979.

Grunwald, J. and T.M.S. Chang, "Immobilization of alcohol dehydrogenase and dextran-NAD+ within nylon-polyethyleneimine microcapsules: preparation and cofactor recycling", J. Mol. Catalysis, 11:83, 1981.

Gu, K.F. and T.M.S. Chang, Biomat. Artif. Cells Artif. Organs, 15:289, 1987.

Gu, K.F. and T.M.S. Chang, Biotech. and Bioeng., 32:363, 1988.

Guarino, J.R. and L.J. Guarino, Science, 115:285, 1952.

Guyton, A.C., Textbook of Medical Physiology, Saunders B. Company, 7th ed., 1986, p.454.

Haynes, R. and K.A. Walsh, "Enzyme Envelopes on Colloidal Particles", Biochem. Biophys. Res. Comm., 36:235, 1969.

Helfferich, F., Ion Exchange, McGraw Hill Series in Advanced Chemistry, 1962, pp.6, 62.

Herron, N. and C.A. Tolman, J.A.Chem.Soc., 109:2837, 1987.

Hoenich, N.A., et al., "Comparison of membranes used in the treatment of end-stage renal failure", Kidney Int., 33:S44, 1988.

Holt, L.E. and P.H. Holz, J. Pediatr., 63:306, 1963.

Horvath, C., "Pellicular Immobilized Enzymes", Biochim. Biophys. Acta, 358:164, 1974.

Ittel, T.H., B. Buddington, N.L. Miller and A.C. Alfrey, "Enhanced gastrointestinal absorption of aluminum in uraemic rats", Kidney Int., 32:821, 1987.

Jindal, K.K., A. Manuel and M.B. Goldstein, "A simple and accurate method to estimate Kt/V Urea", Trans. Am. Soc. Art. Int. Organs, 33:286, 1987.

Kiil, F., Acta Chir. Scand. Suppl., 253:143, 1960.

Kijma, Y., T. Sasaoka and M. Kanayama, N. Engl. J. Med., 296:515, 1977.

Kjellstrand, C., H. Borges, C. Pru, D.L. Gardner and D. Fink, "On the clinical use of microencapsulated zirconium phosphate-urease for the treatment of chronic uremia", Trans. Am. Soc. Artif. Intern. Organs, 27:24, 1981.

Klibanov, A.M., "Immobilized Enzymes and Cells as Practical Catalysts", Science, 219:722, 1983.

Kolff, W.J. and H.T.J. Berk, Geneesk. Gids., 5:21, 1943.

Kolff, W.J. and H.T.J. Berk, Acta Med. Scand., 117:121, 1944.

Kolff, W.J. and B. Watschinger, J. Lab. Clin. Med., 47:969, 1956.

Kolff, W.J., Ann. Intern. Med., 62:608, 1965.

Kolff, W.J., "Longitudinal perspectives on sorbent in uremia", Kid. Internat., 10:S211, 1976.

Kolff, W.J., in Artificial Kidney, Art. Liver, Art. Cells, Plenum Press, NY, 1978.

Ku, K., R. Anderson and P. Schoenfeld, "Kinetic modelling of urea in peritoneal dialysis", Dialysis and Transpl., 12:374, 1983.

Kunz, H.J. and M. Stastny, "Immobilized Glucose Oxidase Used to Measure Glucose in Serum", Clin. Chem., 20:1018, 1974.

Laurent, G., et al., "Dialysis related amyloids", Kidney Int., 33:S32, 1988.

Levine, S.N. and W.J. LaCourse, "Materials and design

considerations for a compact artificial kidney", J.Biomed.Mater.Res.,1:275, 1967.

Leung, Y.F., G.M. O'Shea, M.F.A. Goosen and A.M. Sun, "Micro-encapsulation of crystalline insulin or islets of langerhans: an insulin diffusion study", Art. Organs, 7:208, 1983.

Lim, F. and A.M. Sun, "Microencapsulated Islets as Bioartificial Pancreas", Science, 210:908, 1980.

Lim, F., "Microencapsulated hepatocytes as a potential bioartificial liver", Art. Organs, 5:304, 1981.

Lindsay, R.M. and L.W. Henderson, "Adequacy of Dialysis, Mass Transfer Kinetics", Kidney Int., 33:S92, 1988.

Luttinger, M. and C.W. Cooper, "Improved Haemodialysis Membranes for the Artificial Kidney", J. Biomed. Mater. Res., 1:67, 1967.

Maclay, W.N. and R.M. Fuoss, "Electrical properties of solids. XVII. Poly-4-vinylpyridine and some of its derivatives", J. Am. Chem. Soc., 73:4065, 1951.

MacNeil, A.E., A. Mather and W.L. McLaughton, Exhibit of Association of Military Surgeons Annual Convention, 1949.

Man, N-K, T. Drueke, A. Becker, J. Zingraff, P. Jungers and J-L.F. Brentano, "Clinical use of oxystarch", Kid. Intern., 10:S269, 1976.

Manecke, G., and K.F. Bonhoeffer, Z. Elektrochem., 55:475, 1951.

Manis, T., E.I. Feinstein and E.A. Friedman, Conf. Uremia, 2nd: Formulations Expectations, Capri, 1977a.

Manis, T., E.I. Feinstein and E.A. Friedman, Abstr., Am. Soc. Nephrol., 1977b.

Manis, T., S. Zeig, G. Lum, E.I. Feinstein and E.A. Friedman, Am. Fed. Clin. Res., 1978.

Marconi, W. and F. Morisi, in Applied Biochemistry and Bioengineering, L.B. Wingard, E Katchalsky-Katzir, L. Goldstein, Eds. Academic Press, New York, 1979, Vol.2, p.219.

May, S.W. and N.N. Li, " The immobilization of urease using liquid-surfactant membranes", Biochem. Biophys. Res. Comm., 47:1179, 1972.

Meriwether, L.S. and Hildegard M.K., "In vitro reactivity of

oxystarch and oxycellulose", Kid. Intern., 10:S259, 1976.

Mori, T., T. Tosa and I. Chibata, "Preparation and Properties of Asparaginase Entrapped in the Lattice of Polyacrylamide Gel", Cancer Res., 34:3066, 1974.

Mori, T., T. Tosa and I. Chibata, "Enzymatic properties of microcapsules containing asparaginase", Biochim. Biophysic. Acta, 321:653, 1973.

Morrison, A.B., "Experimental Chronic Renal Insufficiency", Meth. Achiev. Exp. Path., 1:455, 1966.

Mosbach, K., "Enzymes bound to artificial matrices", Scientific American, March 1971, p.155.

Neihof, R., "The preparation and properties of strong acid type collodion-base membranes", J. Phys. Chem., 58:916, 1954.

Nikolaev, V.G. et al, "Theoretical Aspects of Carbon Adsorbent Applications for Detoxification", Biomat., Art. Cells, Art. Org., 15(1):59, 1987.

NIH Report: Research Resources for Microbiologic and Immunologic Studies, National Institute of Allergy and Infectious Diseases, NIH, DHEW (April, 1977).

Nilsson, K., "Methods for immobilizing animal cells", TIBTech, 5:73, 1987.

Ortmanis, A., R.J. Neufeld and T.M.S. Chang, "Study of micro-encapsulated urease in a continuous feed, stirred tank reactor", Enz. Microb. Tech. 6:135, 1984.

Payne, C.C. and D.C. Kelly, in Microbial Control of Pests and Plant Diseases 1970-1980, H.D. Burges Ed., Academic Press, New York, 1981, p.61.

Pedrini, L.A., S. Zereik and S. Rasmy, "Causes, kinetics and clinical implications of post-haemodialysis urea rebound", Kidney Int., 34:817, 1988.

Piskin, E. and T.M.S. Chang, "Urea and ammonium removal based on alkalinization and removal of free ammonia", Int. J. Art. Organs, 2:211, 1979.

Piskin, E. and T.M.S. Chang, "Effects of air and fluid flow on removing different concentrations of urea and ammonia from fluid by gas absorption in a capillary haemodialyser", J. Dialysis, 4:31, 1980.

Piskin, K. and T.M.S. Chang, "A new combined enzyme-charcoal system formed by enzyme adsorption on charcoal followed by

polymer coating", Intern. J. Art. Organs, 3:344, 1980.

Platt, R., M.H. Roscoe and F.W. Smith, "Experimental Renal Failure", Clin. Sci., 11:217, 1952.

Poznansky, M.J. and T.M.S. Chang, Proc. Can. Fed. Biol. Soc., 12:54, 1969.

Poznansky, M.J. and T.M.S. Chang, "Comparison of the enzyme kinetics and immunological properties of catalase immobilized by microencapsulation and catalase in free solution for enzyme replacement", Biochim. Biophys. Acta, 334:103, 1974.

Quinton, W.E., D.H. Dillard and B.H. Scribner, Trans. Amer. Soc, Artif. Intern. Organs, 6:104, 1960.

Quinton, W.E., D.H. Dillard, J.J. Cole and B.H. Scribner, Trans. Amer. Soc. Artif. Intern. Organs, 8:236, 1962.

Ringoir, S., et al., "Uraemic Toxins", Kidney Int., 33:S4, 1988.

Rosenthal, A.M. and T.M.S. Chang, "The incorporation of lipid and Na+-K+-ATPase into the membranes of semipermeable microcapsules", J. Memb. Sci., 6:329, 1980.

Ruddleston, J.F. and A. Stewart, J.Am.Chem.Soc.,102:7816, 1980.

Saltzman, M.J., M.M. Beyer and E.A. Friedman, "Mechanism of life prolongation in nephrectomized rats treated with oxidized starch and charcoal", Kid. Intern. 10:S343, 1976.

Schloerb, P.R., "Intestinal dialysis in 1975 perspective: Sorbents and intestinal loop nitrogen transport", Kid. Int., 10:S248, 1976.

Schreiner, G.E. and J.F. Winchester, "Uremia - 1978 perspective", Clin. Nephrol., 11:52, 1979.

Setala, K., "Bacterial enzymes in uremia management", Kid. Int., 13:S194, 1978.

Setala, K., "Treating uremia with soil bacterial enzymes: further developments", Clin. Nephrol., 11:156, 1979.

Setala, K., "The promise of enzymes in therapy of uremia", Nephron, 37:1-20, 1984.

Sherman, J.D., "Ion Exchange Separations with Molecular Sieve Zeolites", in Adsorption and Ion Exchange Separations, J.D. Sherman ed., A.I.Ch.E. Symp. Series, No. 179, 74:98, 1978a.

Sherman, J.D., "Ionsiv F-80 and ionsiv W-85: molecular sieve zeolite NH4+ ion exchangers for removal of urea nitrogen", in Artificial Cells for Artificial Kidney, Artificial Liver and Detoxification, Chang T.M.S. editor, Plenum Press, New York, p. 267, 1978b.

Sherman, J.D. and R.J. Ross, "Selective Ammonium Ion Exchange with Linde F and W zeolite molecular sieves", Union Carbide Corporation, Tarrytown, NY, USA, 10591, 1980.

Sherman, J.D., "Removal of Uraemic Substances with Zeolite Ion Exchangers", European Patent Application, 81106583.8, Aug.25, 1981.

Sherman, J.D., "Ion Exchange Separations with molecular sieve zeolites", Union Carbide Corporation Report, Tarrytown, NY, USA, 10591, 1983.

Sherry, H.S., "Ion Exchange", Marinsky ed., New York, Marcel Dekker, 2:89, 1969.

Shu, C.D. and T.M.S. Chang, "Tyrosinase immobilized within artificial cells for detoxification in liver failure: 1. preparation and in vitro studies, and 2. in vivo studies in fulminant hepatic failure rats", Intern. J. Artif. Organs, 3:287, 1980.

Sinclair, A., D.D. Griffin, J.D. Voreis and S.R. Ash, "Sorbent binding of urea and creatinine in a Roux-Y intestinal segment", Clin. Nephrol., 11:97, 1979.

Skeggs, L.T. Jr. and J.R. Leonards, Science, 108:212, 1948.

Sollner, K. and H.P. Gregor, "The electrochemistry of permselective protamine collodion membranes", J. Phys. Chem., 54:330, 1950.

Sparks, R.E., R.M. Salemme, P.M. Meier, M.H. Litt and O. Lindan, "Removal of waste metabolites in uremia by microencapsulated reactants", Trans. Am. Soc. Artif. Int. Organs, 15:353, 1969.

Sparks, R.E., N.S. Mason, P.M. Meier, M.H. Litt and O. Lindan, "Removal of uraemic waste metabolites from the intestinal tract by encapsulated carbon and oxidized starch", Trans. Am. Soc. Artif. Int. Organs, 17:229, 1971.

Sparks, R.E., "Review of gastrointestinal perfusion in the treatment of uremia", Clin. Nephrol. 11:81, 1979.

Stevenson, W.T.K., R.A. Evangelista, M.E. Sugamori and M.V. Sefton, "Microencapsulation of mammalian cells in a hydroxyethyl methacrylate-methyl methacrylate copolymer:

preliminary development", Biomat. Art. Cells Art. Org., 16:747, 1988.

Stewart, R.D., B.J. Lipps, E.D. Baretta, W.R. Piering, D.A. Roth and J.A. Sargent, Trans. Am. Soc. Artif. Intern. Organs, 14:121, 1968.

Sun, A.M., F. Lim, H. Van Rooy and G. O'Shea, "Long-term studies of microencapsulated islets of Langerhans: a bioartificial endocrine pancreas", Art. Organs, 5:784, 1981.

Sun, A.M., Z. Cai, Z. Shi, F. Ma and G.M. O'Shea, "Microencapsulated hepatocytes: an in vitro and in vivo study", Biom. Art. Cells Art. Organs, 15:483, 1987.

Sundaram, P.V., "The kinetic properties of microencapsulated urease", Biochim. Biophys. Acta, 321:319, 1973

Tenckoff, H. and H. Schechter, Trans. Am. Soc. Artif. Intern. Organs, 14:181, 1968.

Teorell, T., Proc. Soc. Exptl. Biol., 33:282, 1935.

Tosa, T., T. Sato, T. Mori, K. Yamamoto, I. Takata, Y. Nishida and I. Chibata, "Immobilization of Enzymes and Microbial Cells Using Carrageenin as Matrix", Biotech. Bioeng., 21:1697, 1979.

Walser, M. "Urea metabolism in Chronic Renal Failure", J. Clin. Invest., 53:1385, 1974.

Walser, M., Clin.Nephrol.,3:180,1975.

Wineman, R.J., "Overview of chronic replacement of kidney function", AIChE Symp.Series, No.187, 75:1, 1979.

Wolfe, E.A., "Microencapsulated urease and ZP in Uremia", M.Eng. Thesis, McGill University, 1985.

Wolfe, E.A. and T.M.S. Chang, "Orally ingested microencapsulated urease and an adsorbent, zirconium phosphate, to remove urea in kidney failure", Intern. J. Art. Organs, 10:269, 1987.

Wolpert, E., S.F. Phillips and W.H.J. Summerskill, "Transport of urea and ammonia production in the human colon", Lancet, p.1387, Dec.25, 1971.

Wong, H. and T.M.S. Chang, "Bioartificial liver: Implanted artificial cells microencapsulated living hepatocytes increases survival of liver failure rats", Intern. J. Art. Organs, 9:335, 1986.

Yatzidis, H., Nephron, 1:310, 1964.

Yatzidis, H. and D. Oreopoulos, Kidney Int., 10:215, 1976.

Yatzidis, H., Kidney Int., 13,1978.

Yatzidis, H., D. Koutsicos and P. Digenis, "Newer oral sorbents in uremia", Clin. Nephrol., 11:105, 1979.

Yu, Y.T. and T.M.S. Chang, "Ultrathin lipid-polymer membrane microcapsules containing multienzymes, cofactors and substrates for multistep enzyme reactions", FEBS Letters, 125:94, 1981.

Yu, Y.T. and T.M.S. Chang, "Immobilization of multienzymes and cofactors within lipid-polyamide membrane microcapsules for the multistep conversion of lipophillic and lipophobic substrates", Enzyme Microb. Technol., 4:327, 1982.

Yuan, Z.Y. and T.M.S. Chang, "Rat microsomes and cytosol immobilized by microencapsulation in artificial cells", Int. J. Art. Organs, 9:61, 1986.

Young, T. et al., Gastro-Intestinal Dialysis as an Adjunct to Therapy of Renal Failure," Artificial Kidney-Chronic Uremia Program, National Institute of Arthritis, Metabolism, and Digestive Diseases, p.114, DHEW Publ. No. (NIH) 77-1442 (1977).

Zeig, S. and E.A. Friedman, "Potential for oral sorbent treatment of uremia", in Sorbents and Their Clinical Applications, Carmelo Giordano editor, Academic Press, 1980, p.275.

# APPENDIX

**APPENDIX**

Linde Ionsiv F-80 Ion Exchanger

Description

Linde Ionsiv F-80 Ion Exchanger is an alkali metal alumino silicate of the Linde F zeolite type supplied in mixed ionic (Na+, K+) form.

Chemical Formula

$(Na_2O, K_2O) . Al_2O_3 . 2SiO_2 . 3H_2O$

Typical Properties

Particle Size.....Powder (1 to 20 um)

Pore Openings.....3.7 A

Bulk Density.....3.7 lbs/cu. ft. :590 kg/cu.m.

H2O Content.....12-17 wt%

Ion Exchange Capacity.....6.0-6.7 meq/gm. (anhydrous)

Regeneration

Typically, regeneration may be accomplished by the use of NaCl solution as the regenerant fluid.

Typical Application Areas

Linde Ionsiv F-80 exhibits high capacity for $NH_4^+$ ions from mixed cation solution.

## Linde Ionsiv W-85 Ion Exchanger

### Description

Linde Ionsiv W-85 Ion Exchanger is an alkali metal alumino silicate of the Linde W zeolite type supplied in mixed ionic (Na+, K+) form.

### Chemical Formula

$(Na_2O, K_2O) . Al_2O_3 . 3.6SiO_2 . 5H_2O$

### Typical Properties

Particle Size.....Powder (1 to 20 um)

Pore Openings.....4.2 A

Bulk Density.....39 lbs/cu.ft. :630 kg/cu.m.

$H_2O$ Content.....15-20 wt%

Ion Exchange Capacity.....4.8-5/3 meq/gm. (anhydrous)

### Regeneration

Typically, regeneration is accomplished by the use of a mixed salt (Na+, K+, Ca++) solution as the regenerant fluid.

### Typical Application Areas

Linde Ionsiv W-85 Ion Exchanger exhibits high capacity and high specificity (selectivity) for ammonium ions from mixed cation solution.