ARTIFICIAL LIVER SUPPORT
AND RELATED SYSTEMS

by

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This thesis is affectionately

dedicated to my family

'Is Life worth living?' .... he suspects it is, in a great measure, a question of the Liver.

Punch, vol lxxiii, p207, 1877

THE UNIVERSITY OF ASTON IN BIRMINGHAM

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S U M M A R Y

The primary aim of this research has been the investigation of the use of hydrogel polymers as novel haemoperfusion adsorbents in artificial liver support systems. The work was particularly concerned with the design and development of an in-vitro dynamic evaluation technique to enable the adsorption of a selection of nitrogen-based hepatic toxins on the novel hydrogel polymers and other potential adsorbents to be studied.

Initial work was carried out on three types of commercially available adsorbents; activated charcoal/carbon, polymeric neutral resins and ion exchange resins. The adsorption of ammonia, amino acids and false neurotransmitter amines on these materials was examined, using both static and dynamic adsorption techniques and provided information on the effect of factors such as chemical composition, functional groups, particle size, surface area and physical strength, on adsorption. A selection of the adsorbents was encapsulated with hydrogel membranes and the effect of the polymer coating material on adsorption evaluated.

As a result of the knowledge gained from this study, novel macroporous hydrogel particulates were synthesised (as part of a separate project) and evaluated. Unlike the previously described commercially available adsorbents, these hydrogels were biocompatible. Although their adsorption efficiency was not as great as the best of the commercially available adsorbents, it was adequate for haemoperfusion. The novel macroporous hydrogel particulates appear to have considerable potential as haemoperfusion systems in artificial liver support.

An additional area of interest was that of the closely related system of semiconductor-based ammonia sensors. On the basis of the encapsulation studies previously described, hydrogel coatings were selected and used for the encapsulation of the semiconductor-based ammonia sensors. These microchips were then evaluated by Thorn EMI (CRL) UK. These hydrogel coated semiconductor -based ammonia sensors are potentially useful as in-vivo blood monitors and particularly in artificial liver support systems.

KEY PHRASES: Artificial liver support systems
Haemoperfusion systems
Ammonia Amino acids
Novel macroporous hydrogel particulates

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LIST OF ABBREVIATIONS

AA	Acrylic acid
ACR	Acrylamide
Ala	Alanine
BA	Bile acids
BSP	Bromosulphophthalein
Cys	Cysteine
DAA	Diacetone acrylamide
EDM	Ethylene dimethacrylate
EDTA	Ethylene diamine tetra acetic acid
ETA	Ethanolamine
EWC	Equilibrium water content
Gly	Glycine
HEMA	Hydroxy ethyl methacrylate
His	Histidine
HSA	Human serum albumin
Ile	Isoleucine
MAA	Methacrylic acid
Met	Methionine
NMACR	N-Methylolacrylamide
NN'MBA	NN-Methylene bis acrylamide
OTA	Octopamine
PEA	Phenylethanolamine
Phe	Phenylalanine
polyHEMA	Poly(hydroxy ethyl methacrylate)
SEM	Scanning electron microscopy
Tyr	Tyrosine
Val	Valine

CHAPTER 1

INTRODUCTION

1.1 General Introduction

This project is a complex interdisciplinary project, involving aspects of biology, biochemistry and polymer science, and is concerned with the in-vitro evaluation of polymer-based artificial liver support systems. Such a project requires some fairly detailed understanding of the way the normal liver functions, and the problems encountered in liver failure. This knowledge is essential before trying to overcome the constraints produced on designing novel adsorbents necessary for application in artificial liver support systems. For this reason the introduction to this thesis contains the background material appropriate for understanding the normal and abnormal liver functions and its' artificial replacement. A brief overview is given on some of the ways artificial liver support has been tackled in the past, and their unfortunate shortcomings. Some indication as to the nature of this present project is also briefly mentioned in the introduction.

Since there are a host of hepatic toxins implicated in liver failure, this project is centred on the removal of specific nitrogenous hepatic toxins, the removal of which is attempted by the use of adsorbents, in an effort to partly replace the liver's excretory function. The nature of the model hepatic toxins chosen, their role in hepatic failure, the need for their removal and their use in this project are discussed in the first section of Chapter 4. Section 4.2

is devoted to the background of the types of adsorbents employed as potential materials in this project for adsorbing specific toxins. Since the adsorbents used are not themselves biocompatible, and need a biocompatible coating, membranes to serve this function are used for encapsulating the adsorbents. The encapsulation procedure employed in this project and the biocompatibility functions served by such membranes are discussed in Chapter 4.2. In addition to the commercially available adsorbents, this project is involved in the evaluation of purposely made hydrogel particulates. These particulates are designed and developed by combining the advantages observed in the investigations with commercial adsorbents. Such particulates are then employed as adsorbents in this project. The mode of synthesis of these particulate hydrogels, their structure, functions, properties and the use of hydrogels in biomedical applications are also included in Chapter 4.2. The adsorption system employed, the analytical technique and evaluation procedures adapted for this project are outlined in Chapter 3.

As regards to the experimental work, the investigations are divided into three main areas. Firstly, observations of the adsorptive capacity of the adsorbents used (uncoated and coated species) are presented in Chapter 5 with special reference to ammonia, a key cerebral toxin in hepatic failure. Secondly, the observations obtained for other important nitrogenous hepatic toxins, namely amino acids

and false neurotransmitter amines, are presented in Chapter 6.

The knowledge gained from the study of micro-encapsulated adsorbents is then applied to a totally different but related aspect, that of encapsulating semiconductor based ammonia sensors. These chips, because of their small size, may eventually find use in catheters or as implantation devices in the bio-medical field. A more detailed account of the use of such sensors in this project is presented in Chapter 7.

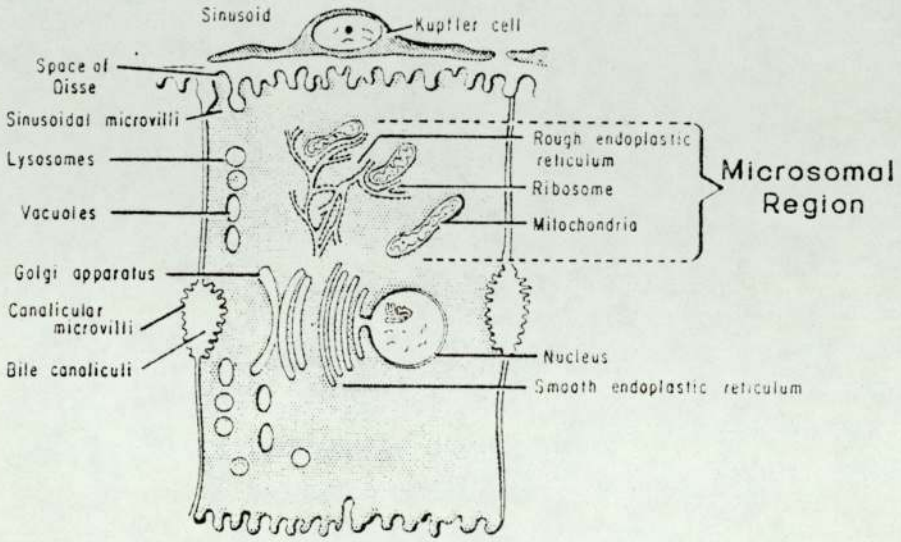
1.2 The Structure of the Liver

The liver, situated in the upper right hand part of the abdomen is the largest single organ in the body. It is divided into hexagonal lobules, consisting of a central hepatic venule, from which radiate plates of cuboidal parenchymal liver cells or hepatocytes, one cell thick. There are about 250 billion such cells in the normal adult organ. There is no division of labour among the hepatocytes and so all hepatocytes have the potential for all functions. The factors that regulate expression of individual hepatic functions are poorly understood, but are partially determined by hepatic microcirculatory influences. Phagocytic Kupffer cells, which serve a protective function in removing foreign bodies and bacteria are found at intervals along the vascular sinusoids or capillaries [1-4].

The liver in adult man constitutes approximately

Fig 1.1

Structure and Subcellular Components of a Normal Liver Cell



2.5% of body weight (or 1500 g); at birth it forms 5%[5]. The liver has a dual blood supply: from the digestive tract and spleen via the portal vein, and from the aorta via the hepatic artery. In cases of obstruction to flow in the liver, portal blood may be shunted around the liver to the systemic venous system[6].

The structural organisation of the parenchymal and vascular elements of the liver is adapted to its' special function as a guardian interposed between the digestive tract (and spleen) and the rest of the body. One aspect of this interposition is the handling of large amounts of nutrient amino acids, carbohydrates, lipids, vitamins and pollutant xenobiotics which enter the body in food and water[1,7,8]. These, and other biochemical functions which occur are governed by the organelles in the hepatocytes (e.g. mitochondria,lysosomes, peroxisomes, endoplasmic reticulum, cytoskeletal filaments and nucleus) Fig 1.1[2].

1.3 Functions of the Liver

The liver is essential for the maintenance of life, and is involved in almost every metabolic function of the body, and is specifically responsible for more than 500 separate activities. Fortunately,it has a large reserve capacity and needs only 10 to 20 per cent functioning tissue to sustain life. Complete destruction or removal of the liver results in death within 10 hours. The liver has an impressive re-

generative ability. Partial surgical removal will, in most cases, initiate rapid replacement of dead or diseased cells with new liver tissue[5].

The functions of the liver are numerous and complex, and are summarized in Fig 1.2[5,6,9]. A major hepatic function involves the effective uptake of substrates from the intestine and their subsequent storage, metabolism and distribution to blood and bile. Another function of the liver is the biotransformation or detoxification of xenobiotic pollutants (such as pesticides, preservatives and dyes ingested in food), drugs (used for treatment) and endogenous metabolites (such as ammonia). Linked to detoxification, the liver is an excretory organ and eliminates many substances from the body, of which the pigment bilirubin is the most obvious, but probably the least toxic [8,9]. The liver also synthesises a number of substances, among which albumin (one of the main plasma proteins) and the blood clotting factors (prothrombin and fibrinogen) are important examples[9]. Other liver functions include a) regulation of blood glucose by storing glycogen. Removal or failure of the liver leads to a fall in blood glucose, hepatic encephalopathy or coma, and ultimately death. b) deamination of proteins - with the formation of ammonia. Surplus amino acids are deaminated in the liver and many other tissues (e.g. kidney, skeletal muscle, peripheral tissue) with the production of ammonia, but only the liver has the capacity to convert ammonia

to urea. Therefore in liver failure, the amino acids and ammonia in blood increase and appear in the urine, and blood urea levels fall. c) the liver is also involved in the formation of bile (from red blood cell breakdown) which is used for emulsification of fats. Any blockage or failure of this pathway leads to accumulation of bilirubin and thus jaundice. d) the liver also stores vitamin B12, fat soluble vitamins A, D, E, K, iron, copper, protein and lipids. e) the liver also has a circulatory function and is involved in the transfer of blood from the portal to the systemic circulation. The activity of its' reticuloendothelial system (Kupfer cells) is involved in immune mechanisms and blood storage (the regulation of blood volume).

The great susceptibility of the liver to damage by chemical agents, appears to be a consequence of its' primary role in the metabolism and disposition of foreign substances. Its' position permits rapid access to the liver of toxins from the alimentary tract. Thus the concentration of foreign chemicals in the liver, the metabolic conversion occurring in the hepatocytes, the position of the liver as a portal to the tissues and the excretion of agents or their metabolites in the bile, all contribute to the vulnerability of the liver to chemical injury[10]. Thus when a patient's liver fails, it is appropriate to think of replacing three major aspects (those of synthesis, detoxification and excretion) of the liver function[9].

Fig 1.2 Major Functions of the Liver [5]

FUNCTION	COMMENTS
Formation and excretion of bile	Bile salts are essential for the digestion and absorption of fats and fat-soluble vitamins in the intestine.
Bile salts metabolism	
Bile pigment metabolism	Bilirubin, the main bile pigment, is a metabolic end product from the processing of old red blood cells. It is conjugated in the liver and excreted in the bile.
Carbohydrate metabolism Glycogenesis Glycogenolysis Gluconeogenesis	The liver plays an important part in maintaining the normalblood glucose level and providing energy for the body. Carbohydrate is stored in the liver as glycogen.
Protein metabolism	Serum proteins synthesized by the liver include albumin, and the alpha and beta globulins (not gamma globulin).
Protein synthesis	Blood-clotting factors synthesized by the liver include fibrinogen(I),prothrombin(II) and factors V, VII, VIII, IX and X. Vitamin K is a necessary co-factor in the synthesis of II, V, IX and X.
Urea formation	Urea is formed exclusively in the liver from NH_3 which is then excreted in the urine and faeces. NH_3 is formed from deamination of amino acids and action of intestinal bacteria on amino acids.
Protein (amino acid storage)	

Fig 1.2 Major Functions of the Liver Contd.

FUNCTION	COMMENTS
Fat metabolism	Hydrolysis of triglycerides cholesterol, phospholipids and lipoproteins (absorbed from the intestine) to fatty acids and glycerol.
Ketogenesis	
Cholesterol synthesis	The liver plays a major role in cholesterol synthesis, most of which is excreted in the bile as cholesterol or cholic acid.
Fat storage	
Vitamin and mineral storage	Fat-soluble vitamins (A,D,E,K,) stored in the liver; also vitamin B_{12}, copper and iron.
Steroid metabolism	Liver in-activates and excretes aldosterone, glucocorticoids, oestrogen, progesterone and testosterone.
Detoxification	The liver is responsible for biotransformation of substances that are potentially harmful into harmless substances which are then excreted by kidneys (e.g. drugs).
Flood chamber and filter action	Liver sinusoids provide depot for blood backed up from venae cavae (right heart failure); phagocytic action of Kupfer cells remove bacteria and debris from blood.

1. Intravenous infusion of clotting factors, glucose and albumin, can partly replace its' synthetic function.

2. Certain drugs need to be avoided, due to the loss of the detoxification function (prolonging drug action)[11].

3. The third function, that of excretion, is the most important, when considering the development of an artificial liver.

1.4 Liver Failure

In liver failure, the amount and activity of the detoxification liver enzymes are greatly reduced by often more than 90%. This results in an accumulation of toxins in the blood and cerebrospinal fluid, which in turn inhibit many other vital enzymes of the liver and other organs and also interfere with the regulation of brain metabolism. As a result, the remaining enzymes of energy production, synthesis, detoxification and regeneration are inhibited. The loss of the primary liver function is also complicated by the metabolic effects of necrosis of much of the liver tissue. Thus the mechanisms of homeostasis are disturbed and then overloaded[12,13]. In this way, a vicious circle of destruction can develop, characterized by progressive and severe mental changes, starting with confusion and rapidly advancing to stupor or hepatic coma and ultimately death[2,14,15].

One possible step of influencing liver regeneration could be the removal of the endogenous toxins.[16]

These toxins include ammonia, mercaptans, free fatty acids and free phenols. In addition, protein-bound anions such as bilirubin and bile acids,normally excreted by the liver, accumulate in liver failure. There are also alterations in the plasma amino acid composition, leading to accumulation of false neuro-transmitters[17], (a detailed discussion of which is given in Chapter 4). It is also common for the coma-producing potential of those toxic substances to be multiplied several fold when they are present together, and they may then act synergistically on the brain[8].

1.4(1) Pathogenesis of Hepatic Encephalopathy

The pathogenesis of hepatic encephalopathy is the term used to describe the origin and degenerative course of cerebral intoxication caused by intestinal contents (e.g. ammonia) that have not been metabolized by the liver in hepatic failure. This leads to neuro-psychiatric syndromes characterized by mental confusion and progressing to unconsciousness or encephalopathy (coma) and then death[5,18]. The condition may occur when there is liver cell damage, due to necrosis or shunting (pathological or surgically created) which permits large amounts of portal blood containing ammonia and other toxins to reach the systemic circulation without traversing the liver[19]. The patho-genesis of hepatic coma is unknown. The disorder is usually classified as metabolic, because the neuro-logical symptoms accompanying both acute and chronic liver failure are potentially fully reversible with

appropriate treatment and, except in cases of chronic hepatocerebral degeneration, are not associated with extensive morphological damage to neurons. Because the liver is the body's principal organ of chemical homeostasis, hepatic encephalopathy could result from failure of the diseased liver to a) release nutrients and cofactors essential for normal brain metabolism and function, or b) remove circulating neurotoxins which may then acculumate in the brain. Much evidence supports the view that hepatic coma is caused by the abnormal accumulation of neurotoxins in the brain. A number of known and suspected toxins are increased in the plasma and cerebrospinal fluid (C.S.F.) of patients with hepatic encephalopathy, and measures that 'cleanse' the plasma (charcoal haemoperfusion, polyacrylonitrile membrane haemodialysis) often improve consciousness in patients with acute hepatic failure[5,20 - 25]. Many toxic factors have been implicated as contributing to the symptoms of hepatic encephalopathy, but the strongest arguments can be advanced for ammonia, mercaptans, short-chain fatty acids, and aromatic amine analogues of central neurotransmitters (Brunner 1975, Zieve 1975). Incidentally the studies of this project are concerned with nitrogen based toxins such as ammonia, amino acids and aromatic amines - false neurotransmitters, more detailed information of which may be found in a subsequent chapter (Chapter 4).

The following general and possible interrelated mechanisms of encephalopathy have been suggested:

1. impaired brain energy metabolism
2. altered neuronal membrane function, and
3. depressed neurosynaptic transmission.

Impaired brain function is a consistent feature of severe liver disease and occurs with two forms of hepatic insufficiency. Fulminant acute hepatic failure causes a rapidly developing delirium, progressing through the stages of delirium, stupor, coma and often death [5]. Chronic cirrhosis with portal-systemic shunting of blood, produces a more insidiously evolving, relapsing encephalopathy, characterized by disturbances of mentation, episodic stupor and abnormalities in cerebral functions. Early diagnosis and treatment of hepatic encephalopathy is crucial, and can substantially reduce the mortality and morbidity of patients with hepatic failure. Hepatic insufficiency results in several relatively distinct neurologic disorders,the characteristics of which depend on the acuteness or chronicity of the liver problem and the anatomy of the affected neurologic structure, as shown in Fig 1.3. A more extensive discussion of the neurological changes has been given by Plum and Hindfelt 1976.

The following section discusses the commonly encountered syndromes of hepatic stupor or coma that accompany fulminant acute liver failure, or acute worsening of chronic liver failure.

As indicated in Fig.1.4 roughly five grades of hepatic encephalopathy can be established in order of severity. Such grading is crucial for identifying the

Fig 1.3 Neurological Complications and Accompaniments of Severe Liver Disease

Hepatic encephalopathy

Hepatic stupor or coma

- Acute (acute inflammation or necrosis due to hepatitis, hepatotoxic agents, Reye's syndrome)
- Subacute or chronic, progressive (advancing hepatitis or cirrhosis)
- Episodic or recurrent portal - systemic encephalopathy (portal bypass plus protein load)

Chronic progressive cerebral degeneration

- Spongiform, with cirrhosis
- Wilson's Disease

Hepatic myelopathy

Hepatic peripheral neuropathy

Fig 1.4 Grades of Severity in Hepatic Encephalopathy[a]

GRADE	SIGNS
1	Mild mental impairment, hypocapnic hyperventilation.
2	Lethargy, confusion, asterixes, hypocapnia.
3	Arousable stupor, pupillary and oculocephalic reflexes present, diffuse muscle paratonia, increased stretch reflexes, extensor plantar responses frequent, hypocapnic hyperventilation.
4	Unarousable coma, pupillary and oculocephalic reflexes present, motor hypertonus, often decerebrate and extensor plantar responses, hypocapnic hyperventilation present.
5	Unarousable coma, pupillary and/or oculocephalic reflexes absent, motor tone flaccid, stretch reflexes suppressed, may have metabolic or even respiratory acidosis.

a Within any grade, all signs may not be present; to estimate prognosis, grading should be based on the single worst-level sign. Muscle flaccidity is only a grade 5 sign if preceded during the earlier course of coma by hypertonus (5)

severity of the disease and comparing patients, and determining the potential effects of therapy [5].

One of the problems encountered when dealing with hepatic failure, by an invasive technique such as haemoperfusion, is deciding at which level of hepatic encephalopathy to administer treatment. If the patient is maintained in full consciousness, and in good metabolic conditions by such a temporary artificial liver support system, then survival is possible due to the capacity of the liver to regenerate . However regeneration also depends on the severity of the initial assault.

Patients in Grade 1-3 coma have a high survival rate, but the mortality is very high for those in Grade 4 and 5 coma. Unfortunately there is no fixed criteria which could be used to predict survival because the syndrome has a strikingly variable course. Some patients in Grade 4 coma do survive after haemoperfusion, others die without regaining consciousness, some at a time when liver function is improving [22].

It is always going to be difficult to assess when regeneration of the patient's liver is no longer possible, so as to give liver transplantation the go-ahead. Also there are no competent means whereby a patient with liver failure can be kept alive until such time as a donor becomes available for liver transplantation. The need for such an artificial liver support system is detailed in the following section.

1.5 Artificial Liver Support

The regenerative capacity of the liver cell is almost unlimited. Therefore after acute liver damage, be it viral, toxic, hypoxic or surgical in origin, restitutio ad integrum, is the usual outcome [22]. In two forms of liver disease, however, this is not the case. The first form consists of those in fulminant hepatic failure. In this case, liver regeneration often is not fast enough to keep the organism alive. When the patient has lapsed into deep coma, the mortality is around 80%, which accounts for 400 deaths per annum in England and Wales, and includes a number of deaths from paracetamol overdose, viral hepatitis[14], exposure to hepatotoxins or drug reactions[8,9,26-29]. The second form includes patients in end stage cirrhosis (irreversible liver failure), where regeneration is disturbed by a hypertrophic architecture of fibrotic tissue. In this situation there is no hope of reversing the course, but transplantation may be possible. This is potentially the largest group of patients, as cirrhosis alone accounts for about 1400 deaths per annum in England and Wales (Dunlop 1975)[9]. For these extreme forms of liver disease, and for critical situations before and after liver surgery a temporary artificial liver support system is needed[30]. Thus the artificial liver would be used like an artificial kidney, both to gain time until a donor organ becomes available and to improve the condition of the recipient.

It is known that kidney transplantation would not be undertaken without dialysis support[31-32].

However, as yet, no equivalent procedure can be carried out before the technically more difficult liver transplant. Likewise at present, there are no means whereby a patient with liver failure can be kept alive until such time as a donor becomes available for liver transplantation[9,33-35]. The major deficiency in available therapy for patients with hepatic failure is an efficient and practical method for temporary liver support. The development of a corresponding artificial liver is made more difficult by the sheer complexity of the biochemical role played by the liver, and the rapid way in which severe impairment of hepatic failure is reflected in disturbances in other organs, notably the brain, as previous stated. On the other hand, these difficulties are to some extent counterbalanced by the ability of the liver, given correct conditions, to regenerate rapidly[36-37]. Thus if functions of the liver can be supplemented during an acute crisis, and the patient maintained with full consciousness and in good metabolic conditions, total recovery and liver regeneration is possible in many cases, provided that the initial assault is not too severe[8,9]. This is the aim of the artificial liver support system. The procedure needs to be quickly available when needed, safe and capable of sustained support for up to 7 days.

1.6 A brief overview of the trials and tribulations with artificial liver support: Past experience and basis for present project

The ultimate aim of hepatic support techniques is to sustain the patient in liver failure while regeneration takes place or while a suitable organ for transplantation can be found. The ideal liver support, therefore, would be one which could take over the functions of the liver which are essential for the survival of the patient over this period. The priority is control of the endogenous intoxication which leads to hepatic coma and death[38]. There have been numerous attempts by many workers at replacing the liver's excretory function in patients with acute liver failure. The range of such techniques employed, includes cross-perfusion techniques, extracorporeal pig or baboon liver perfusion, exchange transfusion of blood or plasma, haemodialysis and haemoperfusion over adsorbents, all of which have been extensively reviewed in the past[8,18,21,22,39-51].

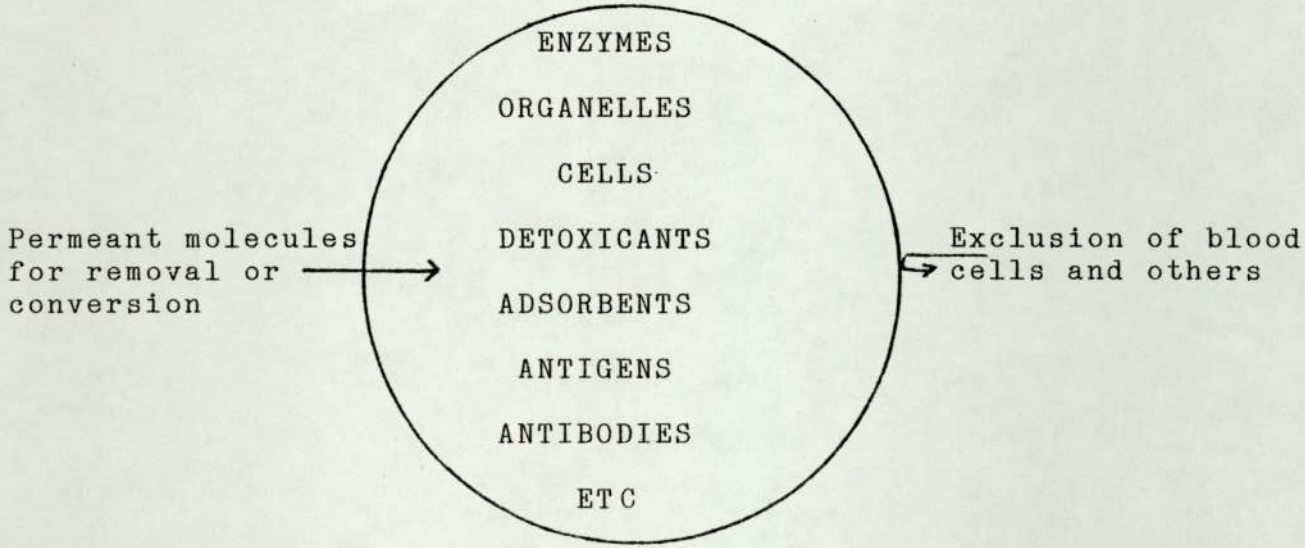
However, none of these methods are entirely satisfactory due to problems largely associated with platelet, erythrocyte and leucocyte depletion, a reduction in fibrinogen, complement and antibody levels in the patients, and also the adsorption of other blood constituents (e.g. glucose, calcium)[16,52]. Most of the haemoperfusion methods employed were used for short-term treatment of acute intoxication and Yatzidis (1964) was the first to report the use of charcoal granules in direct blood perfusion for uraemia and acute intoxication.

Microencapsulation (coating) of activated charcoal using albumin-collodion, first proposed by Chang (1966), prevented the free passage of charcoal particles into the circulation, which had long been recognised as a potential hazard. These problems associated with the use of adsorbents and the measures employed to increase their biocompatibility, are reviewed in a subsequent chapter - Chapter 4. However, it is appropriate at this point to note that following the use of the biocompatible albumin-collodion microencapsulated activated charcoal (by Chang 1972) and the demonstration of its' successful use in acute fulminant hepatic failure, most successful adsorbents have a blood compatible membrane associated with them.

Incidentally the principle of artificial cells can be used to include charcoal, resins, enzymes, antigens and numerous other biologically active materials as shown in Fig 1.5[22,38,53].

Other approaches such as microencapsulating enzymes, immobilized enzymes, liver perfusion, liver slices, liver cell culture, immunoadsorbents and other systems may eventually all be required to make up a complete artificial liver (containing multiple sorbents or reactors). In this present time, new methods like continuous membrane plasma separation and liver cell transplantation into the spleen have been developed. The older methods of haemoperfusion and dialysis have been improved. Enzymological methods and liver transplantation have made good progress[22,54,55].

Fig 1.5 Variation in Contents of Artificial Cells (54)



Unfortunately clinical difficulties continue to arise in obtaining conclusive results in long-term survival of acute fulminant hepatic failure patients. These are related to variations of survival rate with age and etiology, the comparatively small number of patients in any one centre, and the use of haemoperfusion devices of varying blood compatibility and efficiency. Thus an animal model suitable for the statistical analysis of long-term survivaland recovery is desperately required to solve this problem[49,56].

1.7 An Overview of the Present Project

While some adsorbents are known to date to give satisfying results, they still show no selective adsorption. The need exists for a range of biocompatible adsorbents, showing some degree of specificity and selectivity for various blood toxins, to supplement and overcome some of the disadvantages of available adsorbents such as charcoal and ion exchange resins. The use of adsorbents which are themselves biocompatible would present the ideal adsorbent. No attempt has been made by workers to synthesise purposely built biocompatible permselective adsorbents, to meet the desired or 'ideal' specificity and variability for toxins elevated in liver failure. In this project however, such novel hydrogel adsorbents based on the acrylic hydrogel poly(hydroxyethylmethacrylate) or polyHEMA, have been synthesised (although not by the author) in particulate form and evaluated (by the author)

for their selective adsorptive properties. The composition of these adsorbents are also varied by the incorporation of additional copolymers in the structure of the particulates. The synthesis of these adsorbents is detailed in Chapter 4.

Most haemoperfusion trials in the past have been carried out by clinicians who usually use the best empirical approach possible to correct abnormal toxin levels. The major aspect of this study is to obtain more specific selective adsorbents for low molecular weight nitrogenous hepatic toxins, in particular ammonia, and also amino acids and amines, which are chosen as examples of model hepatic toxins in this project.

Charcoal, an important adsorbent material because of its' broad adsorptive spectrum, is initially used uncoated in this project. Studies with poly(2-hydroxyethylmethacrylate)(polyHEMA) coated charcoal species are also performed, to determine whether polyHEMA, (which is used to confer some degree of biocompatibility) can also transport ammonia, as it does water and other small molecular weight substances. Studies of this type have not been performed before. Incidentally, the coating procedure employed and the use of polymers in biomedical studies are discussed in Chapter 4.

A range of conventional ion exchange resins of both the acidic and basic types, and also polymeric Amberlite XAD resins are also used. The structure and properties of these adsorbents are detailed in Chapter 4. These

adsorbents are 'screened' for their potential adsorptive capacity for nitrogen-based toxins in an attempt to arrive at information concerning the effects functional groups and structural specificity have, on the eventual adsorptive capacity of these resins. Again there is no quantitative information on ammonia, amino acid and amine adsorption by ion exchange resins and polymeric resins in the literature. As a measure of biocompatibility these adsorbents are also encapsulated with HEMA and further studies involving the coated species performed. Assessments of the permselectivity and transport properties of the polymer membrane are then obtained by dynamic adsorption techniques (detailed in Chapter 3) which provide the common basis for the evaluation technique of such adsorbents in this project. Using such techniques, the effects that the polyHEMA coating impose on the rate and efficiency of adsorption are investigated. Again this work on the adsorption of ammonia by polyHEMA coated ion exchange resins has not been studied before.

This study is used as a step towards the design of permselective membranes for the specific toxins studied in this project - ammonia being the most important. Information of this type is of great value in designing novel biocompatible particulate hydrogel adsorbents, possessing various structures and specific adsorptive properties, for use as artificial cells in artificial liver support systems: the ultimate aim of this project.

It is appropriate to note that the novel hydrogel particulate adsorbents are not synthesised by the author

the reason being that two such projects are being run in parallel. One using a chemist is involved with the synthesis of novel hydrogel polymers. The other using a biologist (the author) is involved with the initial development of the evaluation and analytical techniques and thus the evaluation of the adsorbents and perm-selective membranes used. The author is also involved in the evaluation of the HEMA - based novel hydrogel beads, synthesised by using and combining some of the advantages of ion-exchange and polymer membrane properties observed throughout the project.

In conjunction with this, the project is involved in the design and modification of permselective polymer membranes for use in the semi-conductor-based gas sensor field, and related applications. Both projects are concerned with membranes and adsorbents that will deal selectively with nitrogenous bases such as ammonia and also amino acids and amines in aqueous solution.

The use of gas sensors by EMI, the collaborating body, has been involved in the detection of water-bound waste, ranging from simple gaseous molecules such as O_2 and CO_2 in a range of atmospheric conditions, to high molecular weight pollutants (e.g. chlorinated hydrocarbons) in aqueous environments (e.g. rivers). These sensors, in order to operate satisfactorily, need to be encapsulated (in this project by a polymeric membrane) for protection and exert permselective control. One aspect of this requirement is reflected in the need for such sensors to operate in aqueous environments

and atmospheric environments of varying humidities. Since the semi-conductor sensors are sensitive to water, it is necessary either to preferentially prevent the access of water to the device (which is difficult because of the low molecular weight of water) or to ensure that the water signal is constant. The hydrogel containing only 'bound' water is one type of membrane having considerable potential in this respect. Since these semi-conductor sensors can detect ammonia, they may be an important asset for use in artificial liver support systems.

1.8 Specific Aims of the Project

In summary, the objectives of this project are therefore severalfold. Firstly it is involved in the evaluation of the adsorptive capacity of conventional adsorbents for nitrogen containing toxins. Secondly, it is involved in the development of polymer based biocompatible membranes for the microencapsulation of these adsorbents. Thirdly it is concerned with the evaluation of the ammonia transport properties of these polymer based membranes used. Fourthly, these studies are conducted in an attempt to obtain guidelines for the ultimate design of novel hydrogel particulate polymers, which could be useful in providing a better understanding of how to reduce hyperammonaemia, encountered in acute hepatic failure.

Novel biocompatible hydrogel particulates, thus synthesised are then evaluated for the selective adsorption of the model nitrogenous hepatic toxins (particularly ammonia) studied. It is hoped that from the range of adsorbents studied, a quantitative and qualitative understanding of the variable specificity and selective adsorption of these toxins will be created. At the same time, it is hoped that the adsorbent will allow the undisturbed passage of other chemical species, not indicated in liver failure. This study of the evaluation of polymer-based artificial liver support systems, is conducted purely on an in-vitro basis, to provide a better understanding of these materials before pursuing the more difficult work involving hazardous animal work and clinical trials.

A selection of biocompatible adsorbents should then be available for use in clinical animal studies and if success permits, applied biomedically in artificial liver support systems. Fifthly, in parallel, this work is expected to provide information that will assist in the design of more ammonia permeable membranes, for the encapsulation of semi-conductor ammonia gas sensors. These sensors may then be useful in artificial liver support systems if they could be implanted and thus be used in conjunction with adsorbents, to monitor the ammonia concentration of the circulating blood at different points.

CHAPTER 2

MATERIALS AND EXPERIMENTAL TECHNIQUES

2.1 Introduction

A record of the materials used and their suppliers are contained in this chapter. In addition, an account of the methods and experimental techniques employed is included. A detailed description of the dynamic adsorption technique and the automated analysis of nitrogen containing compounds is not presented here, since these techniques were developed during the course of this project, and are detailed in Chapter 3.

2.2 Model Toxins

The following model toxins, shown in Table 2.1 were all supplied in solid form, with the exception of ethanolamine, supplied in liquid form.

Table 2.1

CHEMICAL	SUPPLIER
DL -∝ Alanine (2-Amino propionic acid)	Hopkin & Williams Ltd.
Ammonium chloride (AR)	BDH Chemicals Ltd.
Bromosulphophthalein	Koch-Light Laboratories Ltd.
L-Cysteine	BDH Chemicals Ltd.
Ethanolamine	BDH Chemicals Ltd.
Glycine (Aminoacetic acid)	Hopkin & Williams Ltd.
L-Histidine	BDH Chemicals Ltd.
D-L-β-hydroxyphenethylamine (2-Amino-1-Phenylethanol)	Sigma Chemical Company
D-L-Isoleucine	BDH Chemicals Ltd.
D-L-Methionine	Hopkin & Williams Ltd.
D-L-Octopamine hydrochloride (D-L-p-hydroxyphenylethanolamine HCl)	Sigma Chemical Company
L-β-phenylalanine	BDH Chemicals Ltd.
L-Tyrosine	BDH Chemicals Ltd.
Urea (AR)	Hopkin & Williams Ltd.
D-L-Valine	Koch-Light Laboratories Ltd.

2.3 Adsorbents

A number of adsorbent materials were used in this research. They were thoroughly washed before use and are listed in Table 2.2

Table 2.2

MATERIAL	SUPPLIER
Activated charcoal beads	BDH Chemicals Ltd.
Amberlite XAD-2 resin	BDH Chemicals Ltd.
Amberlite XAD-4 resin	BDH Chemicals Ltd.
Amberlite XAD-7 resin	Polysciences Inc.
Amberlite IRC-50 (H^+form) STD grade	BDH Chemicals Ltd.
Amberlite IR-120 (Na^+ form) STD grade	BDH Chemicals Ltd.
Amberlite IR-120 (H^+ form) Analytical grade	BDH Chemicals Ltd.
Amberlite IRN-77 (H^+ form) Nuclear grade	Rohm & Haas Ltd.
Dowex 1-X4 (Cl^- form) STD grade	Polysciences Inc.
Dowex 50W-X8 (H^+ form) STD grade	BDH Chemicals Ltd.
Duolite C255 (H^+ form) STD grade	BDH Chemicals Ltd.
HSA coated Amberlite XAD-7	Kings College, London
Glass Beads ($\triangleq$60 mesh)	BDH Chemicals Ltd.

Additional adsorbent materials used were the particulate hydrogels. These were synthesised during the course of this work by Dr. P.J. Skelly and Mr. U.S. Atwal of this research laboratory. The monomers and cross-linking agents used in their synthesis are shown in Tables 2.3 and 2.4 respectively.

Table 2.3

MONOMERS	ABBREVIATION
Acrylic Acid	AA
Acrylamide	ACR
Diacetone acrylamide	DAA
2-Hydroxyethyl methacrylate	HEMA
Methacrylic acid	MAA
N-Methylolacrylamide	NMACR

Table 2.4

CROSS-LINKING AGENT	ABBREVIATION
Ethylene dimethacrylate	EDM
NN-Methylene bis acrylamide	NN'MBA

2.4 Materials for Preparation and Application of Hydrogel Coatings

Some of the adsorbent materials were also used after the application of an encapsulating membrane of the hydrogel polyHEMA. The polymerisation of HEMA is described in Chapter 4. A list of the materials used in the polymerisation technique is shown in Table 2.5.

Table 2.5

MATERIALS FOR POLYMERISATION		SUPPLIER
Monomer	2-Hydroxyethylmethacrylate	Victor Wolf Ltd.
Initiator	t-Butylcyclohexyl perdicarbonate	Laporte Industries
Solvent	Ethanol	BDH Chemicals Ltd.

The purification of 2-hydroxyethylmethacrylate was carried out by Victor Wolf Ltd. The purified monomer was stored in a refrigerator until required for polymerisation.

The materials for the microencapsulation of adsorbents are shown in Table 2.6

Table 2.6

MATERIALS FOR ENCAPSULATION		SUPPLIER
Polymer	poly-2-hydroxyethyl methacrylate	Prepared in research
Solvents	Ethanol	BDH Chemicals Ltd.
	Acetone	BDH Chemicals Ltd.
	Propan-1-ol	BDH Chemicals Ltd.
Repelcote	2% sol dimethyl-dichlorosilane in 1,1,1-trichloroethane (water repellent-surface treatment)	Hopkin & Williams Ltd.

2.5 Experimental Techniques

This section contains information relating to the experimental techniques used during the course of this research and the materials employed in such techniques.

2.5.(1) Technicon AutoAnalyser

The chief analytical technique used throughout this research project was that of the Technicon Auto-Analyser, used for the assay of nitrogen containing liquid samples. This section contains a list of the reagents used and their suppliers, presented in Table 2.7. In addition, an account of the preparation of the reagents and standard solutions for the analysis is included.

Table 2.7

REAGENT	SUPPLIER
Ethylene diamine tetra acetic acid or EDTA (disodium salt)	BDH Chemicals Ltd.
Sodium hypochlorite solution (12% W/V available Cl)	BDH Chemicals Ltd.
Phenol	BDH Chemicals Ltd.
Sodium hydroxide	BDH Chemicals Ltd.
Sodium carbonate	BDH Chemicals Ltd.
Ammonium chloride	BDH Chemicals Ltd.

All reagents should be of analytical grade. Once prepared all reagents are stable for a considerable time. The preparations of reagents are as follows:

i. Disodium ethylene diamine tetra-acetate (EDTA)

2g EDTA were dissolved in 1 litre of distilled water and 0.1 ml of concentrated Teepol added. The solution was adjusted to pH 11.00 with sodium carbonate solution. The use of EDTA prevents the precipitation of salts, which, if allowed to occur, gives rise to a noisy base line on the recorder. Teepol acts as a wetting agent to promote good bubble patterns.

ii) Sodium hypochlorite

This solution should contain 5 per cent available chlorine and any suspended matter should be removed by filtration through glass wool.

iii) Phenol

83g phenol were dissolved in 100 ml distilled water at room temperature.

iv) Sodium hydroxide (Stock solution)

200g sodium hyroxide were dissolved in 1 litre of distilled water.

v) Sodium phenate

180 ml of the sodium hydroxide were added to the phenol solution prepared as above and diluted to 1 litre with distilled water. This alkaline phenol solution darkens on standing, even when stored in an amber-coloured bottle. Although this has no effect on the analytical system, it can be prevented to some extent by keeping the reagent in the refrigerator, when not in use.

vi) Standard solution of ammonia

0.191g of ammonium chloride was dissolved in ammonia free water and diluted to 1 litre. This solution contains 50 mg of ammoniacal nitrogen per litre. Appropriate standard solutions containing 1, 5, 10,

15 and 20 mg Nl^{-1} were prepared from this solution.

2.5.(2) Dynamic Adsorption System

This experimental technique was developed during the course of the research project and is described in Chapter 3.

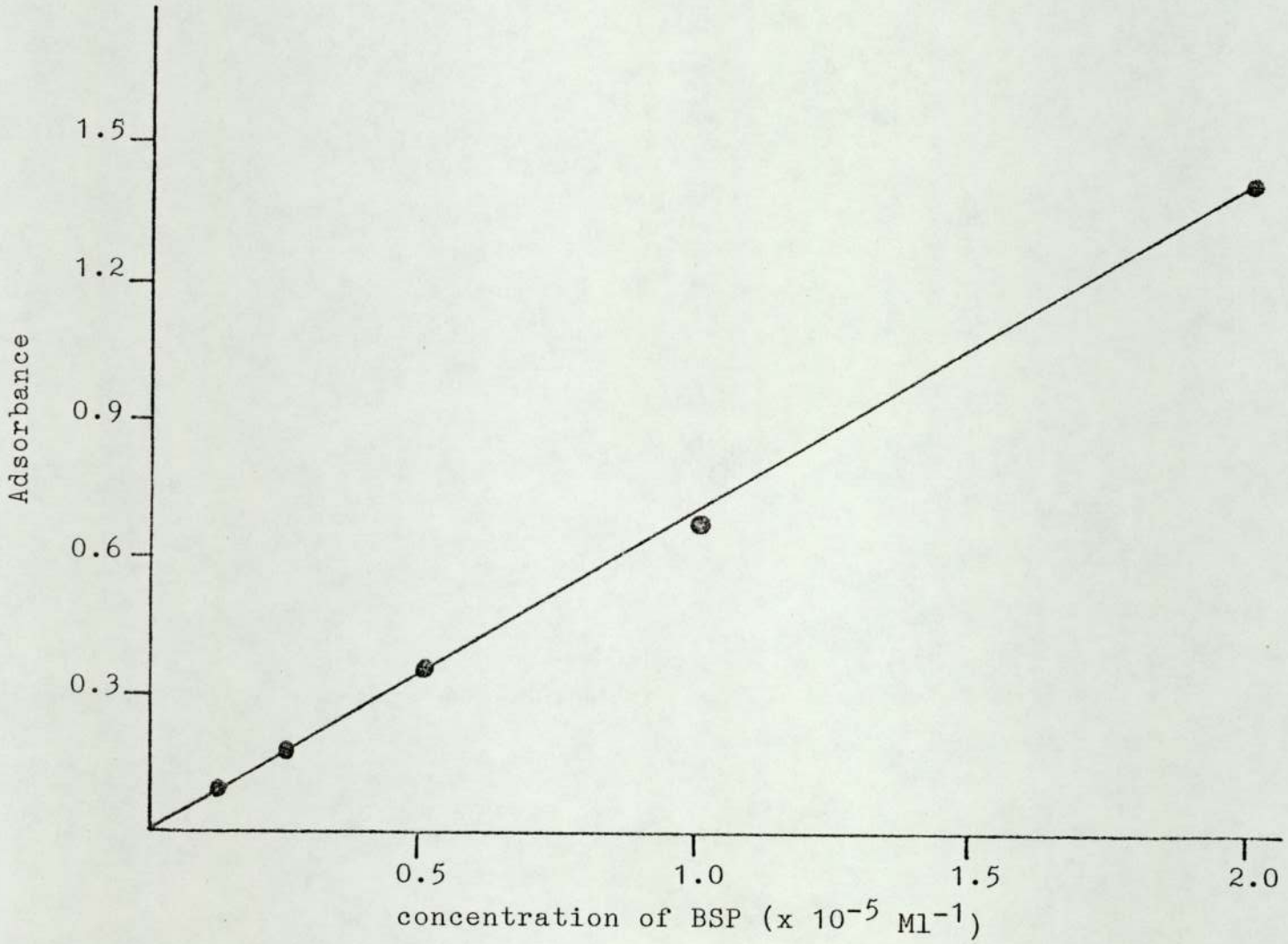
2.5.(3) Scanning Electron Microscropy

The surface morphology of uncoated and coated adsorbents was examined using the Stereoscan S150 Model scanning electron microscope. The electron micrographs of a selection of the uncoated and coated adsorbents are presented here in Plates 2.1 - 2.13 and illustrate the existence of a microencapsulation coating. (The adsorbent materials (dehydrated) were prepared for scanning by coating with gold).

2.5.(4) Ultra Violet Spectroscopy

The analysis of Bromosulphophthalein (BSP) samples was performed using the SP 600 U.V. Spectrophotometer. Samples were read at 580 nm against standard solutions of BSP in 0.1M NaOH. Using a stock solution of 2×10^{-3}M BSP, standard solutions of various concentrations (e.g. 2×10^{-5}M, 1×10^{-5}M, 0.5×10^{-5}M, 0.25×10^{-5}M, 0.125×10^{-5}M and 0.0625×10^{-5}M BSP) were

Fig 2.1 Calibration Curve for Bromosulphophthalein Assay



prepared. The calibration curve obtained for the standards is presented in Fig 2.1

2.5.(5) Atomic Adsorption Spectrophotometry

Atomic adsorption spectrophotometric analysis using the Perkin Elmer Spectrophotometer Model 400 was used for the assay of inorganic ions (Na^+, K^+, Ca^{2+}, Mg^{2+}). This assay was performed by Miss A.Mistry and Mr. K. Murthy of the Geology Department.

2.5.(6) Amino Acid Analyser

An Amino Acid Analyser (Locarte, London) was used in the determination of amino acid concentrations in samples containing a mixture of amino acids. Samples were assayed by Mr. S. Howitt of the Biological Sciences Department.

2.6 Miscellaneous Chemicals

In addition to the previously listed chemicals the physiological media - Krebs Mammalian Ringer Solution (Krebs improved ringer 1)[6] was used. This was prepared using solutions all isotonic with serum. The chemicals for the stock solutions (all of analytical grade) are listed in Table 2.8. The stock solutions were made up individually and then added in series in their appropriate volumes.

Table 2.8

MATERIALS	SUPPLIER
Sodium chloride	Fisons Scientific Apparatus Ltd.
Potassium chloride	Fisons Scientific Apparatus Ltd.
Calcium chloride	Fisons Scientific Apparatus Ltd.
Potassium dihydrogen Orthophosphate	Hopkin & Williams Ltd.
Magnesium sulphate heptahydrate	BDH Chemicals Ltd.
Sodium bicarbonate	Fisons Scientific Apparatus Ltd.
Sodium pyruvate	BDH Chemicals Ltd.
Sodium fumarate	BDH Chemicals Ltd.
Sodium glutamate	BDH Chemicals Ltd.
Glucose	BDH Chemicals Ltd.

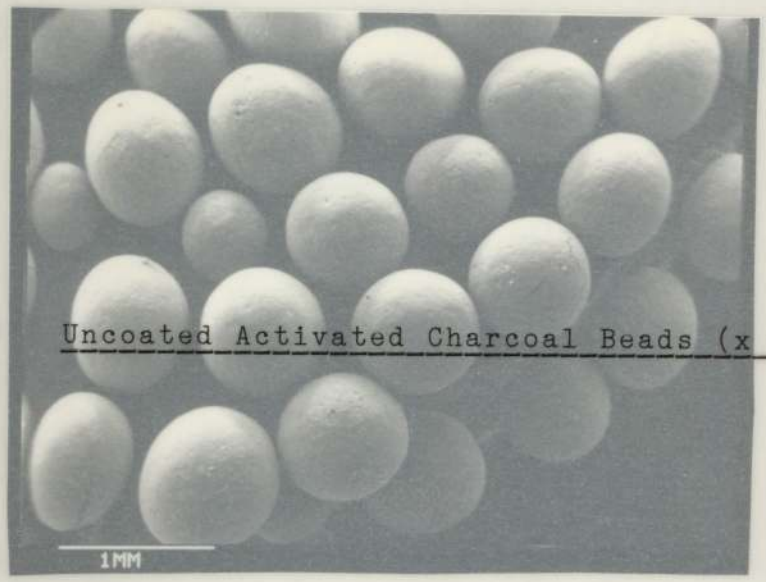


Plate 2.1 Uncoated Activated Charcoal Beads (x 20)

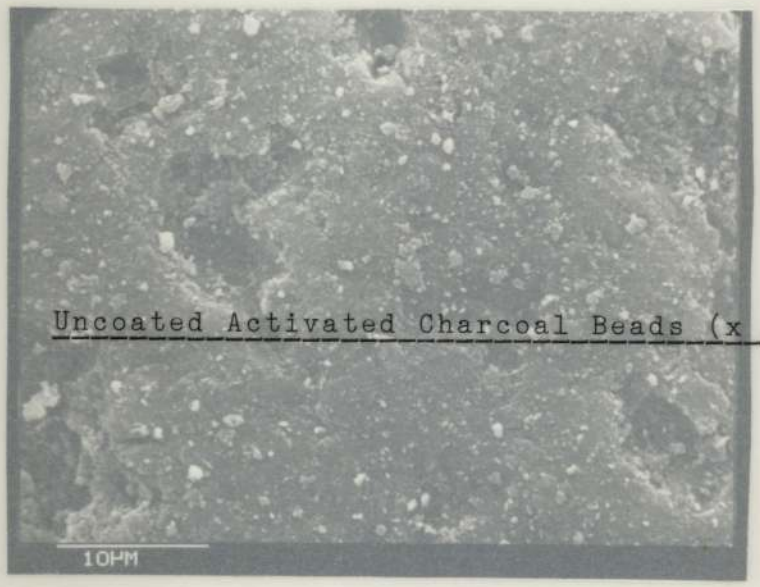


Plate 2.2 Uncoated Activated Charcoal Beads (x 2000)

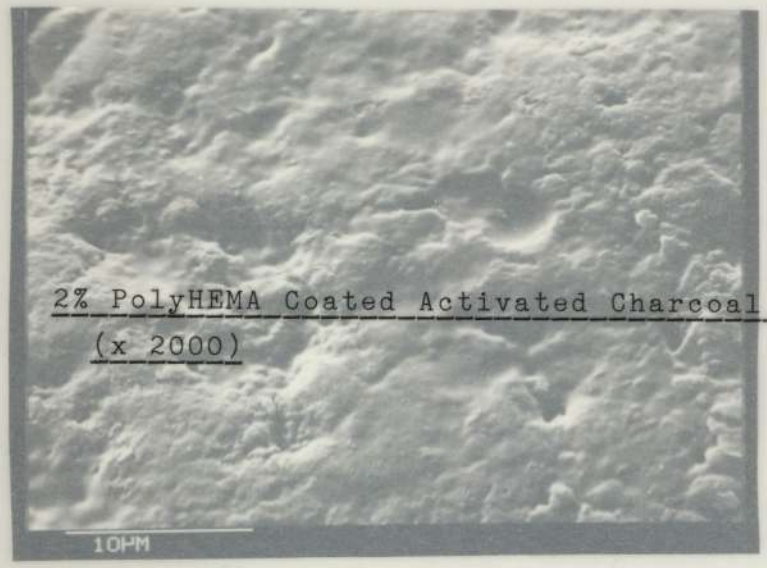
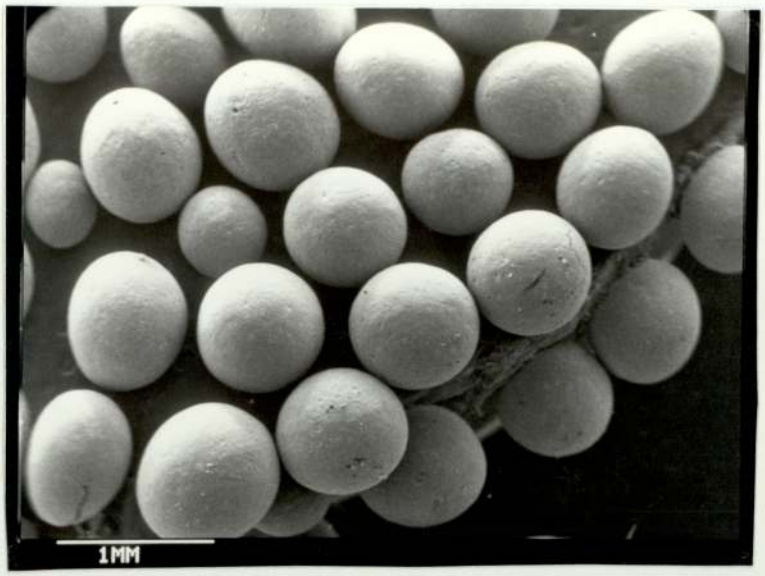


Plate 2.3 2% PolyHEMA Coated Activated Charcoal Beads (x 2000)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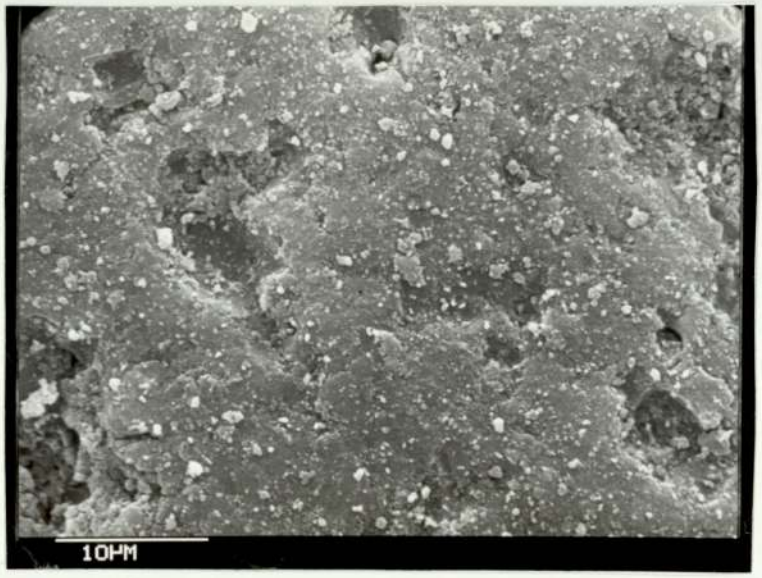

10UM

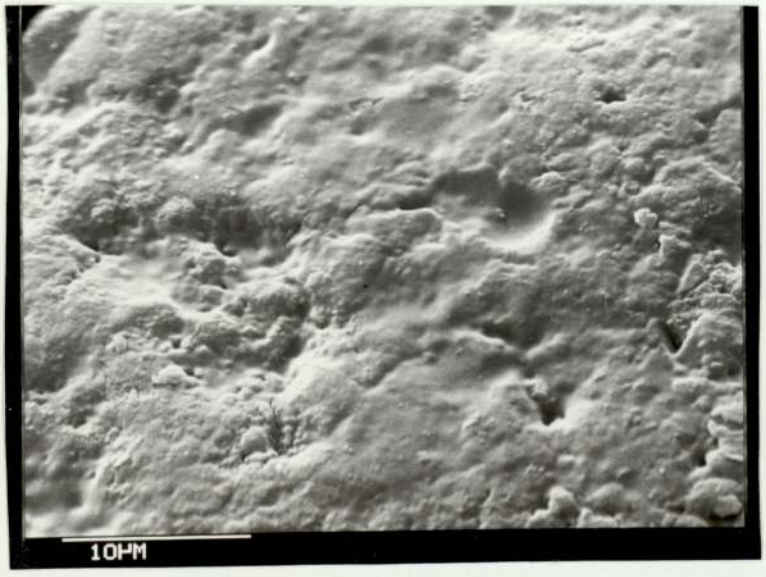
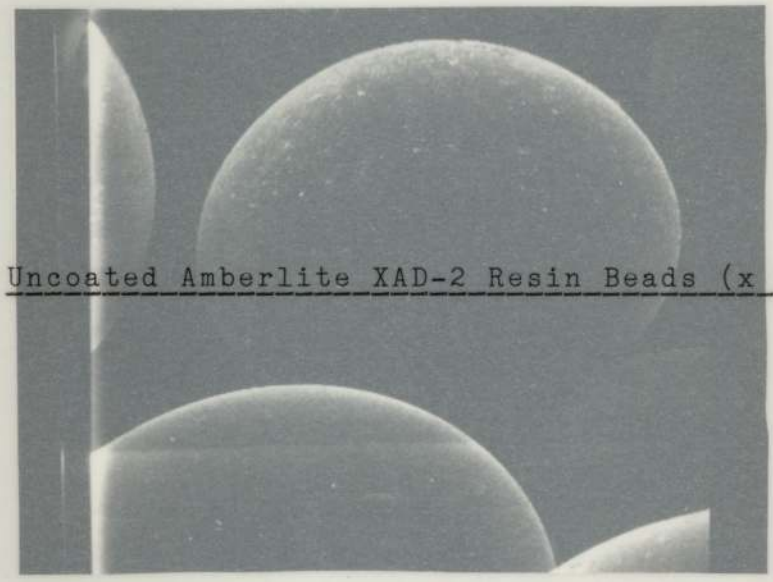
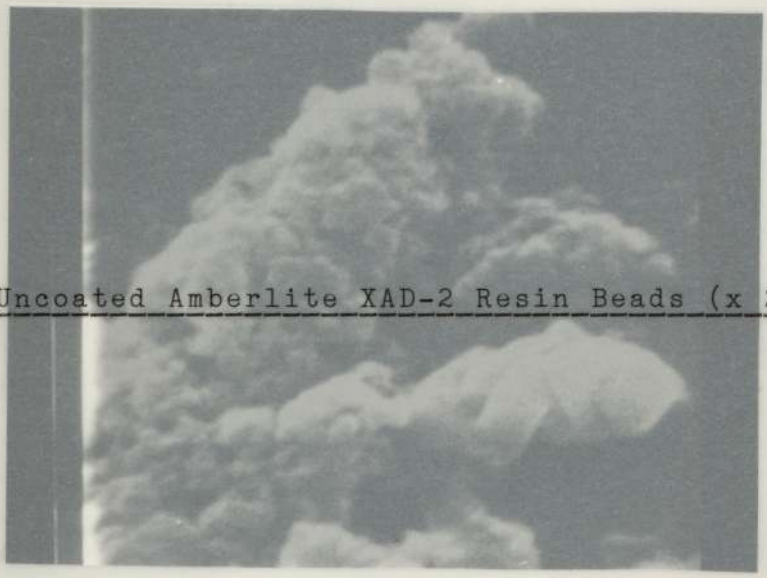

10UM

Plate 2.4 Uncoated Amberlite XAD-2 Resin Beads (x 200)

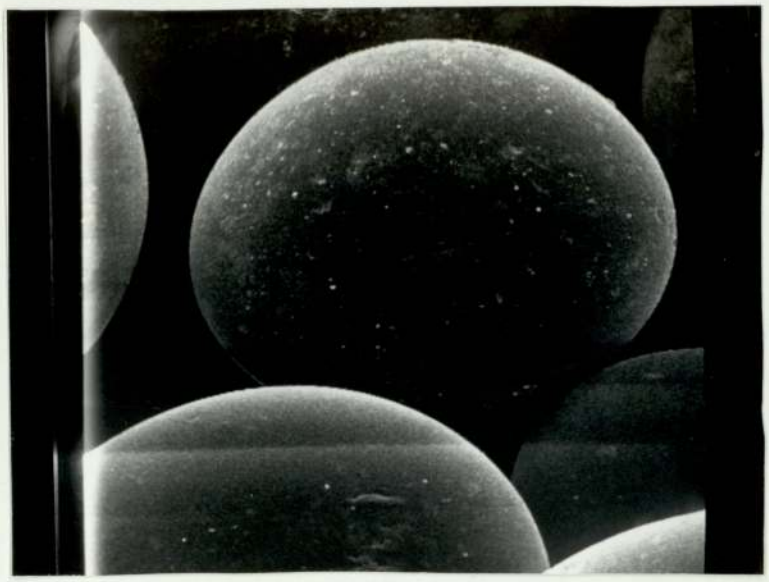


100μm

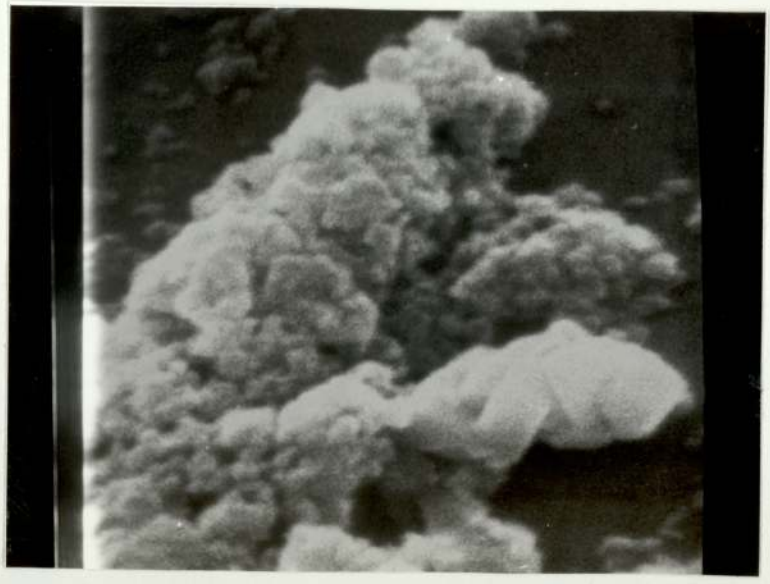
Plate 2.5 Uncoated Amberlite XAD-2 Resin Beads (x 20000)



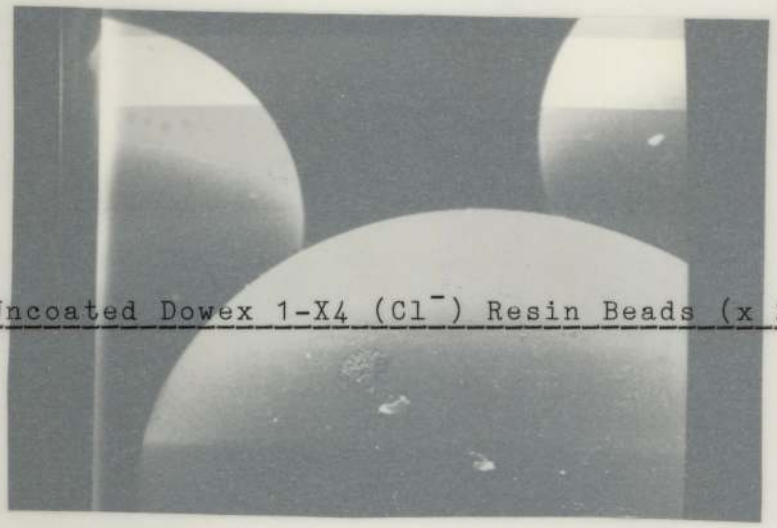
1μm



100μm

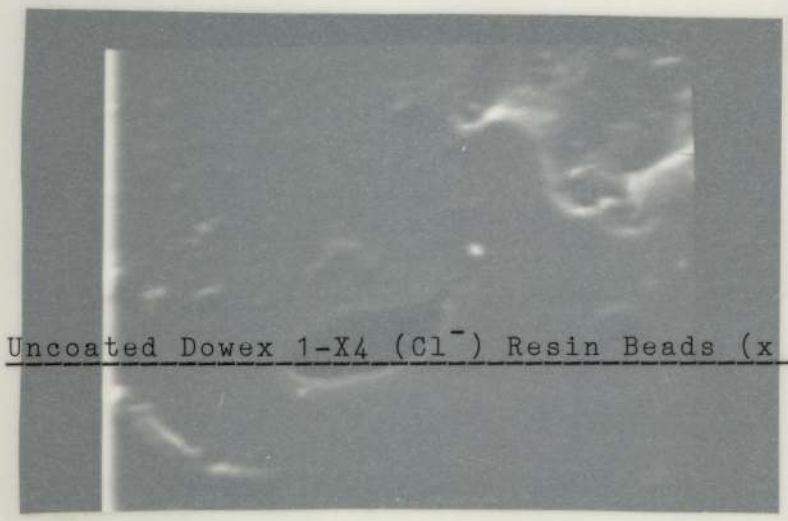


1μm



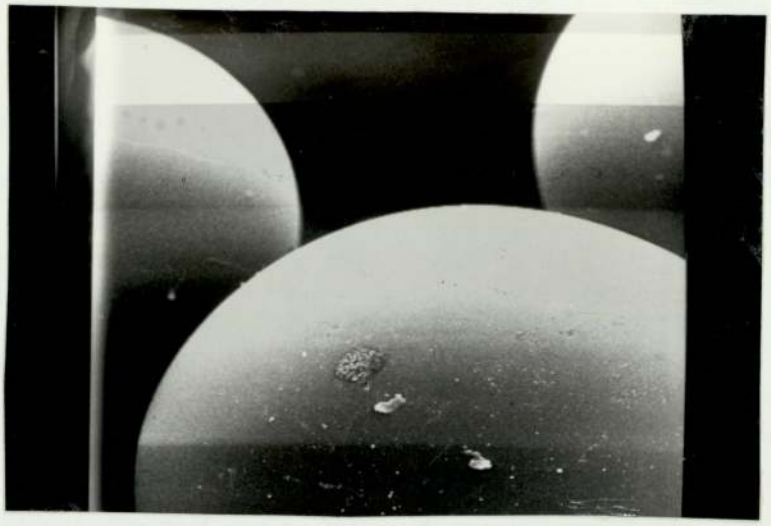
100μm

Plate 2.6 Uncoated Dowex 1-X4 (Cl^-) Resin Beads (x 200)



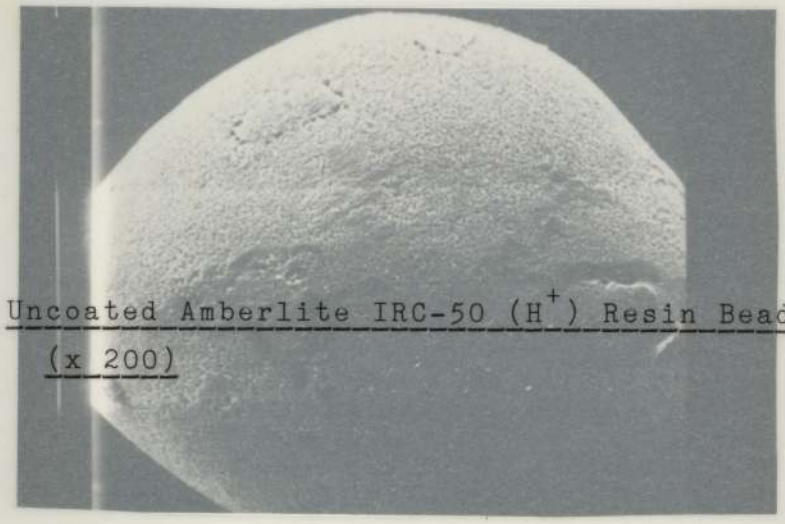
2μm

Plate 2.7 Uncoated Dowex 1-X4 (Cl^-) Resin Beads (x 10000)



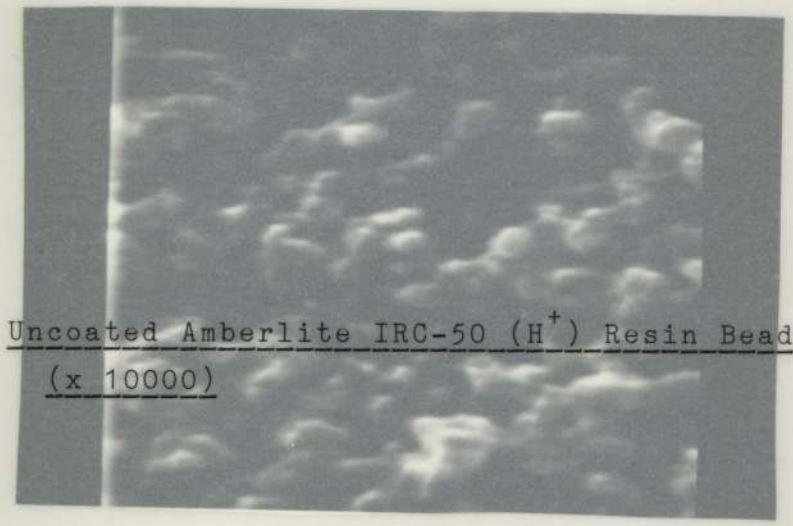
100 μm

2 μm



100μm

Plate 2.8 Uncoated Amberlite IRC-50 (H^+) Resin Beads (x 200)



2μm

Plate 2.9 Uncoated Amberlite IRC-50 (H^+) Resin Beads (x 10000)

100μm

2μm

Plate 2.10 Uncoated Amberlite IR-120 (Na^+) Resin Beads (x 200)

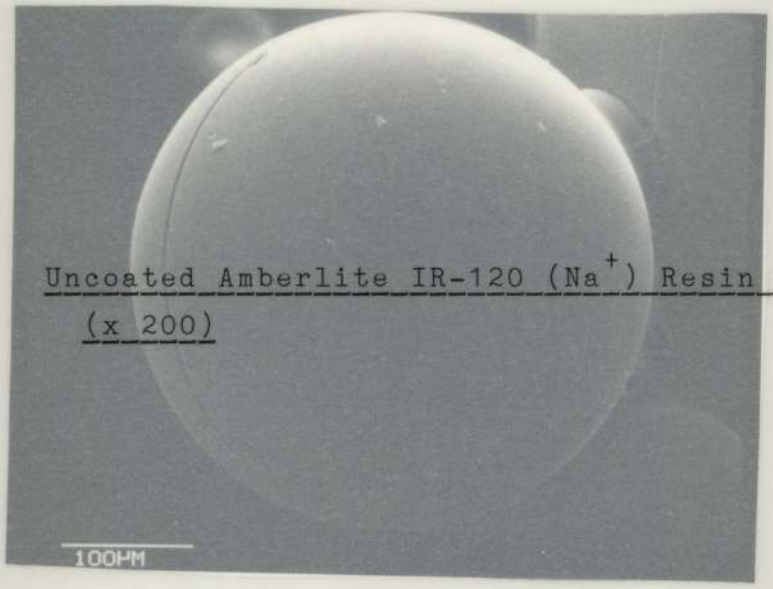
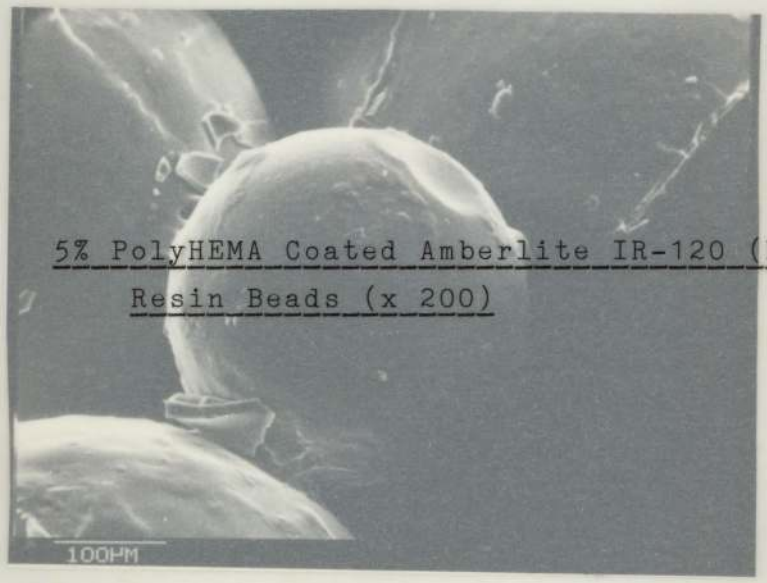
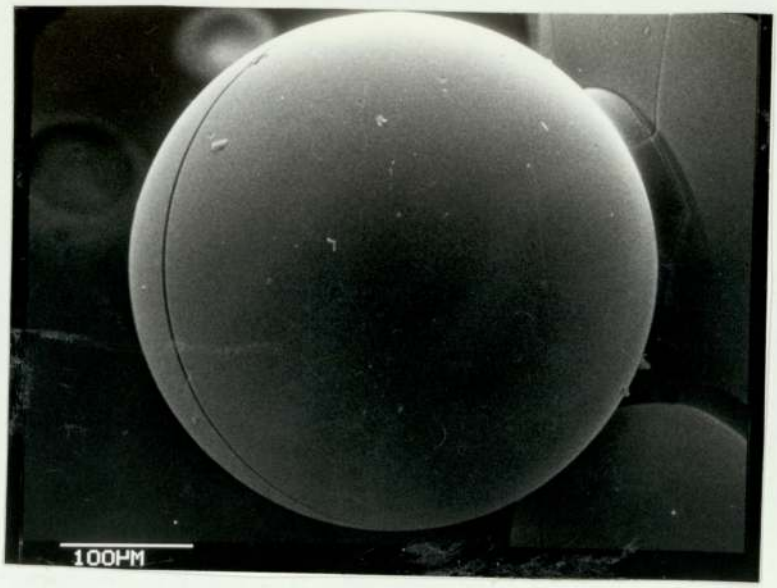


Plate 2.11 5% PolyHEMA Coated Amberlite IR-120 (Na^+) Resin Beads (x 200)




100μM


100μM

Plate 2.12 Uncoated Dowex 50W-X8 (H^+) Resin Beads (x 200)

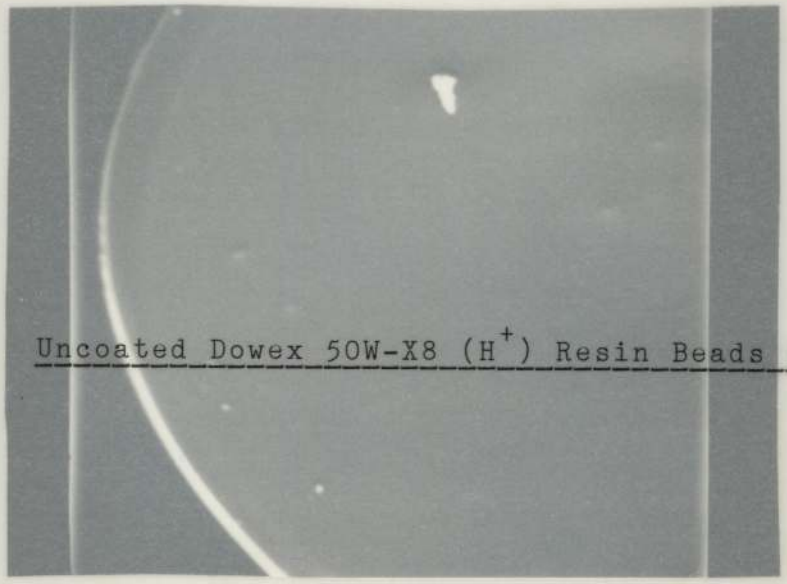
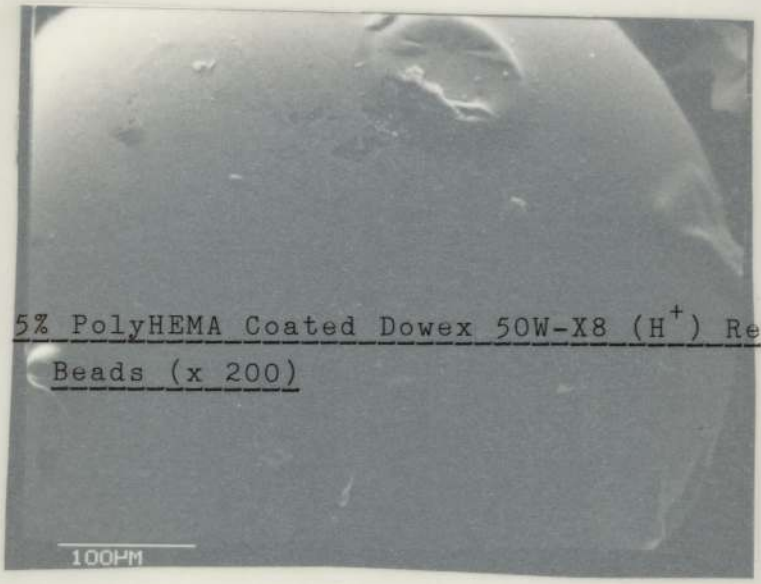
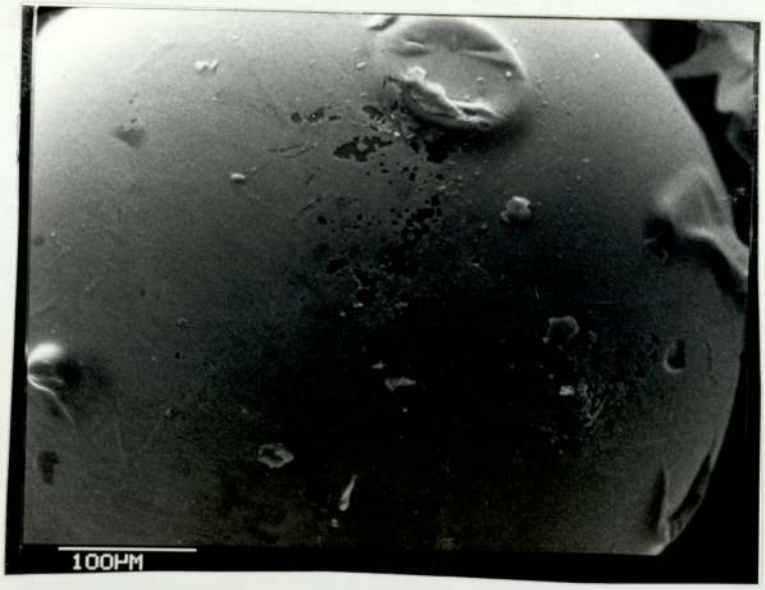


Plate 2.13 5% PolyHEMA Coated Dowex 50W-X8 (H^+) Resin Beads (x 200)



100μm



CHAPTER 3

THE DEVELOPMENT OF A DYNAMIC IN-VITRO EXPERIMENTAL TECHNIQUE FOR THE EVAULATION OF ARTIFICIAL LIVER SUPPORT SYSTEMS

3.1 Introduction

At the outset of this work, the literature did not contain a generally agreed in-vitro dynamic adsorption technique, which could be used to test the potential adsorptive capacity of adsorbents. Experimental animal models had been used, their purpose being to provide results applicable to man. However, the extrapolation of experimental data to man is never without hazard. It is hoped that in time, better animal models will be found, or more desirably, a non-animal model. With this in mind, this chapter is initially concerned with the development of an in-vitro dynamic evaluation technique, consisting of an adsorption system and an analytical system. The details of the dynamic evaluation technique and the systems involved are described in subsequent sections. Briefly three sections are included in the dynamic evaluation technique. The first section on the adsorption system will contain the design of the 'flow' or 'perfusion cell', (which represents the central part of the dynamic apparatus) and the associated constituents used. The operation of the dynamic adsorption system and its' associated fraction collection unit will also be described.

The next section will include the commissioning of the Technicon Auto-Analyser for the analysis of

ammoniacal-nitrogen in samples of nitrogenous solutions. The preliminary experimental work involved in determining correct conditions for this system, the operation of the autoanalyser and the chemical reactions which occur will also be discussed. Finally a discussion on the evaluation and preliminary studies performed is given.

Before beginning these sections, the nature of the conventional 'static' evaluation of adsorbent behaviour will be discussed.

3.2. The Static Technique

The specificity and efficiency of adsorbents are conventionally measured by the construction of adsorption isotherms, using static techniques. Such techniques have been used in this study to obtain some quantitative information about the adsorptive capacity of various potential adsorbents for the model nitrogenous hepatic toxins chosen. The static evaluation of adsorbents involves shaking 1g of washed adsorbent contained in a 10 ml glass phial with a 5 ml aliquot of the desired aqueous nitrogenous solution, for a period of 1 hour or 2 hours at room temperature. The solutions are then decanted off into sample cups for analysis. 'Release' or'desorption studies' are then conducted using distilled water in an identical manner, to determine the strength of adsorptive binding (i.e. the adsorbents' ability to irreversibly bind the adsorbed nitrogenous toxins). Thus, the analysis of the samples gives the amount of adsorbed nitrogen released.

Fig 3.1 shows a representative of such an adsorption isotherm, by this technique. In this case the model toxin was Tyrosine and the adsorbent Duolite C255(H^+) (an ion exchange resin) whose nature is described in Section 4.2(4).

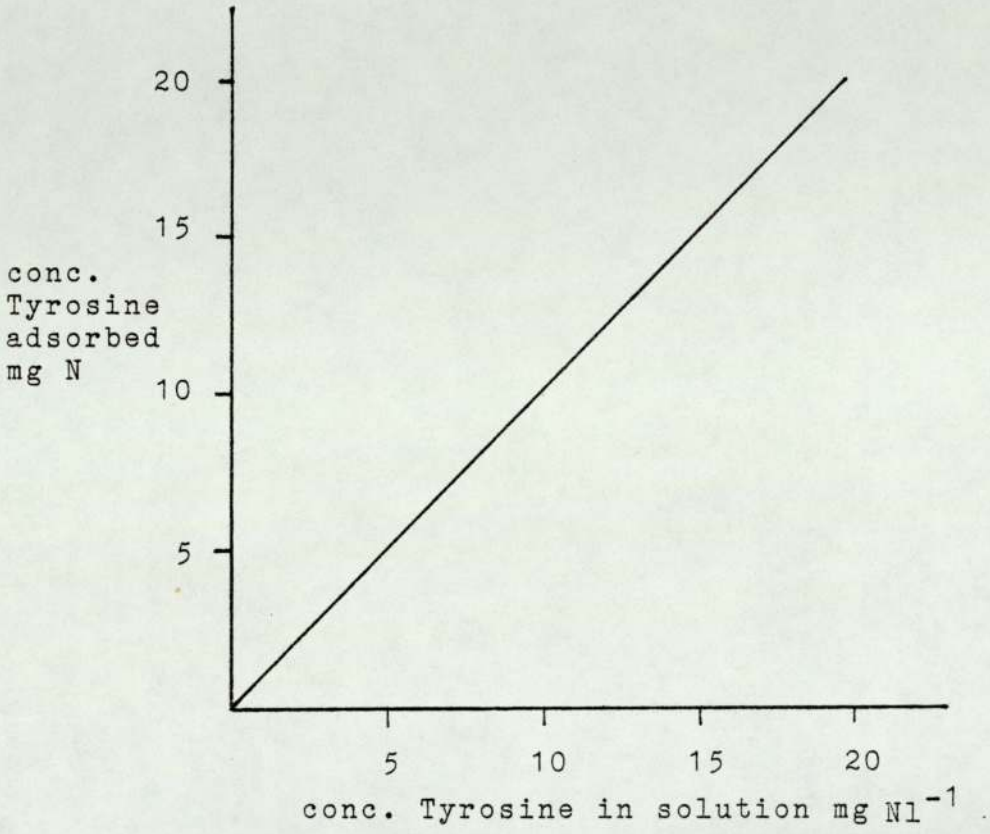


Fig 3.1 Adsorption isotherm constructed for adsorption of nitrogenous hepatic toxin - Tyrosine - by the adsorbent Duolite C255(H^+)

Nitrogenous solutions containing 15 mg ammoniacal-nitrogen per litre (referred to as 15 mg NH_3-Nl^{-1}) are adopted throughout the study for various reasons. The use of a higher nitrogenous concentration produces better discriminatory results and minimises errors which would be

encountered in the use of low concentrations of model toxin. In addition the rate and adsorption capacity of adsorbents can be more satisfactorily studied, and the ammoniacal nitrogen concentration employed throughout the study is within a convenient range of sensitivity on the analytical system.

The time intervals used for static adsorption studies were 1 hour and 2 hours because it had been noted in preliminary experiments with ion exchange resins that contact periods of 15 minutes, 30 minutes or 60 minutes did not produce a significant difference in the results obtained. Thus a 1 hour and 2 hour experimental time was adopted to allow a safety margin and ensure that equilibrium adsorption had been achieved under the conditions stated. Initial experiments with the static adsorption technique and performing experiments in duplicate and triplicate, enabled conditions to be set identically for further static experiments. Thus any errors previously encountered in early experiments, were reduced to negligible levels of $\pm$2% and no attempts were made to record statistical means and the standard errors of the mean ($\pm$S.E.M) in the results.

3.3 Dynamic Adsorption Technique : Adsorption System

3.3.(1) Continuous Flow Apparatus

The continuous flow apparatus of the adsorption system, shown in Plate 31 consists of three parts - the reservoir, the feed system and the flow cell. These are made out

Plate 3.1 The Continuous Flow Apparatus for Dynamic Experiments

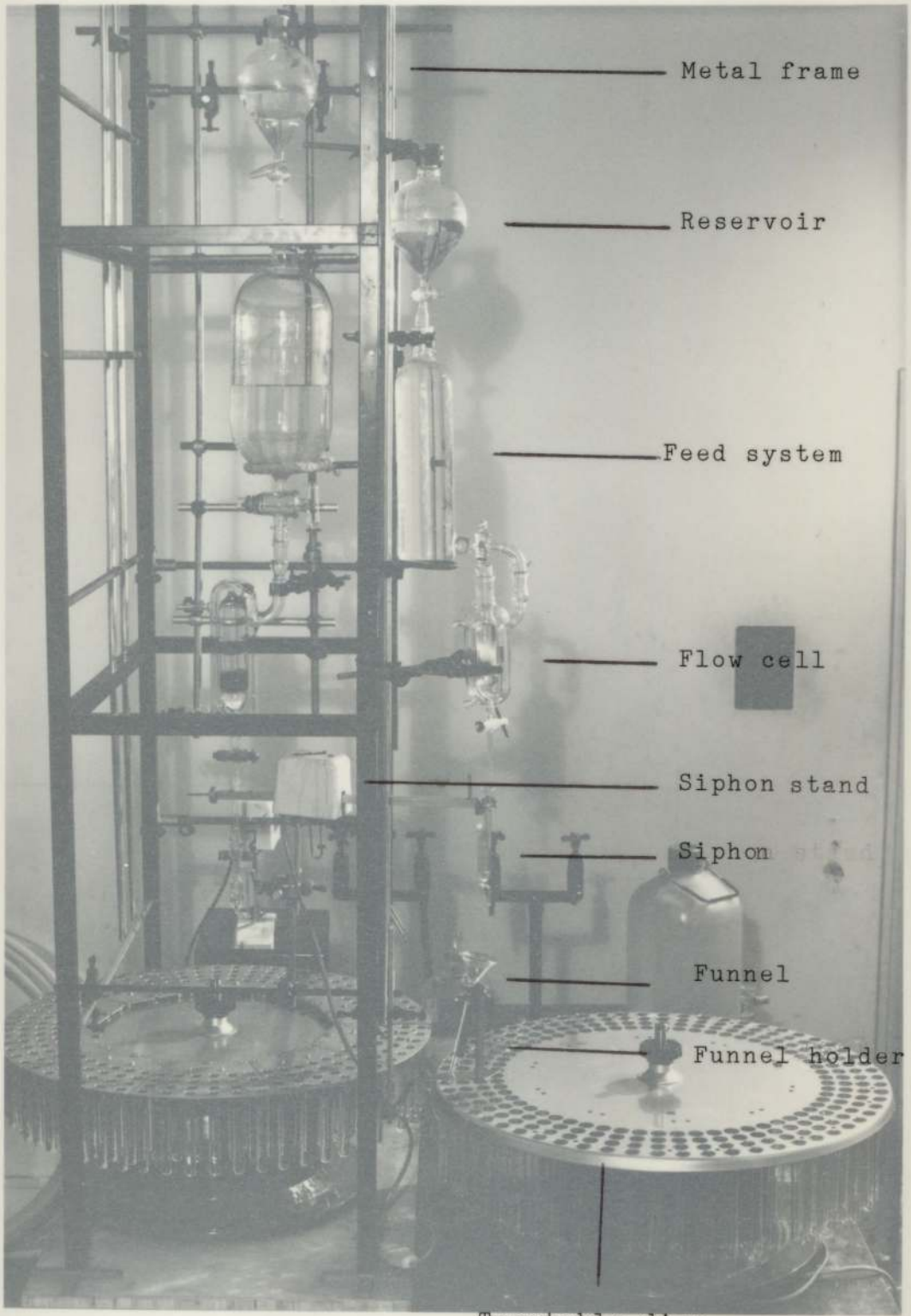


Plate 3.1 The Continuous Flow Apparatus for Dynamic Experiments

Plate 3.2 Flow Cell of Continuous Flow Apparatus

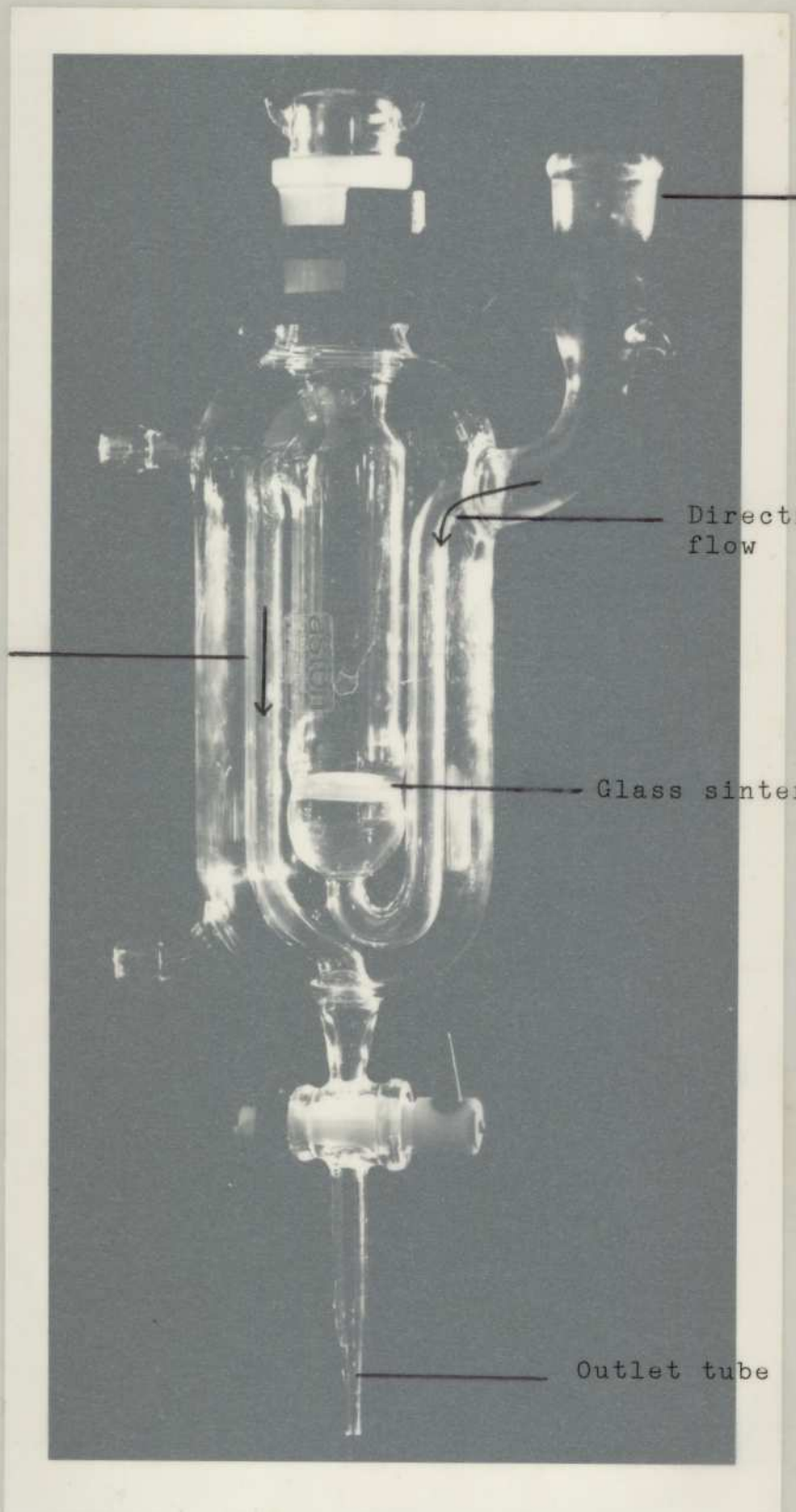
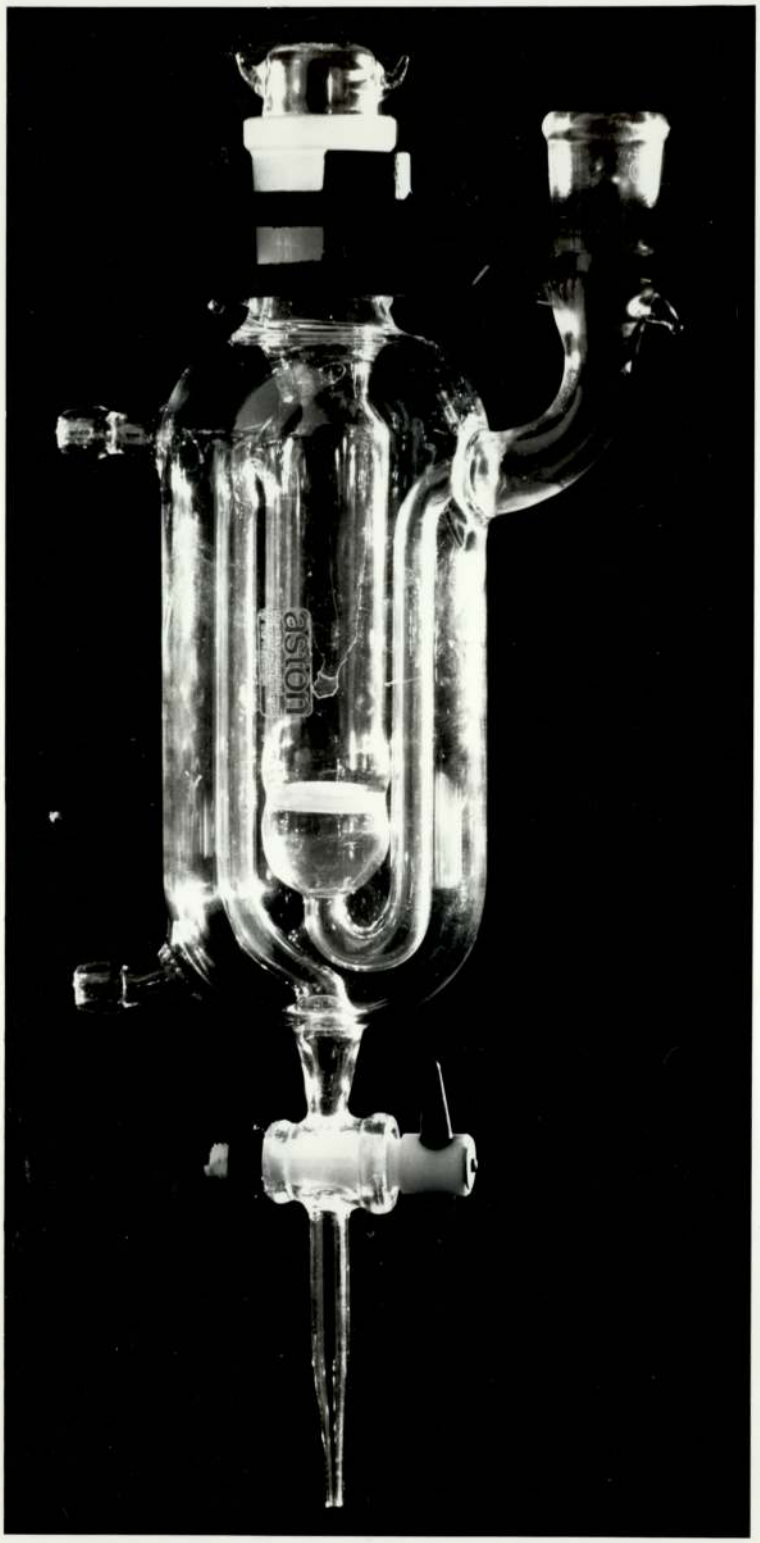


Plate 3.2 Flow Cell of Continuous Flow Apparatus



of glass and can be readily sterilized by autoclaving.

i) The reservoir is a large glass receptacle, used as a 'storage' for the model toxin solutions,

ii) The double-walled feed system into which the model toxin solutions flows to before reaching the third component, comprisesthe intermediate component,

iii) The double-walled flow cell, into which the adsorbents for analysis are placed, forms the third component. The flow cell column (Plate3·2) is 2.5 cm. in diameter, with chambers of 2 cm. below and 8 cm. above the glass sinter (porosity 1).

Adsorbents for evaluation are placed above the glass sinter, whose purpose is to prevent entrainment distally of the adsorbent particles. Jacketing of the feed-system and flow cell, enables the circulating test fluid to be temperature controlled if desired. Both components are fitted with stop cocks, thus flow rates can be controlled. All components are sealed with high vacuum grease and secured tightly together with wire. The design of the flow cell, the central part of the adsorption system, will now be discussed.

The flow cell was designed and initially developed for the fractionation of polymers[57] (B.J.Tighe 1965) and was based on the well characterized apparatus devised by Duveau and Piguet (1962)[58]. It was altered further for this research project, to produce a miniaturized flow cell, suitable for a dynamic adsorption system, in the following way.

Adsorbent weight, flow rates and blood volumes[8,21,26,59,60-65] used in animal and clinical work by various authors were initially compared. These values were then scaled-down to arrive at a suitable adsorbent weight and flow rates for the miniaturized flow cell. Thus the conditions encountered using this system closely matched the conventional haemoperfusion techniques and were in accordance with the range of space velocities which satisfy clinical situations. The space velocity is derived from the general mathematical expression:

$$\text{Space Velocity (time}^{-1}) = \frac{\text{Volume per unit time}}{\text{Volume of adsorbent}}$$

Conventional perfusion systems used for animals and patients have space velocities in the range of 0.02 - 1.5 min^{-1}. The system employed in this study arrives at 0.4 - 1.0 min^{-1} space velocities, using a flow rate of 2 mls/min - 5 mls/min and adsorbent volume of 5 mls.

3.3.(2) Fraction Collector

The collecting system employed is that of an automated fraction collector, consisting of a siphon and siphon stand, a funnel and holder and a turntable disc, containing test-tubes to collect the liquid fractions. The whole dynamic adsorption apparatus is assembled (assembling time 45 mins) and supported by a 7 ft. metal frame, which can accommodate the use of two such continuous flow systems.

3.3.(3) Operation of Continuous Flow Apparatus

The desired adsorbent is washed with distilled water (and 0.9% physiological saline, if preferred),

drained, dried with filter paper and 5g transferred to the adsorption cell, where it is supported by the glass sinter. To prevent the dislodging of adsorbent particles (which can eventually pass out of the flow cell) a piece of glass wool is placed over the adsorbent. The permeant or test fluid is placed in the glass reservoir which is connected to the feed system. This then leads to the inlet tube of the flow cell (Plate 3-1). Below, the flow cell, siphon, funnel and test tubes are positioned for the collection of successive fractions without spillage. Perfusion is conducted in an antigravity direction to eliminate streamlining and to ensure uniform and efficient adsorption. In this way a continuous stream of test fluid flows from the feed system, down the inlet tube of the flow cell, and then up through the glass sinter and bed of adsorbent. The circulation is regulated by means of a stop-cock at the outlet tube and can be maintained at any flow rate (usually 2-5 mls per minute) for the desired length of time. The perfusate then flows to the outlet tube, where the liquid fractions are collected dropwise in the siphon. When filled, the contents of the siphon (capacity range used: 3 - 10 mls) are delivered into the test tubes via a funnel. The automated fraction collector rotates after each sample/fraction collected, thus presenting a clean tube for successive sample collection. Fractions collected are loaded on to the autoanalyser for analysis, after one passage through the adsorption system. A detailed description of the autoanalyser system is given in the next section.

3.4. Dynamic Adsorption Technique : Analytical System

3.4.(1) Introduction

The analytical system needed is one which can cope with the analysis of the range of nitrogenous compounds to be used in this study, and at the same time provide accuracy, reproducibility and reliability of results. Thus, in this case, a group reaction for nitrogenous compounds is indicated (discussed later in this section). The Technicon autoanalyser is chosen as the preferred system, because normal colorimetric procedures are automated with the continuous monitoring of samples and the results are displayed permanently by a recording system. The operation of the system is simple and rapid to execute, i.e. suitable for routine use (over 300 analyses can be performed in an 8 hour working day) without the need for exotic equipment or techniques. Its' sensitivity, specificity, working range and economical low cost per analysisare also very attractive. The components of the autoanalyser and its' operation will now be discussed[66-68].

3.4.(2) Operation of AutoAnalyser

The components of the autoanalyser consist of a sampler, proportioning pump and manifold, dialyzer (not essential), heating bath, colorimeter and recorder, see Plate 3.3. Each component and its' mode of operation will now be discussed.

Plate 3.3 Technicon AutoAnalyser

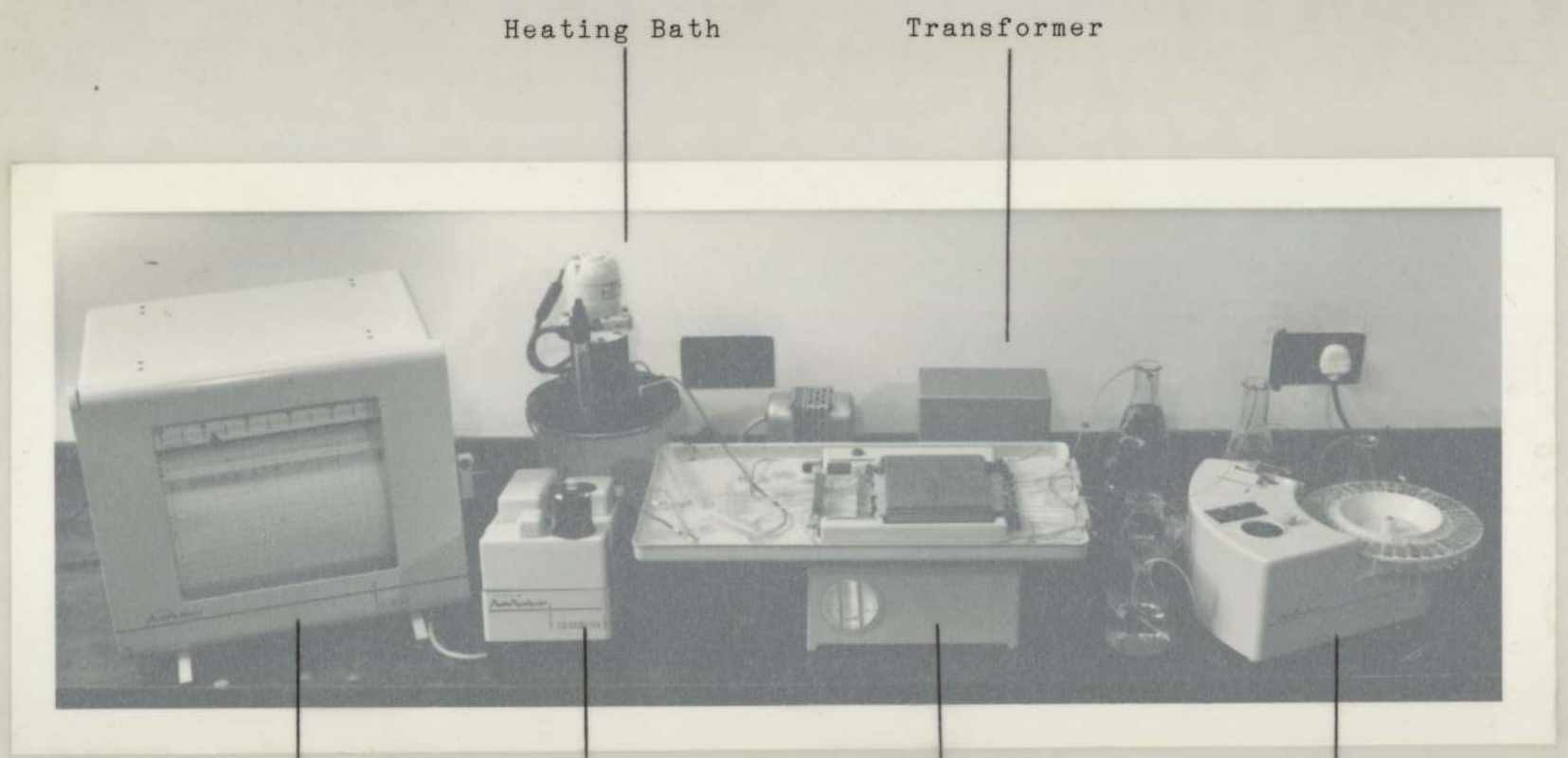


Plate 3.3 Technicon AutoAnalyser

i) Sampler

The sampler contains a tray which holds up to 40 sample cups. As the loaded sampling plate rotates, at the pre-set rate of speed (40 samples/hour) a metal probe dips into the cup presented, and picks up its' contents for a given time period. The probe then lifts out of the cup, aspirates air for about 1 second, and dips into a wash receptacle, where liquid wash (distilled water) is aspirated. Again, after a pre-set interval, the probe rises from the wash receptacle, aspirates air and moves into the next sample. (For analyses of 40 samples/hour, sample:wash ratio is 2:1). This in-and-out action is repeated until all the samples on the plate have entered the system. The combination of air bubbles and liquid wash, form an insulating barrier between samples. Thus each sample travels through the auto-analyser as a separate entity, barred from contact with its' neighbours by air segments and wash fluid. In addition, the rapid descent of the sample probe eliminates any possible differences in the analytical results, due to variations in fluid level from sample cup to sample cup. Samples then pass on to the proportioning pump and manifold.

ii) Proportioning Pump and Manifold

The proportioning pump consists of rollers occluding plastic tubes which are thus stretched taut on a platten (Plate 3·4). As the rollers move forward, fresh fluid is drawn in from behind, while fluid is pushed forward. The rate of pumped fluid is dictated entirely by the internal diameter of the pump tube. The system of pump tubes, interconnecting tubes, mixing coils, glass and plastic pieces is called the Manifold. From here samples move on to the oil heating bath.

iii) Heating Bath

In this system, the reaction is not fairly rapid, and the mixing coils do not give enough time for the colour reaction to be developed, thus the liquid must be held for a longer period. Therefore the fluid is passed through a heating bath at 90°C, containing a 'time delay coil' of about 40 feet of glass tubing wound into a spiral coil. The fluid takes about 5-15 minutes to pass through the coil, depending on the volume of the coil and the pumping rate, within which time the colour is sufficiently developed to be measured colorimetrically.

Plate 3.4 Manifold Platten of AutoAnalyser

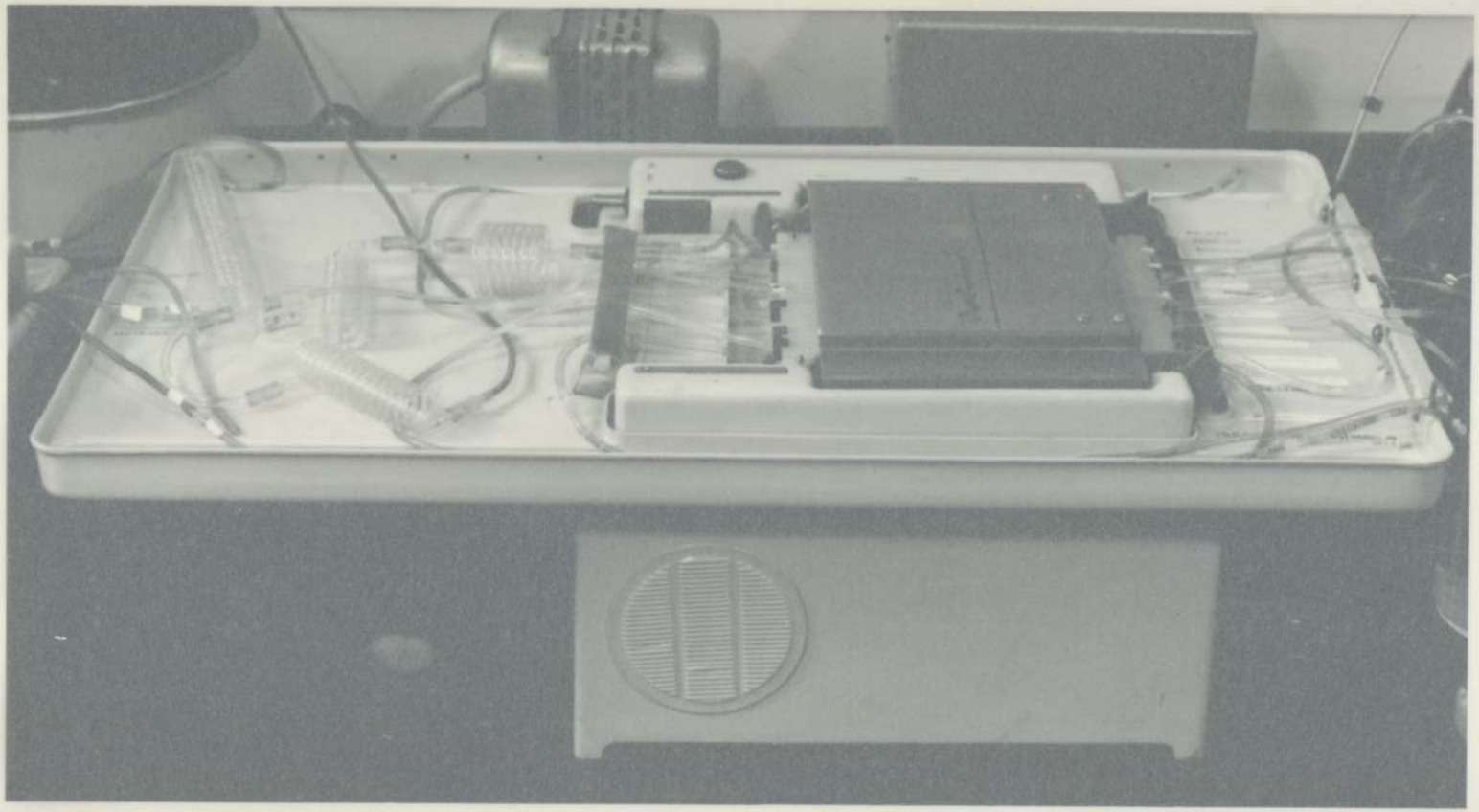
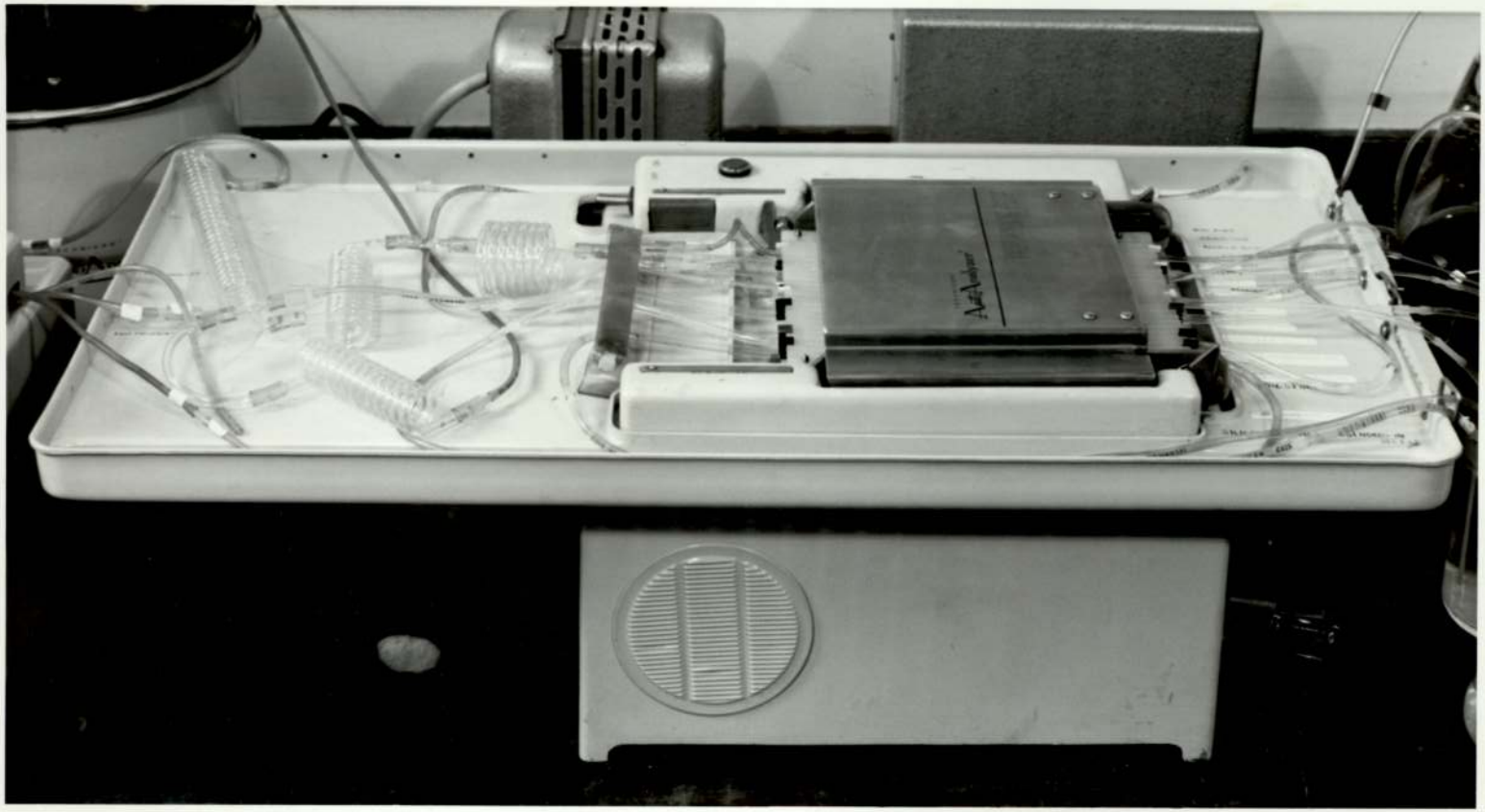


Plate 3.4 Manifold Platten of AutoAnalyser



iv) Colorimeter

The liquid stream is pumped through the colorimeter flow cell, for determination of the optical density of the fluid. Air bubbles are vented and the air-free or non-segmented liquid passes through a tubular flow cell. The concentration of ammoniacal-nitrogen in samples varies proportionally with colour intensity, resulting in an increase or decrease in transmitted light.

v) Recorder

The results are displayed by the recording system. The recorder chart paper moves continuously at a fixed rate, and peaks appear for the changing optical density of the continuously flowing reaction mixture. Peak heights are determined with the use of a transparent chart overlay and are translated into meaningful values using the Apple Computer II. It would be of great value in the future to interface the autoanalyser to a computer system. This would enable a direct computer print-out of peak values to be presented immediately.

3.4.(3) Reagents and Conditions Required

The chemical reaction chosen for the analysis of the nitrogenous solutions is the 'indophenol' reaction[69,70,70a] a modificiation of the original Folin Technique and the use of Nessler's reagent. Reagents used for this reaction are disodium ethylene diamine tetra acetate (or more commonly EDTA), sodium hypochlorite (NaOCl) and sodium phenate, the concentrations of which are included in Chapter 2. To arrive at the correct specific conditions for the desired colour formation, the reagents are used in various dilutions and volumes, at different temperatures and time periods. The information from the best conditions within this 'test tube' preliminary work is then transformed into significant expressions on the autoanalyser, by converting the volumes of reagents to flow rates of the various channels (determined by the bore of the individual tubes). (The flow diagram for the determination of ammoniacal nitrogen is shown in Fig 3.2. The instrument is then stabilised for 30 minutes and standard solutions of ammonium chloride at concentrations of 1, 5, 10, 15, 20 mg. ammoniacal-nitrogen per litre (NH_3-N/l) are analysed. A calibration curve (Fig 3.3) is then constructed and samples are analysed.

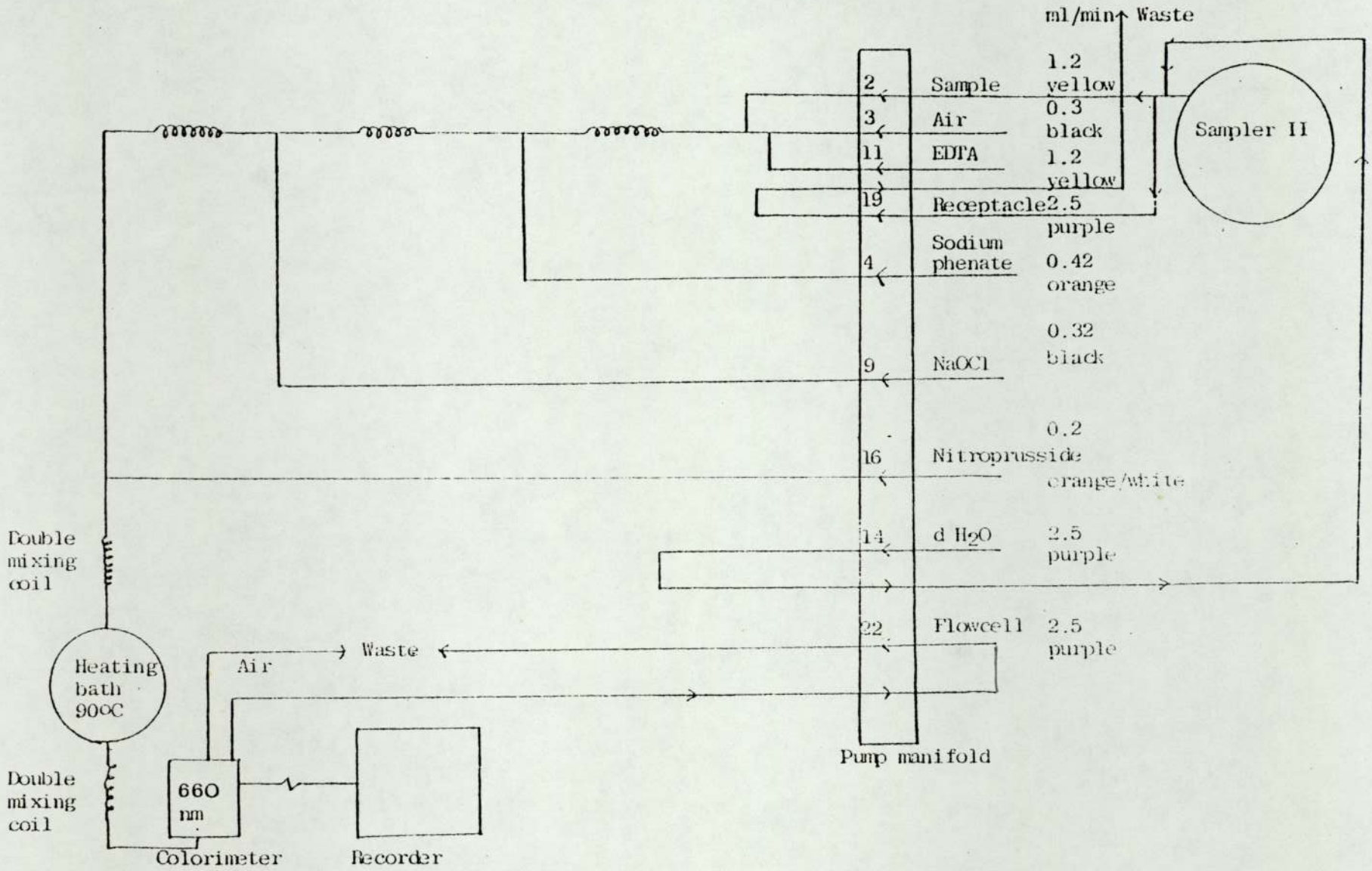
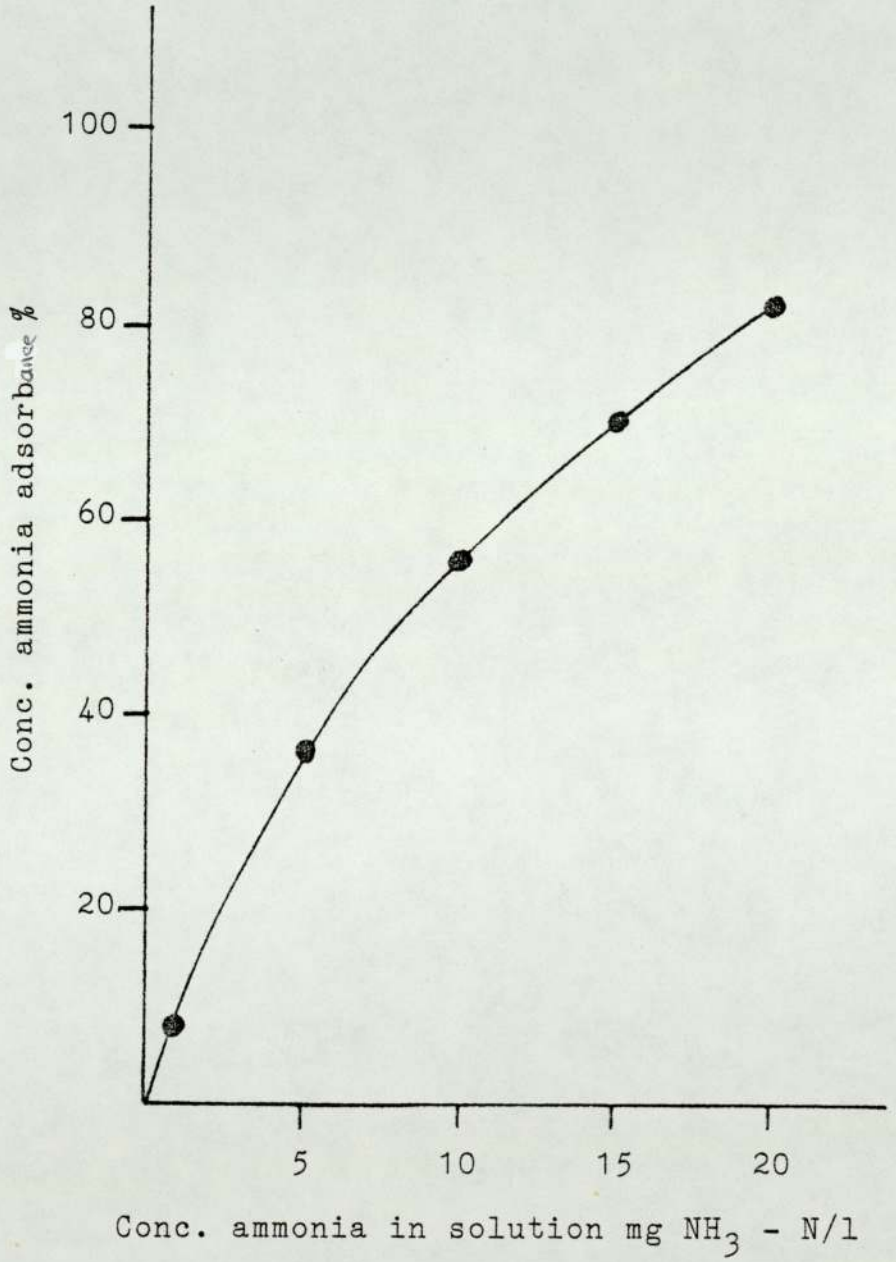


Fig 3.2 Flow diagram for determination of ammoniacal nitrogen

Fig 3.3 Calibration Curve for Determination of Ammoniacal Nitrogen

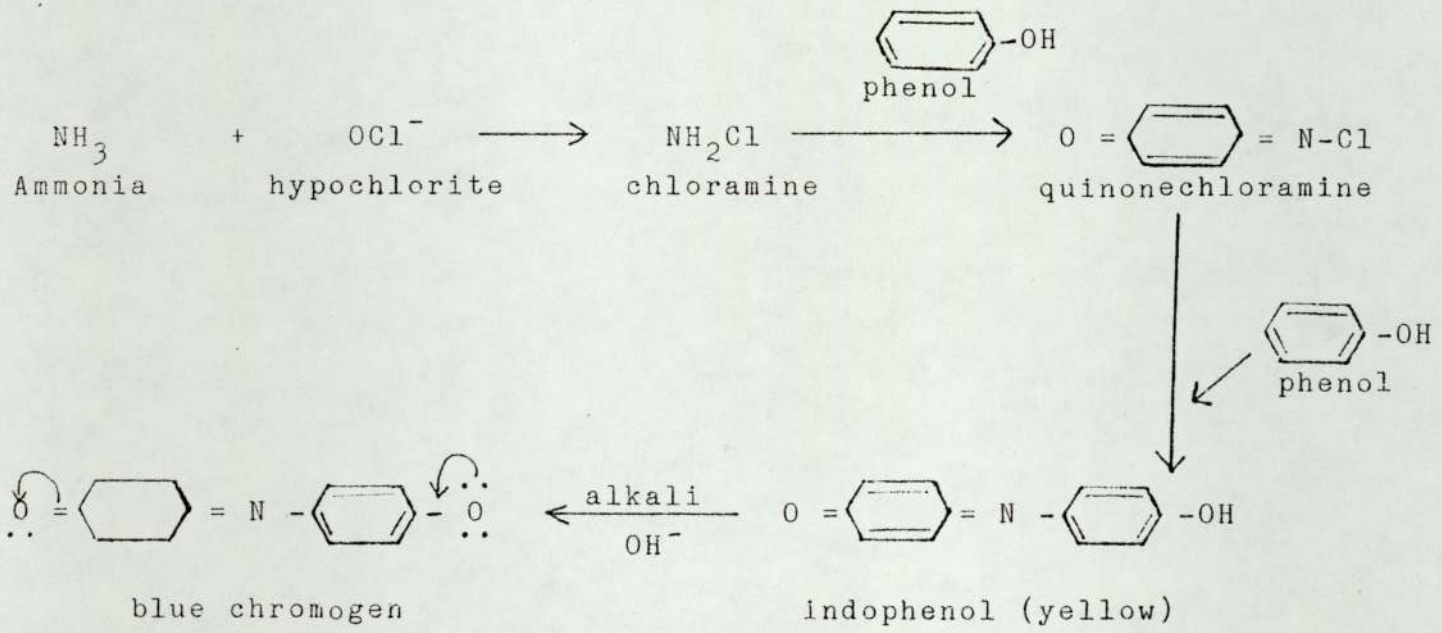


3.4.(4) Colour Reaction

Samples introduced into the autoanalyser system are firstly treated with an alkali EDTA/NaOH buffer solution maintained at pH 11.0 to ensure that all the ammonium ions are converted to free ammonia. (Alkaline samples slowly lose ammonia, but this 'closed' analytical system prevents this). The EDTA chelates with any interfering metal ions (such as Cu, Ni, Hg, Ca), thus preventing the precipitation of their salts in the system. This is important especially when body fluids are being analysed.

Sodium phenate and sodium hypochlorite in turn are then added to the system and mixing takes place. Ammonia and hypochlorite react together under these alkali conditions to give chloramine which then reacts with the phenol to form quinonechloramine. Quinonechloramine couples with another mole of phenol and forms the yellow associated indophenol which then dissociates in an alkali medium, to give a blue chromogen, indophenol. The chemical scheme for the development of the coloured reaction product is shown in Fig3.4. To obtain the full development of the blue colour, the reaction mixture is heated to 90°C for 5 minutes in the heating bath. The optical density of samples is measured in a colorimeter at 660 nm and recorded on a chart recorder, appearing as a series of peaks on a flat base line.

Fig 3.4 Chemical Scheme for Development of Coloured Reaction Product



3.5. Dynamic Adsorption Technique : Evaluation of Technique and Preliminary Studies

3.5.(1) Introduction

This system will indicate the preliminary evaluation of the dynamic adsorption system modified for this application, and will also contain the preliminary studies performed using such a system.

Although the equilibrium capacity of various adsorbents for many substances of clinical importance is high, the time required to achieve this can be several hours/days, and until the biocompatibility problem is overcome, perfusion is likely to be possible for only a few hours at a time. Thus with this in mind it is therefore necessary to design such a column which makes the best use of the limited time available. The identification of the rate limiting step in the adsorption process is a necessary procedure to improve the design of the column, so that maximum efficiency can be achieved. The adsorption of a metabolite from blood or in this case, nitrogen from a nitrogenous solution, takes place in several stages. Dunlop and workers 1975[9], have indicated thoroughly the implications of these steps in dynamic systems. They claim that the diffusion through the external boundary or stagnant layer of fluid adjacent to the adsorbent particles, is the only step in the adsorption process that can be significantly altered by the column design[71]. The dynamic adsorption tech-

nique in this study is essentially a basis for making comparisons. Certain simplifications are adopted, especially where surface area of adsorbents is concerned. Adsorbents differ in surface area. However, the same or equivalent volume of adsorbent is used throughout the study, but due consideration is given to the surface areas, when evaluating the adsorbent's efficiency. Although in principle, it is possible to change the significance of the stagnant layer, in clinical usage, certain factors such as volume of adsorbent and blood flow rate, can only be varied in certain limits. In this work, the question of flow rate and volume of adsorbent have been decided on the basis of common experience in the clinical evaluation of adsorbents. Thus blood flow rates of 100-200 mls/min and adsorbent volumes of 200-500 mls are common. By scaling down (as previously described in Section 3.3.(1)), whilst maintaining equivalent 'space velocities', an adsorbent volume of 5 mls and flow rates in the order of 2-5 mls are found to be acceptable. This volume of adsorbent and flow rate of perfusate are employed in this study.

3.5.(2) Preliminary Evaluation Studies

In order to assess the time period over which mixing occurs when solutions are passing through the adsorption (or perfusion) cell in conditions of no

Fig 3.5

Fig 3·5

TO SHOW THE DEGREE OF MIXING OF SOLUTIONS

CONDITIONS: $[NH_4Cl]_o \equiv 15$ mg Nl^{-1}; distilled H_2O; flow rate = 2 mls/min for 5½ hours

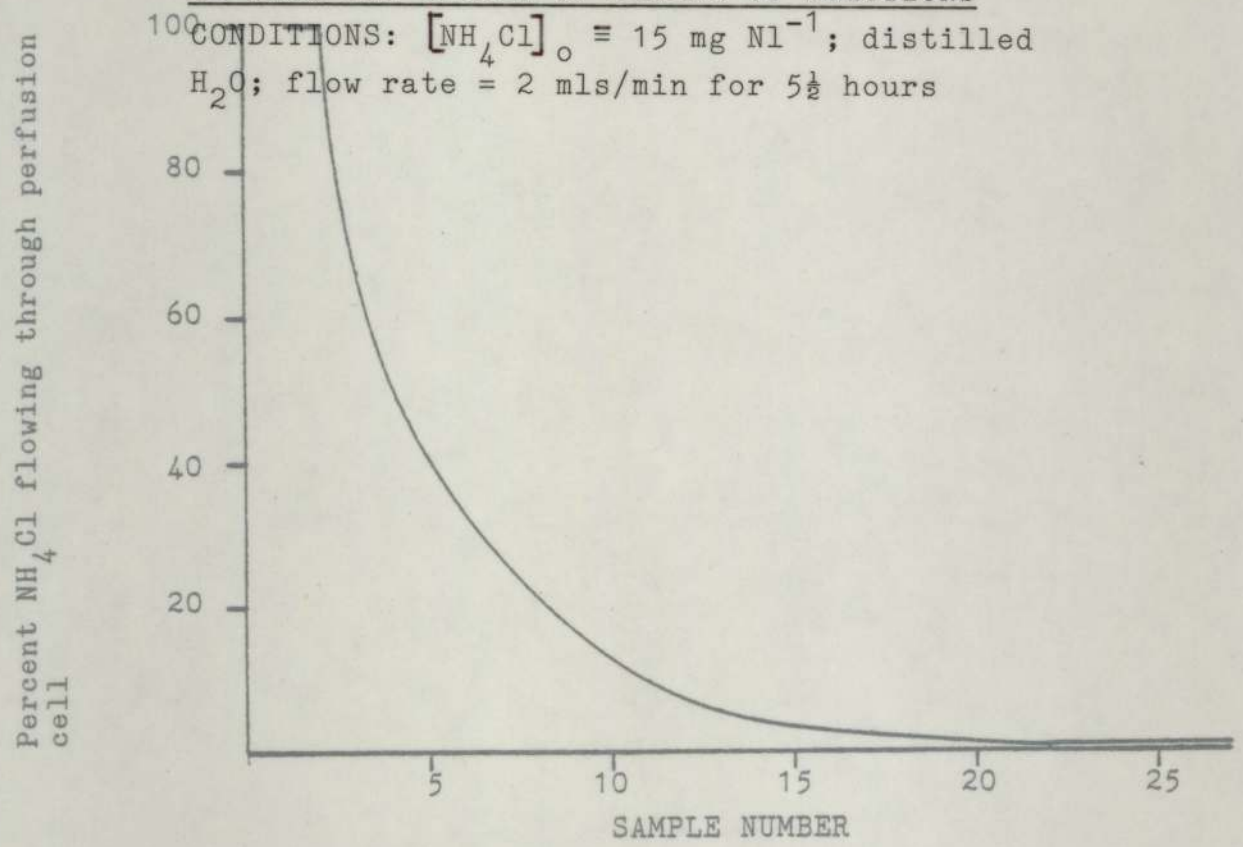


Fig 3.6

Fig 3·6

TO SHOW DEGREE OF MIXING OF SOLUTIONS

CONDITIONS: Distilled H_2O; $[NH_4Cl]_o \equiv$ 15 mg Nl^{-1}; $[Phe]_o \equiv 15$ mg Nl^{-1}; 5 ml glass beads

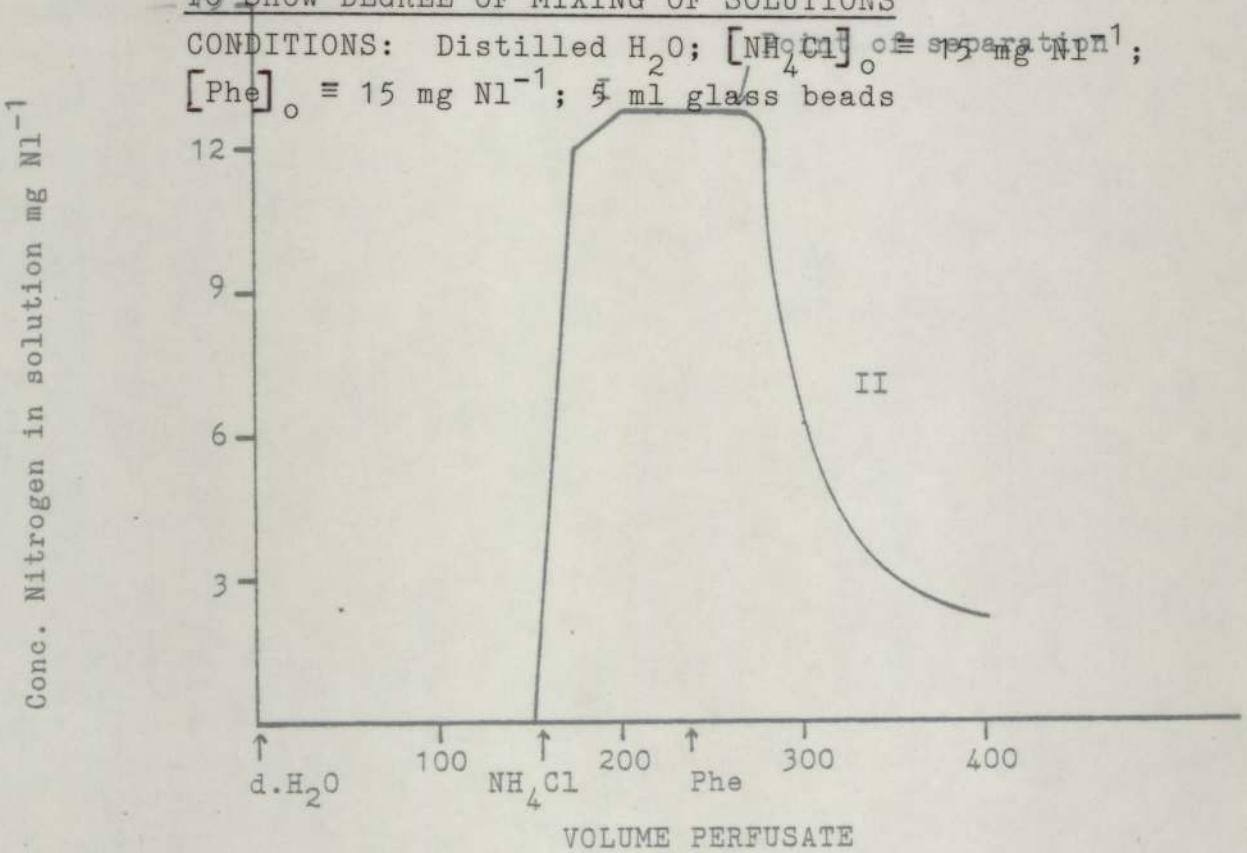


Fig 3.5

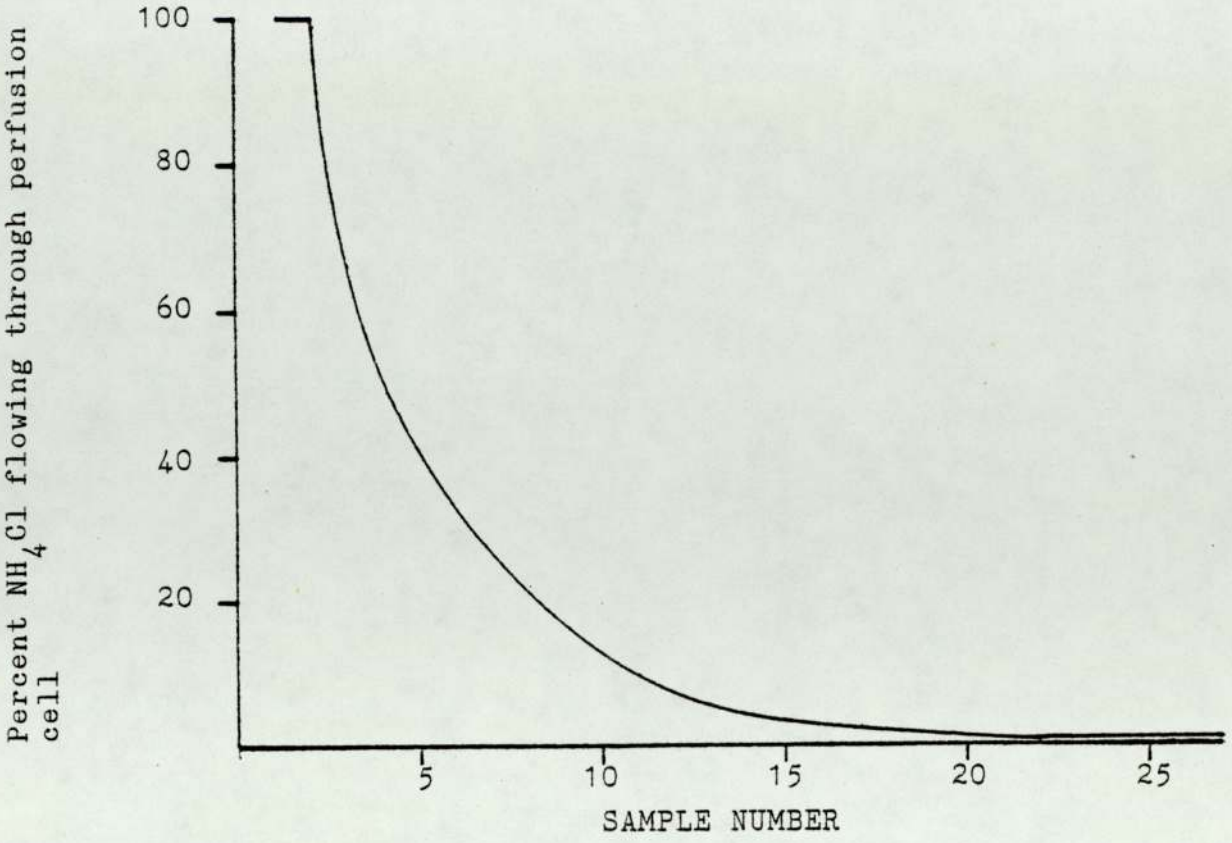
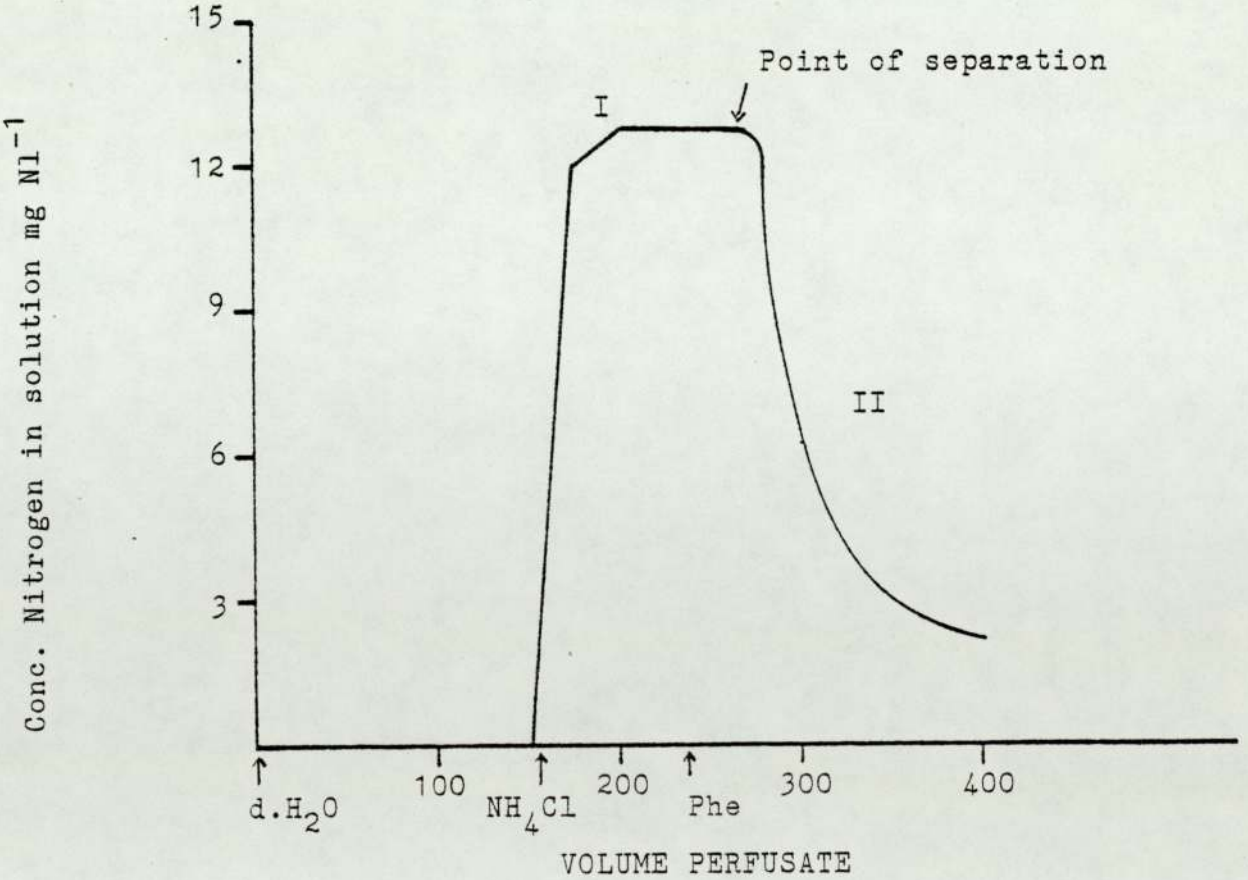

Percent NH_4Cl flowing through perfusion cell
100
80
60
40
20
5
10
15
20
25
SAMPLE NUMBER

Fig 3.6


Conc. Nitrogen in solution mg Nl^{-1}
15
12
9
6
3
Point of separation
I
II
d.H_2O
100
NH_4Cl
200
Phe
300
400
VOLUME PERFUSATE

adsorption, two experiments are performed, as follows:

i) A standard solution of ammmonium chloride (NH_4Cl) containing 15 mg NH_3-N/l, that is 15 mg ammoniacal-nitrogen per litre is placed in the perfusion cell (Plate 2) in the chamber above the glass sinter to its' full capacity: 35 mls. Distilled water is then perfused at a rate of 2 mls/min for 5½ hours, through the cell, and fractions (8 mls/fraction) collected and analysed. Results are then expressed by means of a graph as presented in Fig 3.5.

ii) A second experiment is performed using 5 ml of glass beads as a blank (in the place of an actual adsorbent) which are placed above the glass chamber of the flow cell. An ammonium chloride solution containing 15 mg NH_3-N/l is firstly perfused through the system and then a solution of phenylalanine (an amino acid) also containing 15 mg N/l. Samples collected are analysed, and results expressed in the form of a graph as shown in Fig 3.6.

3.5.(3) Discussion

These two studies indicate that on perfusion, a certain degree of mixing of the perfusate occurs. Thus there are no stagnant flow regions in the column. The extent of this mixing, or more strictly, the rate of diffusion of the perfusate into the solution of the flow cell, which represents the 'boundary or stagnant

layer', is indicated in Figs 3.5 and 3.6.

It is clear, that in altering the concentration or the nature of the perfusate, there is a gradual change in the graph obtained. Thus an abrupt switch or 'step-change' in the graph is very rare and does not occur in practise. Figures 3.5 & 3.6 are combined and presented in Fig 3.7 where the curve obtained for Fig3.6 is separated at the point of addition of the phenylalanine solution, and the curves expressed individually, as curves I & II - (curve I representing the first section and curve II the second section of the graph). Curve III and also its' mirror image curve IV are superimposed on curves I & II.

By comparing the curves, it can be seen that the changes taking place, occur within a certain 'dead space volume', equivalent in all cases. For curves I & II, however, one must allow for the fact that a volume of 5 mls of the perfusion cell is occupied by the glass beads. The dead space volume is approximately equal to a volume of 120 mls - 140 mls.

Because these results are consistent, and the time involved for the complete change from one regime to another is in the order of 60 mins - 70 mins, one is not going to be particularly anxious about a change in adsorption capacity in that time period, since this is very small in comparison to the time period of 2-4 days employed for some experiments.

Because adsorption is assessed by rapidly filling the

Fig 3.7 TO SHOW DEGREE OF MIXING OF SOLUTIONS

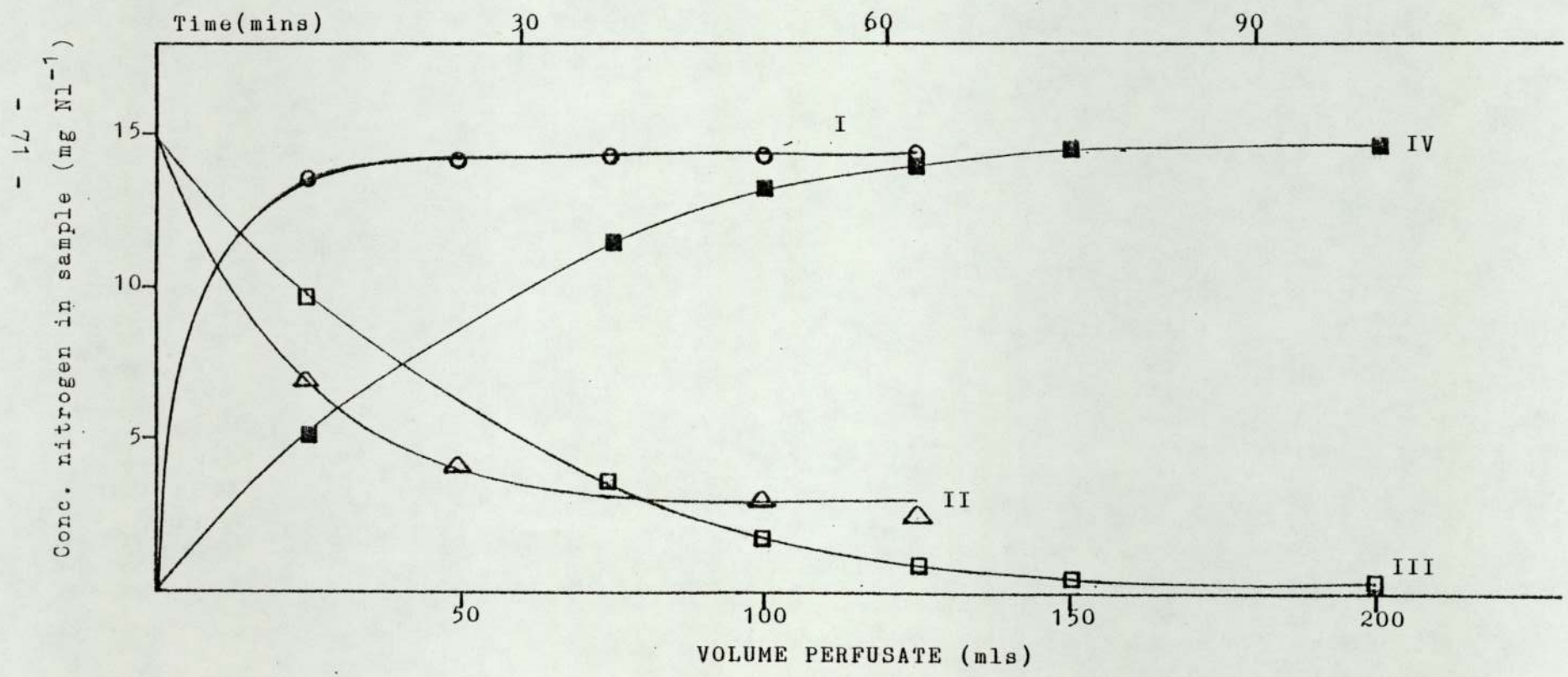
CONDITIONS: d.H_2O; $[NH_4Cl]_o \equiv 15$ mg Nl^{-1}; $[Phe]_o \equiv 15$ mg Nl^{-1}; 5 ml glass beads

H_2O-NH_4Cl (I) =

NH_4Cl-H_2O (III) =

Glass beads, NH_4Cl-Phe (II) =

Mirror Image NH_4Cl-H_2O (IV) =



cell from the reservoir, and then allowing perfusion to take place at the desired flow rate, information of this kind on the dimensions the 'dead space volume' is important in confirming that no abnormal adsorption effects are occurring.

CHAPTER 4

MODEL TOXINS AND ADSORBENTS USED IN STUDY

4.1. Model Toxins Used in Study

4.1.(1) Introduction

Although the identity of the actual toxins producing hepatic coma remains shadowy, many of them are nitrogen-containing. They form part of a wide spectrum of various compounds, ranging from ammonia and amino acids, to free fatty acids, phenols, mercaptans and other sulphur containing species[8,9,22,72-75]. In this work, however, attention has been confined to the nitrogenous toxins. This section of Chapter 4 aims to introduce the hepatic toxins studied and to justify reasons for their choice in the project. Their physiological significance will be dealt with first, followed by a discussion on their pathological roles in liver failure.

It is important at this stage to state that the type of adsorbent required for an artificial liver support system, is not necessarily a very aggressive super-efficient adsorbent, because other non-toxic nitrogenous compounds would then also be removed. Also the complete removal of a toxin could be disastrous, especially if it serves vital functions at physiological levels.

Since the chemical species of interest in this project possess a dual role (i.e. a physiological role at normal levels and a pathological role at elevated levels), it is vital that levels lower than physiol-

ogical levels are not encountered on adsorption. However, it is not altogether easy in the clinical situation to distinguish between physiological and pathological levels, especially in the case of individual amino acids, because the level at which some amino acids become toxic is only broadly defined, and is spread over a wide range for different individuals, for the various causes of liver failure[76].

The model hepatic toxins employed in this study (all nitrogenous in origin) include ammonia, amino acids and amines. These will now be discussed in turn.

4.1.(2) Ammonia

4.1.(2)1. Production

Under normal physiological conditions, most of the blood ammonia (NH_3) is derived from small intestinal digestive processes of dietary protein and from bacteria, which metabolise urea and products of protein digestion. Other tissues (liver, muscle, nerve tissue, brain, kidney and red blood cells) also generate significant quantities of ammonia[1, 17, 77-82].

4.1.(2)2. Metabolism

Because of the low permeability of the blood brain barrier to ammonium ions, transport of ammonia from blood to brain is diffusion limited[83]. Ammonia is metabolised in the brain according to the following pathway - shown in Fig 4.1.

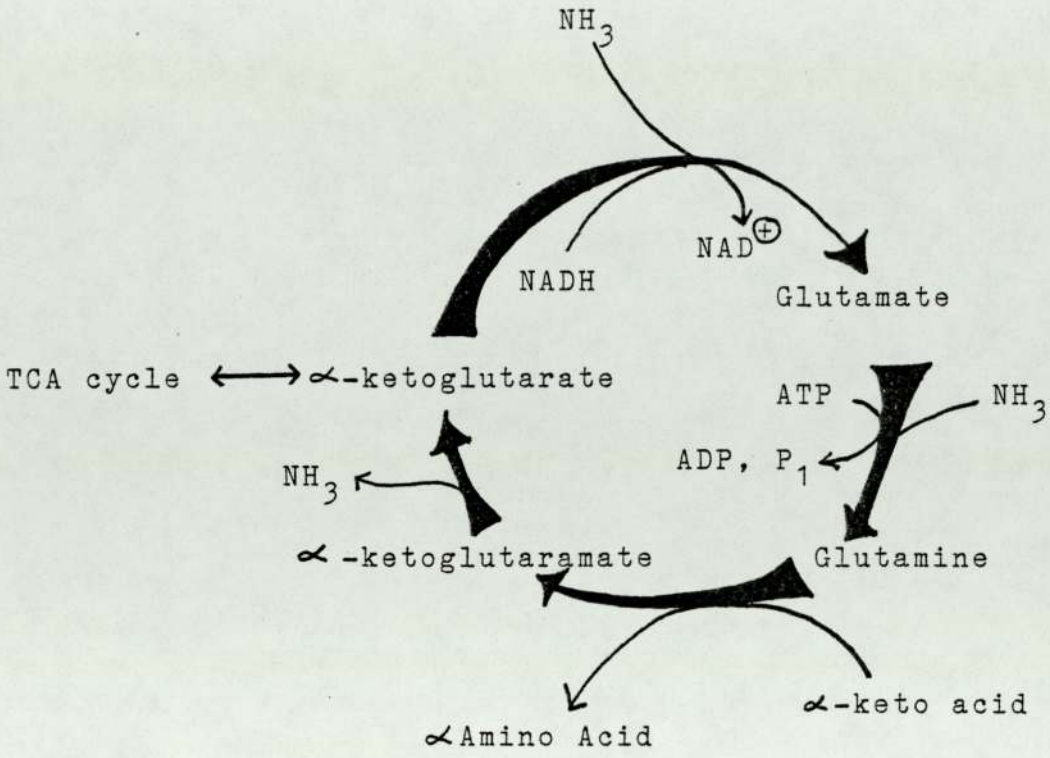


Fig 4.1 Pathway of Ammmonia Metabolism in Brain

Reductive amination of α-ketoglutarate and energy-dependent amidation of glutamate are the major routes of ammonia removal in brain. Although conversion of glutamine to α-ketoglutaramate and subsequent hydrolysis of α-ketoglutaramate to α-ketoglutarate does occur in brain, the flux through this portion of the cycle is thought to be low in normal individuals. In patients with hepatic encephalopathy, increased concentrations of glutamine and certain α-keto acids (phenylpyruvate) in brain tissue may promote glutamine transamination leading to α- ketoglutaramate formation.

Skeletal muscle metabolises large quantities (50%) of ammonia in normal individuals, and plays a normal role in ammonia homeostasis. The kidney and brain are only minor sources of ammonia in normal animals and humans[81].

4.1.(2)3. Function

Ammonia adjusts the acid-base balance of the body by being excreted as ammonium ions. If ammonia (as ammonium ions) were not available, the excretion of acids would involve the removal of the basic ions of the blood and so endanger blood neutrality. Blood ammonia levels are normally in the range of 20-70 μg NH_3-N per 100 mls[84-86].

4.1.(2)4. Excretion

Ammonia from any source is converted to urea in the liver by enzymes of the Krebs-Henseleit cycle[6, 8, 87], according to the following simplified chemical scheme:

$$2NH_3 + CO_2 \longrightarrow C{=}O(NH_2)_2 + H_2O$$

Urea

Most urea is excreted in the urine by the kidney, but about 25% diffuses into the gastro-intestinal tract. Normally ammonia generation by the kidney involves the metabolism of glutamine and the deamination of other amino acids. Ammonia thus liberated combines immediately with hydrogen ions (H^+) derived from ionized carbonic

acid to form ammonium ions (NH_4^+). The latter are then exchanged for sodium (Na^+) and potassium (K^+) ions in the urine. Thus, this production of ammonium ions allows the excretion of excess anions and hydrogen ions, without the corresponding loss of fixed base (Na^+ and K^+)[81]. The mechanism and chemical scheme of ammonia excretion are shown in Figs 4.2 and 4.3 respectively[6].

4.1.(2)5. Liver failure

In liver failure, ammonia is poorly metabolised, and ammonia laden portal blood bypasses the liver to enter the systemic circulation. (This is portal systemic shunting)[8,17,77,78,88-93]. Ammonia is therefore elevated in the blood and cerebrospinal fluid (C.S.F.), and there is an increase in the permeability of the blood brain barrier to ammonia (ammonium ions)[5,83]. This is highly neurotoxic, and leads to hepatic encephalopathy or coma. The ammonia concentration in the brain is closely linked to the level of neural activity. See Fig 4.4 for ammonia in hepatic encephalopathy[94]. Hyperammonaemia also contributes to encephalopathy indirectly by raising the brain concentration of neutral amino acids which alter neurotransmitter metabolism by toxic effects on neuronal metabolism[82,91,95-98]. Up to the 1950's ammonia intoxication was poorly understood, to the extent that patients with failing liver from any cause were given a high protein diet, if necessary by tube when they were unconscious[78]. This is now known to be detrimental to the health of the

Fig 4.2 Mechanism of ammonia excretion (6)

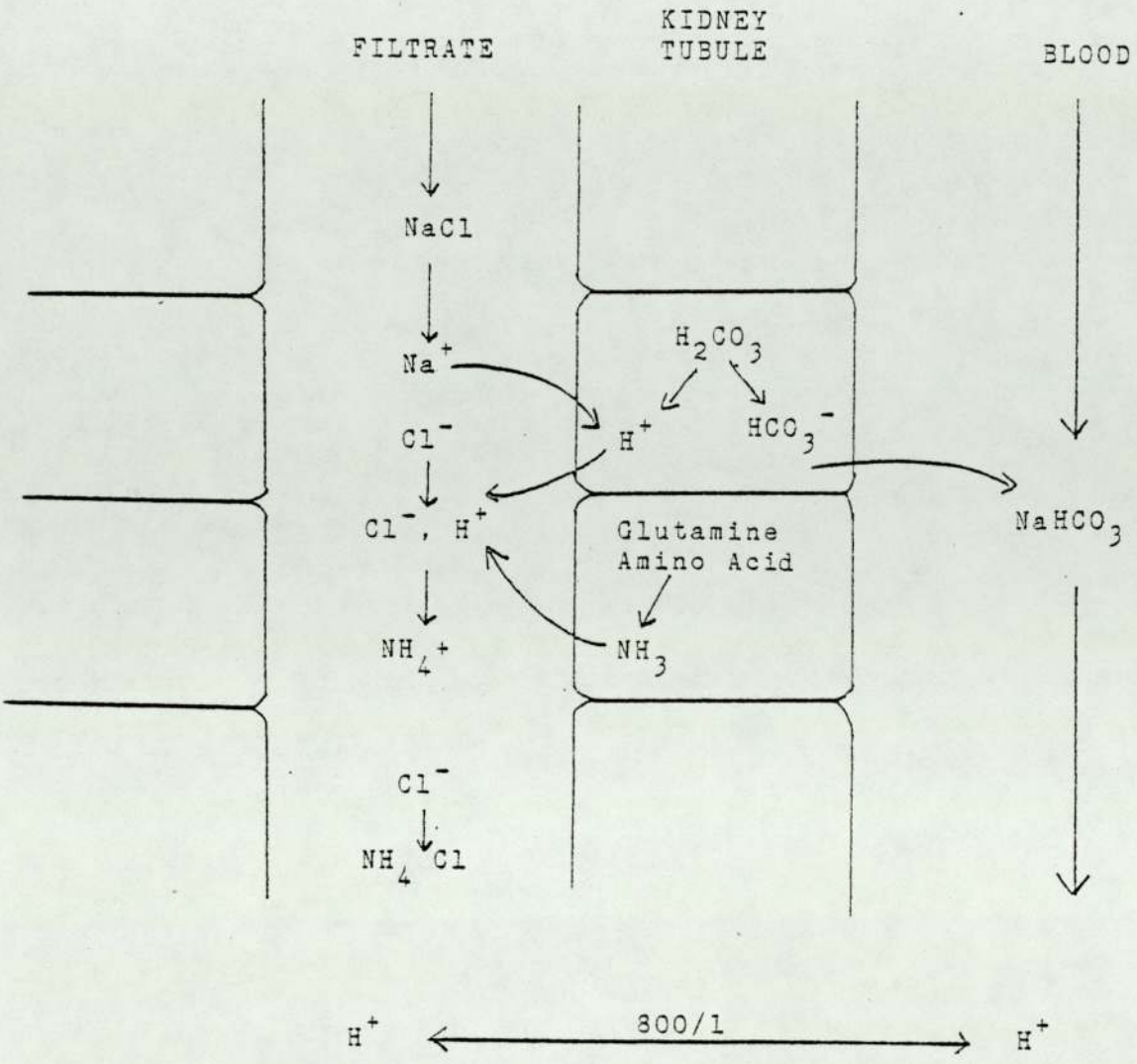
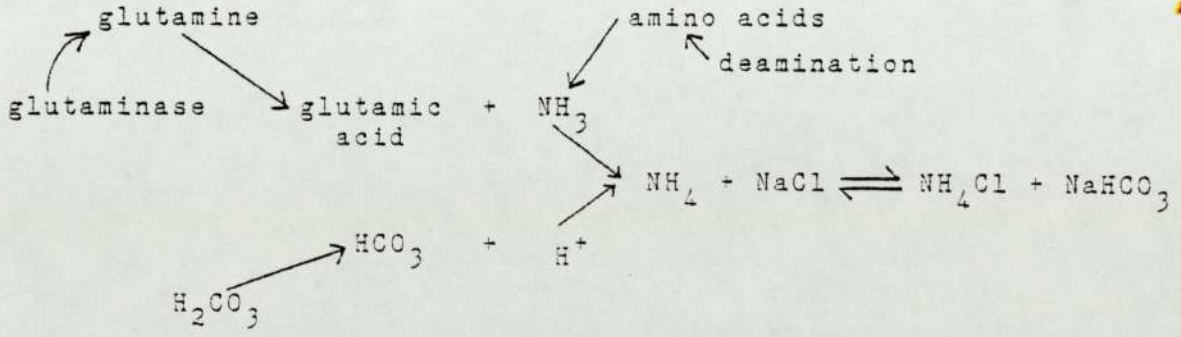


Fig 4.3 Chemical scheme for ammonia excretion (81)



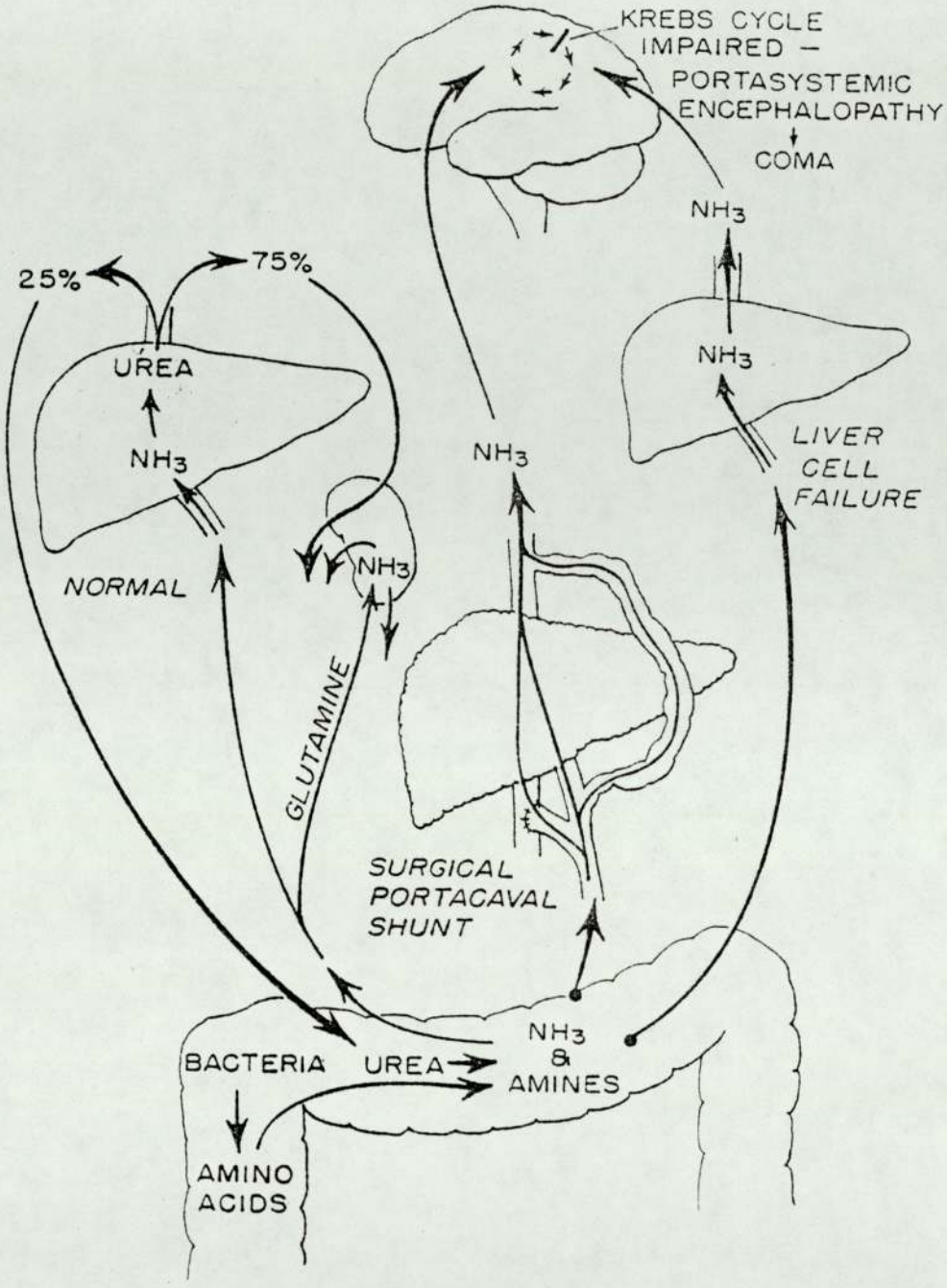


Fig 4.4 Normal and Abnormal Circulation of Ammonia

patients. However, little progress has been made to date in the removal of ammonia in hepatic encephalopathic patients.

4.1.(2)6. Use of Ammonia in this Project

Ammonia is chosen as a model toxin in this study because of its' obvious implications in liver failure as already stated, and also because its' elevated levels, up to 180 μg NH_3-N/100 ml in some cases, also contribute to increased levels and neurotoxic effects of other potentially toxic nitrogenous hepatic toxins - namely the amino acids and amines (false neurotransmitters)[99-104]. Ammonia, a weak base, is structurally and chemically the simplest toxin implicated in liver failure, but plays a central role in the pathogenesis of hepatic coma. In choosing a nitrogenous model toxin from the vast range available, it therefore seemed appropriate to begin with ammonia. Also the solubility of ammonia in water is an important asset for the in-vitro evaluation procedures to be used in this study, and its' analysis could be easily met using the analytical methods adopted.

4.1.(3) Amino Acids

4.1.(3)1. Function

Amino acids are the fundamental components of proteins[87, 105-108], and take part in all living processes. In addition to the protein-bound amino acids,

the cellular tissue and plasma of living organisms contain a permanent reservoir of free amino acids ('amino acid pool') which take part in numerous metabolic reactions and biosynthetic pathways. The amino acid distribution patterns of blood, plasma and urine are of great diagnostic significance in medicine.

4.1.(3)2. Amino Acids in Liver Failure

A convenient finding in acute liver failure is that amino acids are elevated to abnormal concentrations in plasma, cerebrospinal fluid (C.S.F.), urine and brain. Amino acids also play a role in the pathogenesis of hepatic coma, and they may even act synergistically to potentiate the effect[5,8,23,82,109,110]. The concentration of amino acids producing hepatic encephalopathy (coma) in liver failure differs with the individual, such that whilst the level of an amino acid in one patient may produce coma, this level may have no significant detrimental effect on another patient. In chronic liver failure without encephalopathy, many of the straight chain amino acids are increased in the plasma, and with the occurrence of encephalopathy they increase further [82]. In acute fulminant hepatic failure, the plasma concentration of all except the branched chain amino acids is increased [111-117].

Among the most prominent changes in plasma are increased concentrations of aromatic amino acids, methionine (more than tenfold), phenylalanine, tyrosine and unbound/free tryptophan (most toxic, about three to

five fold), and also aspartate, glutamate and citrulline and others (less than three fold). Decreased concentrations are found of the branched chain neutral amino acids, leucine, isoleucine and valine as are threonine and arginine, to levels often below 50% of the normal plasma concentration (23,82,118-122). Correlations have been observed between the rise and fall of plasma amino acids, and the progression or improvement of the encephalopathy. The reasons for this altered pattern are still incompletely understood. A cerebral imbalance of aromatic and branched chain neutral amino acids, reflected by a decreased plasma [(leucine + isoleucine + valine)/(tyrosine + phenylalanine)] ratio may precipitate encephalopathy, possibly by promoting the synthesis of toxic aromatic amines. The molar concentration ratio of the above mentioned amino acids in plasma, correlates inversely with the severity of neurological impairment in patients with hepatic encephalopathy. Nevertheless, it seems unlikely that an imbalance between branched chain and aromatic amino acids in either plasma or brain causes the encephalopathy(8,22).

4.1.(3)3. Use of Amino Acids in This Project

Amino acids have been chosen as examples of model nitrogenous hepatic toxins from the range available because of their importance in hepatic encephalopathy and because their selective adsorption is virtually unknown. Several other factors have been important in selecting

amino acids as model toxins.

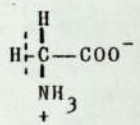
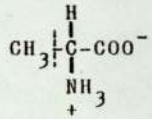
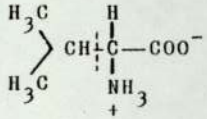
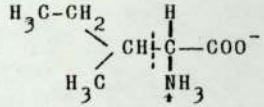
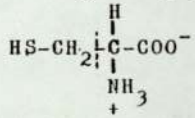
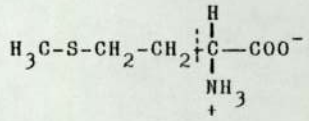
Firstly, they differ chemically and structurally from ammmonia. Their molecular weights are greater than that of ammonia, and they are suitable toxins to use in order to expand on the ammonia study.

Secondly, amino acids (general formula R-CH(NH_2)COOH) are alike in containing an α carboxyl group (-COOH) and an α amino group (CH(NH_2)), but their chemical nature is dependent on their side chain residue - R. This residue may be acidic, basic or neutral depending on their isoelectric points[105-107]. Thus different amino acids with varying structures could be chosen from the range of amino acids available.

Thirdly, it is this R-group which is important in deciding the size and solubility of the amino acids. The nature of this substitute affects not only the biochemical role of the molecule physiologically, but governs the pathological role of the amino acid as a toxin (e.g. phenylalanine). This chemical variability may also be important in governing the nature of adsorption and may also be useful in obtaining a certain degree of selectivity in adsorption of amino acids.

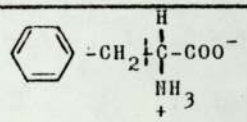
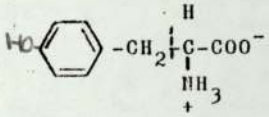
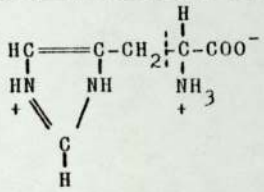
Thus the amino acids appear to be a useful group of toxins chosen for investigation, as their structure enables them to be studied not only as a large group of toxins, but also as a range of different compounds within that group. The amino acids used in this particular study are listed in Fig 4.5.

Fig 4.5 Amino Acids Used in This Study

CLASS	AMINO ACID	ABBREV-IATION	MOLE-CULAR WEIGHT	FORMULA	ALTERNATIVE CLASSIFICATION	METABOLISM
I Aliphatic Amino Acids A) Monoamino-monocarboxylic Acids	1) Glycine	Gly	75		neutral (uncharged) polar R-group	Glucogenic
	2) Alanine *	Ala	89		non-polar (hydrophobic) R-group	Glucogenic
	3) Valine	Val	117		non-polar (hydrophobic) R-group	Glucogenic
	4) Isoleucine (Essential)	Ile	131		non-polar (hydrophobic) R-group	Glucogenic and Ketogenic
B) Sulphur Containing Amino Acids	5) Cysteine	Cys	121		neutral (uncharged) polar R-group	Glucogenic
	6) Methionine	Met	149		non-polar R-group (hydrophobic)	Glucogenic

continued ...

Fig 4.5 Amino Acids Used in This Study

	CLASS	AMINO ACIDS	ABBREV-IATION	MOLE-CULAR WEIGHT	FORMULA	ALTERNATIVE CLASSIFICATION	METABOLISM
II	Aromatic Amino Acids	7) Phenylalanine	Phe	165		non-polar (hydrophobic) R-group	Glucogenic and Ketogenic
		8) Tyrosine	Tyr	181		neutral (uncharged) polar R-group	Glucogenic and Ketogenic
III	Heterocyclic/ aromatic Amino Acids	9) Histidine (Essential)	His	155		positive charged R-group Basic Amino Acid	Glucogenic

* Alanine is the least hyrdrophobic member of the class of hydrophobic amino acids, as it is near the border line between non-polar amino acids and those with uncharged polar R-groups.

4.1.(4) Amines

Intimately connected with the amino acids and their chemistry are the neurotransmitters and false neurotransmitters. A number of compounds meet many of the criteria necessary for chemical neurotransmission.[123,124] Amines are one set of such compounds which act as false neurotransmitters in hepatic encephalopathy. Before discussing these amines, however, it is appropriate to consider the normal neurotransmitters.

4.1.(4)1. Neurotransmitters

The actions of neurotransmitters are ubiquitous, influencing a variety of tissues and physiological functions. For a compound to be considered a neurotransmitter, it should be present in nerves, together with its' biosynthetic enzymes. When the nerves are stimulated, the transmitter should be discharged from nerve endings. Once liberated, the transmitter should be recognised by and bind to its' specific receptor on the postjunctional cell membrane and produce a biological response characteristic of that cell. Mechanisms should be available to rapidly terminate the actions of the neurotransmitter[125].

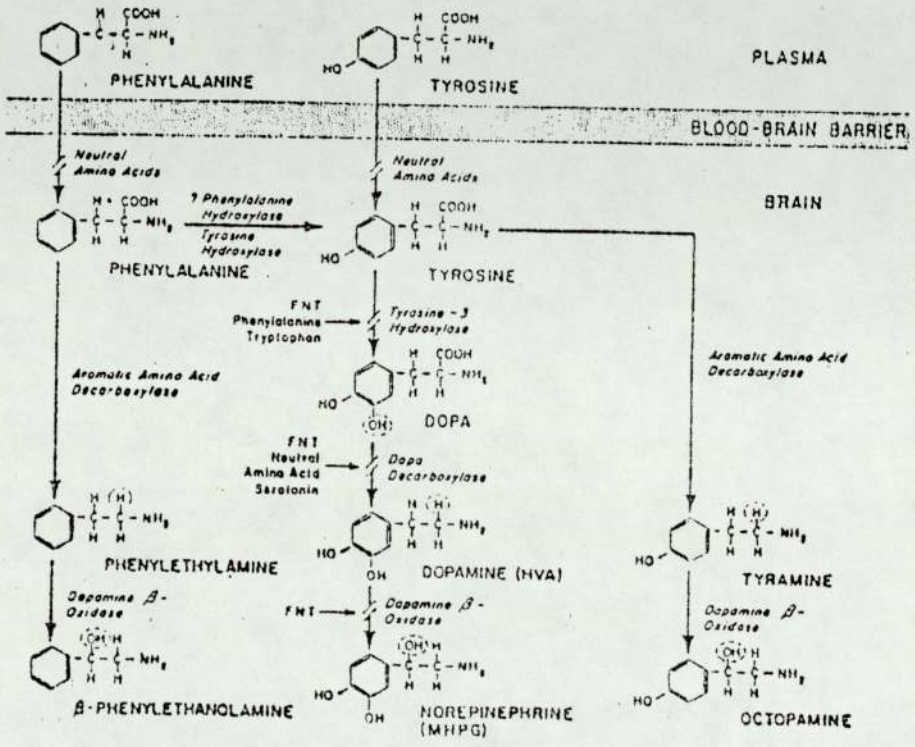
The neurotransmitters are substances such as norepinephrine and serotonin, histamine and possibly even several highly neuroactive amino acids such as glycine, alanine, taurine, γ amino butyrate, glutamate and asparate, which in addition to acetylcholine may play important roles in normal synaptic transmission

in the brain and spinal cord. The role of amines as false neurotransmitters will now be discussed.

4.1.(4)2. Amines as False Neurotransmitters

The role of amines is far less recognised and obvious than that of the amino acids and ammonia. The strongest evidence implicating amines as toxins, relates to their role as false neurotransmitters[5,227]. This occurs with the failure of hepatic function. In this case the blood brain barrier selectively allows incorrect amounts of neurotransmitter precursor amino acids such as phenylalanine and tyrosine to enter the central nervous system. This then leads to altered neurotransmitter synthesis. Fig 4.6 shows the synthesis of some false and true neurotransmitters[8]. Phenylalanine and tyrosine are precursors of the normal cerebral neurotransmitters - norepinephrine and dopamine, as well as several neuroactive substances such as phenylethylamine, tyramine and octopamine, which are normally present only in small amounts[16,82,122,127]. The precursor amino acids and aromatic amines, are produced in the gut by protein degradation and by bacterial action along with ammonia and mercaptans. They are normally efficiently removed by the liver[128,129] by monoamine oxidase (MAO) and cleared from portal blood. But when hepatic function is impaired and blood is shunted around the liver, precursors accumulate in the blood and flood the central nervous system. They are then locally β-hydroxylated by relatively non-specific enzymes and replace normal

Fig 4.6 Synthesis of Some False and True Neurotransmitters[8]



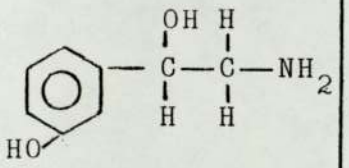
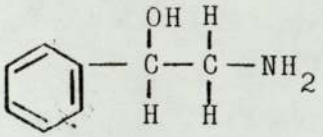
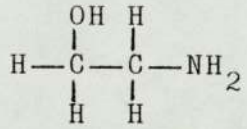
A scheme of synthesis of catecholamines and phenylethylamines has been suggested and is shown above. Blocks in the synthetic function are indicated by lines and suggest that there are numerous sites at which catecholamine synthesis can be blocked. If tyrosine, for example, cannot go to DOPA, it is primarily decarboxylated to tyramine and then to octopamine, thus perhaps explaining the increase in brain octopamine while brain norepinephrine is decreased on the basis of diminished synthesis.

transmitters, acting as false neurotransmitters[5,127,130]

4.1.(4)3. Amines Used in This Project

The range of nitrogenous toxins implicated in liver failure is vast. This study looks at a cross-section of model toxins selected from that range. Already discussed in the previous sections are ammonia (a basic toxin), and amino acids (which can be both acidic and basic). These have been chosen as suitable representatives within that range. Amines have also been chosen as model hepatic toxins in this study because they complete the range of water soluble nitrogenous hepatic toxins selected from the large range available, and because of their importance as cerebral toxins in liver failure. The adsorption of false neurotransmitter amines in artificial liver support systems is a desirable goal in the treatment of liver failure patients. The analysis of amines poses no problems as they can also be analysed by the system used for ammonia and amino acid. They have not been studied in great detail in the past, and their relative adsorption is virtually unknown. The amines studied in this project are octopamine, phenylethanolamine and ethanolamine, the structures of which are shown in Fig 4.7. Of these, octopamine, one of the most important false neurotransmitters, has received much attention by many workers, and is discussed in the next section,

Fig 4.7 False Neurotransmitters Studied

STRUCTURE	Mwt	NAME	ABBREV-IATION	PRECURSOR	ANALOGUE OF
	189.6	Octopamine	OTA	Phenylalanine Tyrosine	Norepinephrine
	137.2	Phenylethanolamine	PEA	Phenylalanine	Octopamine
	61.08	Ethanolamine	ETA		

together with phenylethanolamine, its' analogue.

4.1.(4)3.(i) Octopamine

The sympathomimetic amine octopamine, (1-[p-hydroxyphenyl]-2 aminoethanol or β-hydroxyphenylethanolamine) has the properties of a neurotransmitter in man (and invertebrates)[125]. It has been proposed (Fischer 1971, 1972, 1976) that octopamine is involved in the pathogenesis of hepatic encephalopathy (HE), acting as a false neurotransmitter (or co-neurotransmitter), i.e. a synaptic competitor to the physiological adrenergic transmitters[121,131-133]. Octopamine is synthesised from intestinally derived tyramine (for which there is a relative blood-brain barrier) and also from amino acids in the brain. Brain levels of octopamine and tyrosine correlate closely in animals with hepatic insufficiency[128]. Octopamine is taken up by central nerve endings and stored in granules and competes with a normal physiological adrenergic central neurotransmitter dopamine or norepinephrine for uptake and release in central nerve endings. This therefore leads to encephalopathy, and increased urinary levels of octopamine[131]. Elevated levels of octopamine precursors, phenylalanine and tyrosine, with lesser elevations of other amino acids have been found. Normally these precursors are converted successively to dopa, dopamine, noradrenaline and adrenaline in nerve endings.

However, in liver failure, the enzyme converting tyrosine to dopa (tyrosine hydroxylase) is inhibited and tyrosine and phenylalanine accumulate, and are directed into synthesis of tyramine, then octopamine by relatively non-specific enzymes[17,125].

Normal subjects have octopamine blood levels below 1 ng/ml (N=70), while patients with grade 3 or grade 4 encephalopathy show values above 3.2 ng/ml (N=70)[131].

4.1.(4)3.(ii) Phenylethanolamine

The aromatic amine, β-phenylethanolamine or β-hydroxyphenethylamine (2-amino-1-phenylethanol) is basically octopamine minus an -OH group. It is able to undergo transmethylation in a competitive manner with octopamine, and play a role in hepatic encephalopathy, either synergistically or as an alternative to octopamine itself. When phenylethanolamine levels are increased in the brain, it can be released from sympathetic nerves and thus act as a false neurotransmitter. Blood levels of phenylethanolamine have been elevated sometimes to levels as high as 1 μg/ml in some patients[8,127,131].

4.2. Adsorbents Used in Study

4.2.(1) Introduction

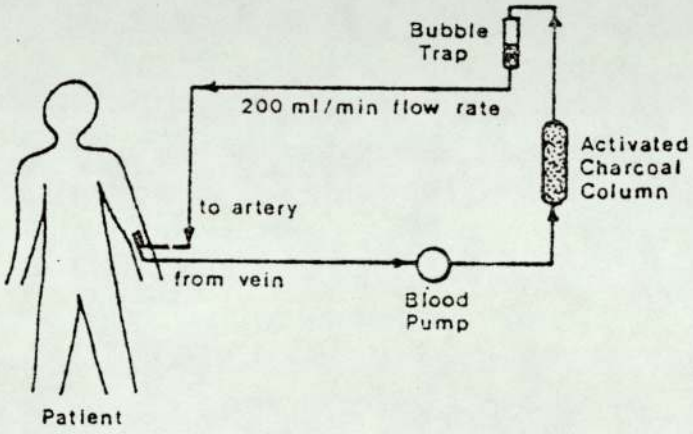
A variety of adsorbents is employed in this study. These range from neutral adsorbents such as activated charcoal, through to polymeric neutral uncharged resins of the Amberlite XAD type and the acidic and basic ion exchange resins. Hydrophilic hydrogels are also used, and are specially synthesised for the purpose of this project. A description of these adsorbents will now be presented.

4.2.(2) Activated Charcoal

4.2.(2)1. Introduction

In the past, the accumulation of a wide range of potentially toxic substances of differing molecular size and physical properties, suggested that a specific approach to artificial liver support, for the treatment of fulminant hepatic failure (FHF)was not yet possible, as the nature of these toxins had not yet been fully determined. Thus, in the past, charcoal, a broad spectrum adsorbent, emerged as the best adsorbent available for use in whole blood haemoperfusion, although it is non-selective and often unpredictable, because it could adsorb a range of toxins due to its' large adsorptive capacity, which is a result of its' large internal surface area[22,60,51,134-139]. A typical haemoperfusion system using charcoal[137] is shown in Fig 4.8.

Fig 4.8 A Typical Haemoperfusion System



Activated charcoal is used as an adsorbent in this study. The structure and properties of activated charcoal will now be discussed briefly, followed by its' use in medicine.

4.2.(2)2. Structure and Properties

Activated charcoal (carbon) is a highly porous material, prepared by carbonisation and activating - organic substances, mainly of biological origin, such as wood, petroleum, coal, peat and coconut shells[51,60,137]. It has a very large surface area because of its' micro-porous structure, but as a result of this structure it is a relatively soft, fragile material, which presents problems associated with biocompatibility, when used for haemoperfusion purposes. These problems will be discussed in Section 4.2.(2)4.

4.2.(2)3. Use of Activated Carbon in Medicine

The concept of flowing blood over activated charcoal granules to remove unwanted substances, originated with Yatzidis (1964), who used such a column to remove creatinine, uric acid, phenolic compounds, guanidine bases and organic acids[8,26,60,140]. Yatzidis mentioned the possible use of such a system in removing endogenous toxins (as in kidney or liver failure) as well as exogenous toxins (drugs or poisons). Side-effects noted were the removal of some blood cells (especially platelets) from the blood, hypotension and the embolism of fine charcoal micro-particles into the blood. These are then deposited in various organs (e.g. lungs, spleen, liver, kidneys),

and plug organ capillaries which causes tissue necrosis. Fine particle generation can be brought to a minimum by extensive preliminary washing procedures before use. Before using an activated carbon for haemoperfusion the minimum evaluation tests that should be performed are microparticle generation, adsorption capacity and ion elution. A summary of these tests is outlined in Fig 4.9 .

Fig 4.9 Summary of Tests to Evaluate Carbon

1. Cleanliness
2. Washability
3. Attrition resistance
4. Surface morphology
5. Adsorption capacity
6. Microparticle generation
7. Ion elution
8. Blood compatibility
9. Sterility, toxicity, pyrogenicity

At present, however, the majority opinion is that coating of the carbon is essential before use in haemoperfusion systems, in order to decrease the incidence of thrombus formation, by reducing the friability of the adsorbent[63,135,140-143]. Thus the embolism of fine carbon microparticles into the blood is prevented, which in effect promotes biocompatibility of the charcoal with blood, and so blood cell loss during haemoperfusion is reduced to tolerable levels.

4.2.(2)4. Use of Materials for Coatings

A large number of membrane materials for micro-encapsulation of charcoal, which includes nylon, cellulose acetate, heparin-complexed materials, collodion, albumin-collodion coating and others, have now been studied for use in uraemia, intoxication and liver failure (Chang) [8,135,144,145]. These are listed in Fig 4.10.

Fig 4.10 Biomedical Coatings Used For Activated Charcoal

1. Albumin (adsorbed)
2. Albumin (adsorbed on cellulose nitrate)
3. Albumin (crosslinked)
4. Cellulose acetate
5. Cellulose triacetate (deacetylated)
6. Cellulose nitrate (collodion)
7. Dextran (adsorbed)
8. Haemoglobin (adsorbed)
9. Heparin complexed cellulose nitrate
10. Hydroxyethyl cellulose
11. Methacrylate copolymers
12. Nylon
13. Polyhydroxyethyl methacrylate
14. Poly(acrylic acid and stryrene copolymer)
15. Poly(acrylonitrile) and Acrylonitrile methyl acrylate copolymer
16. Poly(acrylic acid Butyl methacrylate)

The coatings used, however, are not entirely satisfactory, as they are non-specific and thus not permeable to a wide range of toxins[51]. Thus for the long-term goal of a total artificial liver, additional systems would be required for the removal of toxins not removed

by activated charcoal. Within the last few years, a few haemoperfusion columns based on coated activated charcoal have been developed, and they became commercially marketed[8,137]. These devices and their major features are listed in Fig 4.11. A picture of one of these devices is shown in Fig 4.12.

Fig 4.11 Commercial Haemoperfusion Devices

Name	Manufacturer	Adsorbent	Adsorbent size (mm diameter)	Adsorbent coating
Haemocol	Sander Ltd 1974	300g Charcoal	2 - 4	5 μm acrylic hydrogel
Adsorba 300C	Gambro Inc	300g Charcoal	1 (2mm)	3-5 μm cellulose
Hemodetoxifier	Becton-Dickinson	94g Charcoal	0.3-0.84	None

However, whether these devices are still being used is not known. They may be employed in acute intoxication cases, but not in artificial liver support systems. Activated charcoal has been used for adsorption purposes and also as a base material in this project, for applying and testing the microencapsulation technique, the coating procedure of which will be explained subsequently.

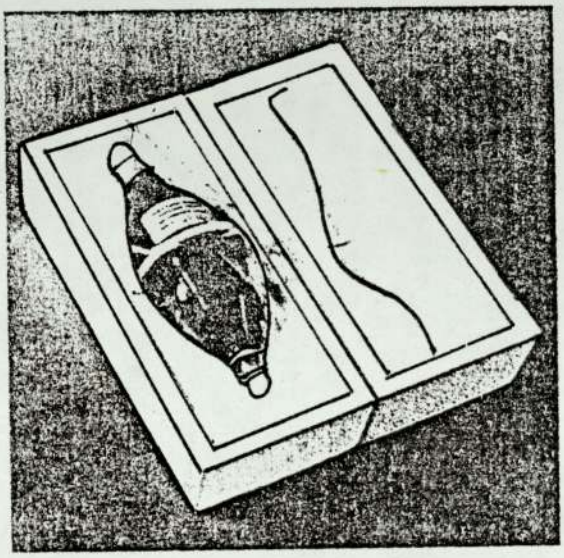


Fig 4.12 'Haemocol' Column : Finished Product in Opened Pack

4.2.(2)5. Characteristics of Coating Material

Coatings applied to adsorbents for biocompatible reasons can be purposely made to confer some degree of permselectivity for the adsorbent, and thus control the nature of the materials allowed access to the adsorption sites of the adsorbent[51]. It is desirable to select a polymer coating that has a high affinity for certain specific toxins and a low affinity for the other non-toxic compounds present. The characteristics of an 'ideal' coating system have been defined by Bruck as being:-

1. The coating must be strong enough to eliminate fragmentation and generation of microemboli.

2. The coating must be freely permeable to the toxins of interest.

3. The coating must be blood tolerable, particularly with respect to adhesion of blood cellular elements.

4. The coating must permit good flow and low pressure drops in the column, i.e. coated particles must not adhere or aggregate in the column.

5. The coating must be readily sterilizable, non-toxic and non-pyrogenic.

In addition to these properties, the coating might also be used to impart a degree of selectivity to the charcoal[145]. With these points in mind the preparation and application of a coating material is investigated.

4.2.(2)6. Preparation and Application of Coating Material

Activated charcoal is encapsulated in this project to confer some degree of biocompatibility. The coating material used is an acrylic hydrogel such as poly-hydroxyethyl methacrylate, commonly abbreviated to poly HEMA . The function and properties of HEMA will be discussed in section 4.2.(5) on hydrogels. However, it is necessary at this point to state how polyHEMA is prepared, before giving details on the coating procedure.

4.2.(2)6.(i) Polymerisation of HEMA

Poly(2-hydroxyethyl methacrylate), polyHEMA, is prepared by solution polymerisation of the monomer HEMA (200g) using t-Butylcyclohexylperdicarbonate (1g) as initiator, and ethanol (2 l) solvent, in a nitrogen atmosphere. Reaction time and temperature are 36 hours and 50°C respectively, with continuous stirring of the reaction mixture. Precipitation of the prepared polyHEMA solution is accomplished by the dropwise addition of the solution to diethylether, which has the advantage of also eliminating any residual monomer that may be present. The poly-HEMA is then oven dried at 40°C for 36 hours, before use.

4.2.(2)6.(ii) Microencapsulation of Adsorbents

In this project, microencapsulation is performed using activated charcoal, and ion exchange resins as adsorbents [26,134,144,146,147]. Initially, dried polyHEMA (1g) is

dissolved in 49g of a solvent mixture, containing ethanol and acetone in a 90:10 ratio (weight). This mixture is then introduced into a glass rotaflask (previously treated with Repelcote, 2% solution of dimethyldichlorosilane in 1,1,1-trichloroethane to produce a 'non-stick' silicone surface), containing 20g adsorbent particles (resin particles are dried before coating). This mixture is then rotated for 10 minutes to produce electrostatic charges on the beads, and so prevent sticking to one another. The rotaflask is then placed in a water bath at 30°C, the solvent 'evaporated off' (15-90 minutes) and the coated adsorbent removed from the reaction vessel.

In later coatings, propan-1-ol is used as the preferred solvent instead of acetone in the solvent mixture. This method produces a 5% polyHEMA coating. This percentage of HEMA coating is an expression involving the ratio of the weight of HEMA to the weight of beads encapsulated. To produce other percentages by weight of HEMA, the weight of HEMA or adsorbent used is altered accordingly. The surface morphology of the uncoated and coated adsorbent particles is then examined with a Stereoscan S150 Model Scanning Electron Microscope. The electron micrographs are shown in Plates 2.1 - 2.13 in Chapter 2.

4.2.(3) Polymeric Uncharged Resins

4.2.(3)1. Introduction

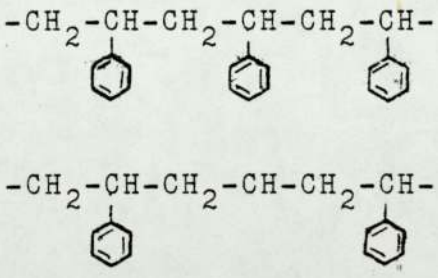
Alternatives for carbon are being sought and are

largely centred on available polymer particles, such as the polymeric neutral uncharged resins. This project employs such resins for the adsorption of the model hepatic toxins (nitrogenous-based) used in this study. From this class of resins, those of concern in this particular project are discussed below.

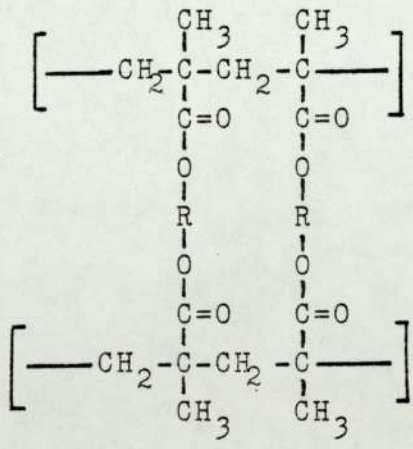
4.2.(3)2. Structure and Properties of Polymeric Uncharged Resins

The macroreticular polymeric adsorbents of the Amberlite XAD-resin type such as Amberlite XAD-2, XAD-4 and XAD-7 are employed in this study. Amberlite XAD-2 and XAD-4 resins are hydrophobic aromatic adsorbents of polystyrene divinyl benzene copolymers. Amberlite XAD-7 resin is a polar aliphatic adsorbent of acrylic ester. The chemical structure of these Amberlite XAD resins are shown in Fig 4.13[62, 148-149].

Fig 4.13 Chemical Structure of Amberlite XAD Resins



Amberlite XAD-2, 4



Amberlite XAD-7

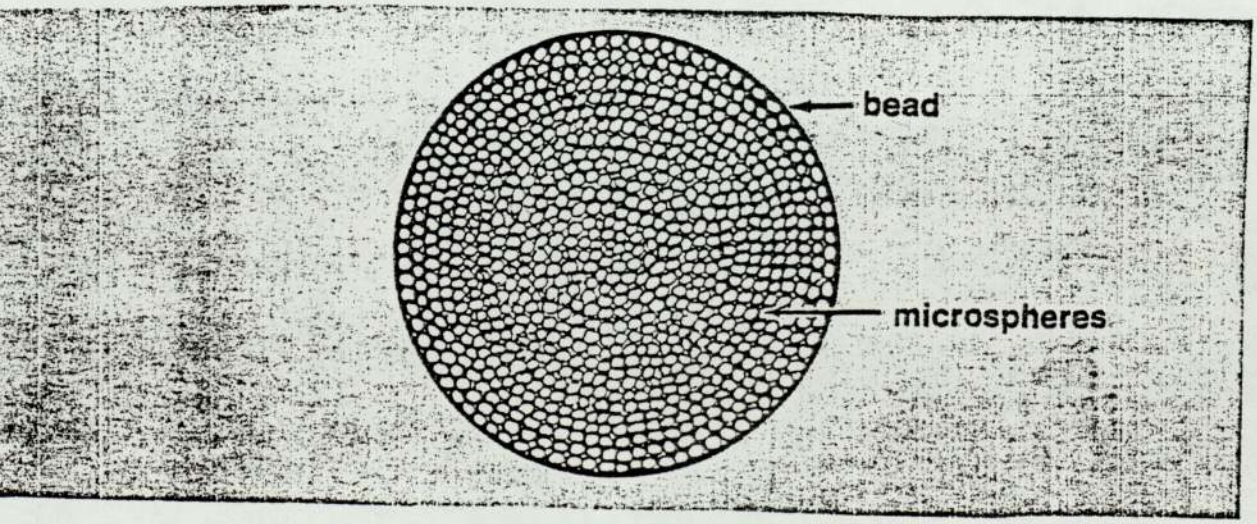
Fig 4.14 Amberlite Polymeric Adsorbents[148]

GRADE	Mwt RANGE	CHEMICAL NATURE	POROSITY VOLUME (%)	TRUE WET DENSITY (g/cc)	SURFACE AREA (m^2/g)	AVERAGE PORE DIAMETER (Å)	SKELETAL DENSITY (g/cc)	PARTICLE SIZE (mm)
XAD-2	1000 or less	Poly-styrene	42	1.02	330	90	1.07	0.25-1.2
XAD-4	20000 or less	Poly-styrene	51	1.02	750	50	1.08	0.25-1.2
XAD-7	60000 or less	Acrylic Ester	55	1.05	450	80	1.24	0.25-1.2

As it can be seen from the chemical structure of Amberlite XAD-7 in Fig 4.13, the precise nature of this material has not been given and there is great reluctance on the part of the manufacturers to completely describe it and reveal the nature of the group R, which they simply describe as a 'polyfunctional aliphatic residue' (145, 149).

The properties of the polymeric adsorbents used are shown in Fig 4.14[148].There it can be seen that in the case of the Amberlite XAD-2 and XAD-4 there is an inverse relationship between surface area and pore size with the smaller pore size adsorbent having the greater surface area. These resins occur in the form of white, hard, insoluble polymerised beads. The individual beads may each be considered to be an agglomeration of a large number of very small microspheres, fused together into a spherical agglomeration[148]. This is depicted in Fig 4.15 below:

Fig 4.15 Sketch of an Individual Amberlite XAD Resin Bead



This macroreticular structure results in a continuous solid phase, as well as a continuous pore phase. In the absence of functional sites, the polymeric adsorent derives its' adsorptive properties from its' combination of macroreticular porosity, pore size distribution, high surface area and nature of its' structure.

Adsorption in these Amberlite XAD resins involves van der Waals' forces, which bind the sorbate to the solid surface, leading to surface adsorption. Water or other solvents then very rapidly penetrate the pores. Many types of interactions such as hydrophobic bonding, dipole-dipole interaction and hydrogen bonding are important[148].

Albumin-coated Amberlite XAD-7 Resin

The use of human serum albumin (HSA) has been investigated as an alternative coating material to synthetic polymers, by the Liver Unit team of the Kings' College Hospital and Medical School. This protein has been shown by several workers (Packham, Chang) to reduce platelet adhesion to foreign surfaces and to improve the blood compatibility of such surfaces. HSA-coated Amberlite XAD-7 has been used in the development of an artificial liver support system for the removal of bilirubin, the bile acid chenodeoxycholic acid, bromosulphophthalein (BSP) and phenols[150,151]. This clinically used adsorbent is used in this project for the adsorption of the model toxins detailed in section 4.1, (e.g. ammonia, amino acids, amines). Amberlite XAD-7

has a higher affinity for human serum albumin (HSA) than the other resins of the Amberlite series and HSA can be tightly bound to Amberlite XAD-7. The coated resin used also contained 0.1% Azide as an antibacterial agent.

4.2.(4) Ion Exchange Resins

4.2.(4)1. Introduction

While it is difficult to predict or vary the properties of charcoals, ion exchange resins have clearly defined structures and many variations have been synthesised commercially. Thus, ion exchange resins are employed in this study because, by the use of the different classes available, a comparative study of the adsorptive capacity, for the range of nitrogenous toxins of interest can be achieved. Also the type of groups capable of adsorbing the model nitrogenous toxins can be investigated.

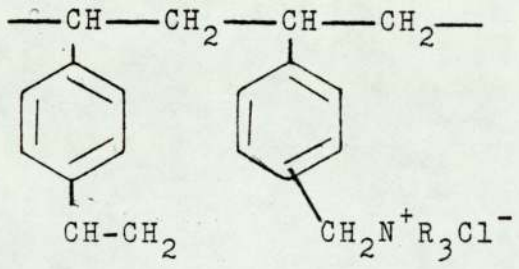
An ion exchange material may be broadly defined as an insoluble matrix of fixed ionic groups, containing labile or mobile ions of the opposite charge, which are free to wander throughout the resin interstices, and are thus capable of exchanging with ions in the surrounding medium, without any major physical change taking place in its' structure[148,152,153]. In general, the ion exchangers used are spherical particles and approximately one-half millimetre in diameter. The structure and properties of the various types of ion exchange resins used will now be discussed.

4.2.(4)2. Structure and Properties of Ion Exchange Resins

4.2.(4)2.(i) Basic Anionic Ion Exchange Resin

The strongly basic anion exchanger of polystyrene divinylbenzene copolymers, substituted with quaternary ammonium active functionality such as Dowex 1-X4 is used. This resin derives its' properties from the amino group in the resin structure. The basic strength of the resin depends partly on the nature of the active group and also on its' position. The quaternary ammonium group ($-NR_3^+ OH^-$) gives rise to a strongly basic resin, the functional (ionogenic) group of which is shown below, Fig 4.16[62,148, 152-154].

Fig 4.16 Strongly Basic Functionality (quaternary ammonium)



The properties of this anion exchange resin are presented in Fig 4.20.

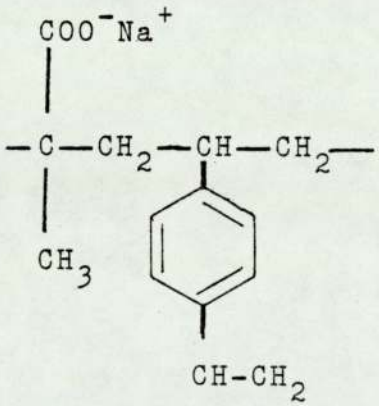
4.2.(4)2.(ii) Acidic Cationic Exchange Resins

Weakly acidic and strongly acidic cation exchange resins are also employed in this study. These will be discussed in turn.

a) Weakly Acidic

Weakly acidic cation exchangers of a cross-linked polyacrylic-divinylbenzene matrix with carboxylic (-COOH) functionality, e.g. Amberlite 1RC-50, is used. The functional (ionogenic) group of this resin is shown below, Fig 4.17 (62,148,152-154).

Fig 4.17 Weakly Acidic Functionality



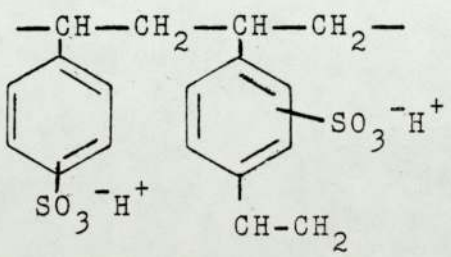
This ion exchanger is in the form of white opaque, spherical particles and is insoluble in water. The exchange capacity and selectivity of this resin are derived from the carboxylic acid groups and are also a result of its' physical and chemical properties. The properties of this resin are presented in Fig 4.20. The macroreticular structure of Amberlite 1RC-50 resin imparts high adsorptive capacities, and the rough physical

structure permits its' use without the pre-treatment steps of extraction and clarification, required by other adsorbents, e.g. Amberlite XAD-7[148,152,154]. These pre-treatment steps are given in Chapter 4.2.(5).

b) Strongly Acidic

The strongly acidic cation exchangers of a cross-linked polystyrene-divinylbenzene matrix with sulphonic acid functionality, e.g. Amberlite 1R-120, Dowex 50W-X8, Duolite C255, and Amberlite 1RN-77 (Nuclear grade ion exchange resin derived from parent resin, Amberlite 1R-120) are employed in this project. The properties of these are also presented in Fig 4.20. Cation exchange resins are generally in two forms, the free acid or hydrogen form and the salt form, often the sodium form. The functional (ionogenic) group on these cation exchangers is shown below (hydrogen form), Fig 4.18[62, 152-154].

Fig 4.18. Strongly Acidic Functionality

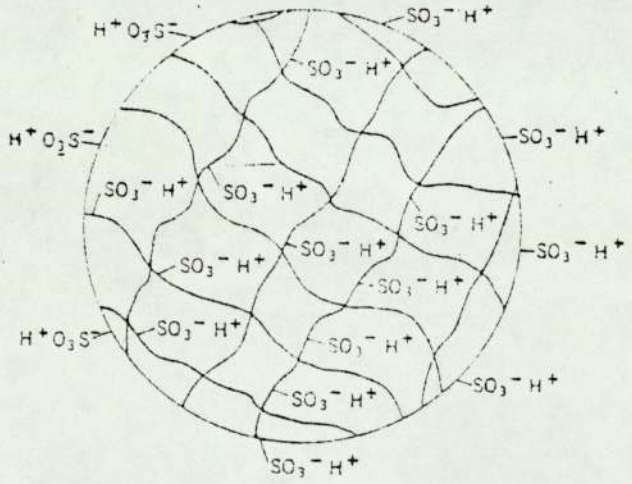


Conventional ion exchange materials contain ion-active sites throughout their entire structure with a grossly

uniform distribution of activity. Thus in Fig 4.19 a cation-exchange resin with a negatively charged matrix and exchangeable positive ions (cation) is indicated[153]. The hydrogen form will absorb* cations and release an equivalent amount of hydrogen ions into solution, whereas with the sodium form, cations are absorbed and the equivalent amount of sodium ions released.

The exchange potentials for cation absorption on to a strongly acid resin are influenced by a number of

Fig 4.19 Cation-Exchange Resin Schematic



*The word 'adsorb' and 'absorb' are used here with their usual connotation, i.e. 'adsorb' refers to processes which involve the sorption of one material at the surface of another, while 'absorb' is used to describe other types of sorption.

factors, the most important being molecular weight, size, valency and concentration. In general, for efficiency in ion exchange, the affinity of the ion to be absorbed must be substantially greater than that of the ion already in the resin[152].

4.2.(4)3. General Use of Ion Exchange Materials

Ion exchange materials have been used for water treatment in the past and still find wide application in water softening. The use of ion exchange in medicine and the pharmaceutical industry at present, utilises many facets of ion exchange technology[154,155].

It is now being used in many discrete areas; the use of ion exchangers and related materials as artificial organs remains the immediate interest area in this project. It is hoped that the use of the range of ion exchange resins outlined, will provide clinically useful comparative information about the adsorption of nitrogen containing toxins, as there is still no widely accepted method of treatment available at present for the reduction of such toxins in hepatic failure.

4.2.(4)4.Pre-treatment of Adsorbents

It is known that the use of uncoated adsorbents in haemoperfusion results in the production of fine micro-particles in the haemoperfusion system, and thus bio-incompatibility of the adsorbents. However, when charged resins are used and an ion exchange capacity is introduced,

Fig 4.20 Ion Exchange Resins[148]

RESIN	MATRIX TYPE	GRADE	FUNCTIONAL STRUCTURE	IONIC FORM	EFFECTIVE SIZE (mm)	MOISTURE CONTENT (%)	TOTAL EXCHANGE CAPACITY a) meq/g dry b) meq/ml wet
STRONGLY BASIC ANION EXCHANGE RESIN							
Dowex 1-X4	Styrene DVB	Standard	$-N^{+}(CH_3)_3-$	Cl^{-}	0.45-0.6	43	a) 3.5 b) 1.33
WEAKLY ACIDIC CATION EXCHANGE RESIN							
Amberlite 1RC-50	Meth-acrylic DVB	Standard	-COO-	H^{+}	0.33-0.5	43-53	a) 10 b) 3.5
STRONGLY ACIDIC CATION EXCHANGE RESIN							
Amberlite 1R-120	Styrene DVB	Standard	$-SO_3-$	Na^{+}	0.47-0.62	44-48	a) 5 b) 1.9
Amberlite 1R-120	Styrene DVB	Anal-ytical	$-SO_3-$	H^{+}	0.47-0.62	44-48	a) 5 b) 1.9
Amberlite 1RN-77	Styrene DVB	Nuclear	$-SO_3-$	H^{+}	0.45-0.6	55	a) 1.75
Dowex 50W-X8	Styrene DVB	Standard	$-SO_3-$	H^{+}	0.5-0.6	53	a) 4.8 b) 1.7
Duolite C255	Styrene DVB	Standard	$-SO_3-$	H^{+}	0.47-0.55	—	b) 2

the loss of essential ions from the circulation, (e.g. Na, K, Ca, Mg) occurs[62,156,157]. Resin haemoperfusion also results in the adsorption or disruption of formed blood elements, removal of vital substances from the blood, and the addition of toxic substances to the blood from the resin material itself, as well as the embolism of resin material into the circulation. Usually, this problem is solved by applying a biocompatible coat to the adsorbent surface. However, in this study, uncoated species of adsorbents are employed, to firstly determine their adsorptive capacity, and no account is taken of the biccompatibility of the adsorbent material in the initial stages of preliminary screening of adsorbents. Therefore, in order to obtain some idea of the levels of interfering species imparted to the test solution by the adsorbents, in the event of adsorption studies, the adsorbents were treated with distilled water as follows:

10g of adsorbent are shaken with 50 ml distilled water for 2 hours, and the samples analysed for any detectable species, using the Auto-Analyser. On addition of d.H_2O to the adsorbent, the colour imparted to the water was noted, and the pH of the solution recorded (using an Orion pH meter). The adsorbent and d.H_2O are then subjected to gentle agitation as experienced in the static adsorption technique used in this project. The colour of the water is again noted, the pH taken and the

Fig4.21 Effect of Addition of Distilled Water to Adsorbents Before and After a 24 Hour Gentle Agitation Procedure (static)

	BEFORE SHAKING		AFTER 2 hrs GENTLE SHAKING			
Adsorbent	colour of solution	pH of solution	colour of solution	pH of solution	impurity detected in solution	Class of Adsorbent
Carbon	clear	10.14	grey-black	10.16	5	neutral
Amberlite IRC-50	cloudy	4.37	cloudy	4.35	0.53	cation exchange resin weakly acidic
Duolite C255	clear	3.61	clear	3.59	0	
Dowex 50W-X8	orange	2.63	orange	2.68	0.53	
Amberlite IR-120 (Na^+)	deep yellow	3.48	deep yellow	3.49	3.38	
Amberlite IR-120(H^+)	clear	4.08	clear	3.93	0	cation exchange resin strongly acidic
Regenerated Amberlite IR-120(H^+)	pale yellow	3.2	pale yellow	3.13	0.32	
Amberlite IRN-77L	deep orange	2.55	deep orange	2.42	1	
Amberlite IRP-69	cloudy	-	cloudy	-	6	
Dowex 1-X4	clear	4.23	slightly cloudy	4.28	3.05	strongly basic anion exchange resin
Amberlite XAD-2	clear	9.4	slightly cloudy	9.2	0	neutral polymeric resin
Amberlite XAD-4	clear	9.07	very slightly cloudy	9.0	0	
Amberlite XAD-7	clear	7.34	cloudy	7.7	9.	

washings analysed for any species detectable by the Auto-Analyser evaluation technique.

The observations from these static 'H_2O-blank' procedures are presented in Fig 4.21 for each adsorbent employed. From these observations, it is clear that some sort of washing procedure is necessary prior to the use of adsorbents. The standard washing procedure adopted will now be outlined.

4.2.(4)4.i) Washing Procedure

1. The adsorbent (10g) is thoroughly mixed with five times its' volume of d.H_2O (50 ml) in a large vessel. At this stage, the adsorbents become swollen with water and the mixture takes the form of a slurry.

2. The water is then decanted off from the adsorbent and the washing repeated twice with additional quantities of water. This is continued until the leaching of the colour from the adsorbent does not occur.

3. The adsorbent is dried with filter paper before being used. Heat is not used for drying the adsorbents because the extremely dry beads which would be formed re-swell very rapidly when placed in aqueous or other polar solutions, thus causing severe strains which may lead to bead breakage.

In the case of activated charcoal, because of its' highly porous nature, many fine particles loosely adherent to the surface of the beads, readily break off during the washing procedure with a decrease in fines as the washing is continued.

The ion exchange resins on the other hand are produced in the form of attrition resistant spherical particles, thus the problem of fines production is not important. However, when these resins, especially the cation exchange resins are placed in water after long storage, a yellow to red-brown colour is imparted to the water[148]. In the case of the anion exchanger a cloudy component is released into the washings. This is due to small amounts of reaction products left within the beads during manufacture. Generally the colour throw ceases after a few washing runs have been made[148].

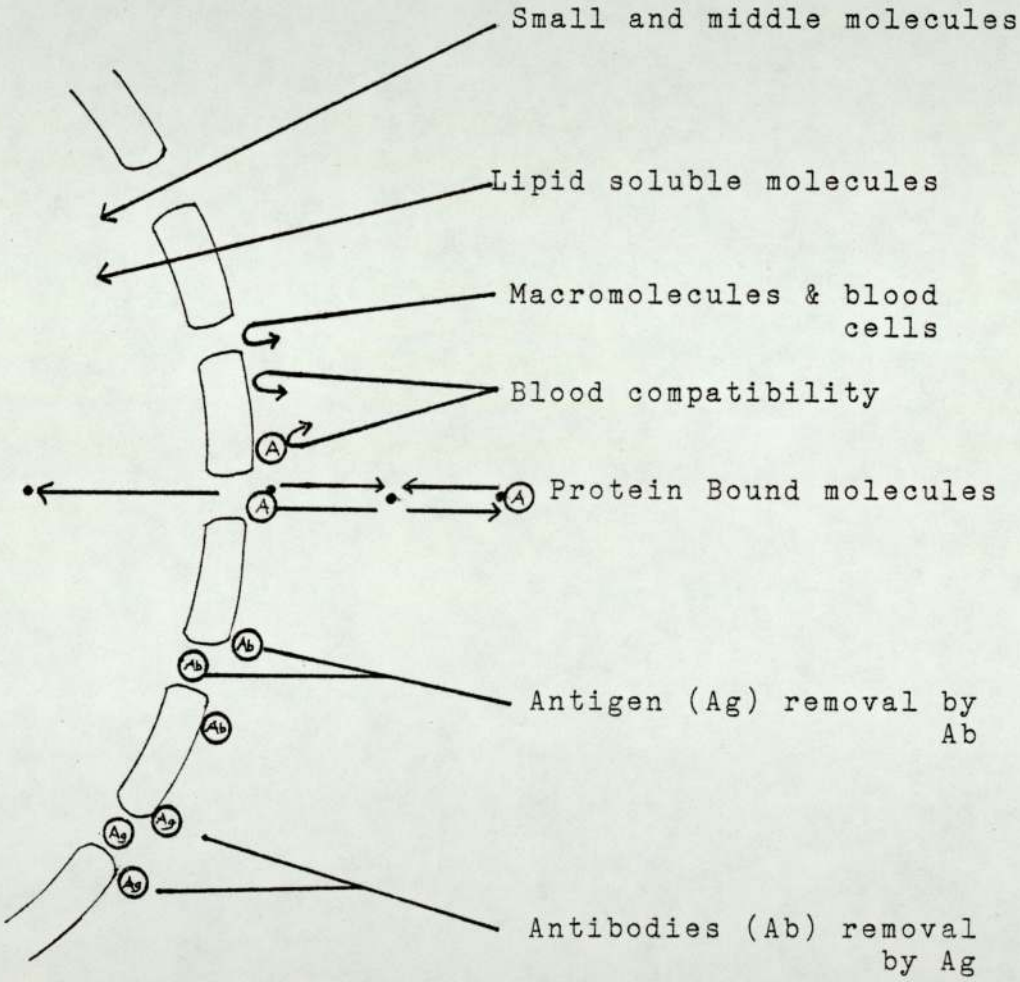
Washing of the Amberlite polymeric adsorbents is essential because normally these adsorbents are rinsed with dilute sodium chloride and sodium carbonate solutions before packaging, to control bacteria and mould growth during storage. These preservative agents and residual monomeric compounds still present in the pores, must therefore be rinsed from the resin with water before use.

4.2.(4)5.Use of Coated Ion Exchange Resins

Attempts to modify the resin surface by using a biocompatible membrane have been made in the past[158] and also in this project, by the method outlined previously. However,

it is common knowledge that the adsorption of toxins to be removed is partly reduced, as they do not readily diffuse across such membrane coatings. By the coating of resins and the examination of the membrane properties of these encapsulated resins, this project aims to eliminate this unfortunate disadvantage encountered with the use of such essential biocompatible coatings.

Fig 4.22 Membrane Properties (22,38,54)



Membrane properties can be extensively varied to selectively remove specific types of molecules. This principle is being extended, modified and developed extensively as expressed in Fig 4.22.

4.2.(5) Hydrogels

4.2.(5)1. Introduction

At present, adsorbents are made biocompatible by coating them with biocompatible membranes. The ideal solution to eliminate the use of bio-incompatible adsorbents, would be to use adsorbents which are themselves biocompatible. Thus, adsorbents composed of the biocompatible membrane material used for the coatings, remain the ideal materials for synthesising such adsorbents. With this in mind, particulate hydrogels, hydrophilic in character are synthesised in a separate project and used as adsorbents in this project. The structure and properties of hydrogels will now be discussed.

4.2.(5)2. Structure and Properties of Hydrogels

i) Definition

A hydrogel is a three-dimensional polymeric network, capable of imbibing large quantities of water (by swelling) without the dissolution of the polymeric network[159].

Three main types of hydrogels have been defined by

B. J. Tighe and co-workers (1971) and consist of non-ionic hydrogels, thermally reversible hydrogels and novel microcrystal hydrogels, all of which have been used in biomedical applications[160-162]. However, the hydrogels used in this study are mainly of the neutral non-ionic hydrogel types. Hydrogels possessing cationic exchange ability are also used. The hydrogels which have received the most attention over recent years have been those derived from polymers of hydroxyl esters of meth-acrylic and acrylic acid, in particular poly 2-hydroxyethyl methacrylate) or polyHEMA. Because polyHEMA was the first synthetic hydrogel used for biomedical applications, it has been the most extensively studied. Bearing this in mind, a review of the structure and properties of hydro-gels must necessarily be mainly concerned with polyHEMA. Particular emphasis is placed on these properties rele-vant to artificial liver support.

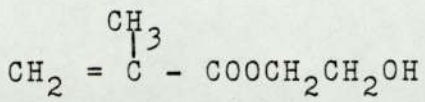
ii) Structure

HEMA (Figs 4.23, 4.24) belongs to the family of mon-omeric esters, having the general structure shown in Fig 4.23, where R = CH_3 and n = 0.

Fig 4.23

$$CH_2 = \overset{R}{\underset{|}{C}} = COO(CH_2CH_2O)_nCH_2CH_2OH$$

Fig4.24



iii) Properties

a) Swelling and Factors Which Govern It

A cross-linked hydrophilic polymer, when placed in water will swell, by absorbing water, until an equilibrium is reached between the osmotic pressure of the polymer segment which causes the swelling and the opposing and retractive forces arising, as the chains between the cross-links elongate. The water uptake at this equilibrium point is known as the equilibrium water content (EWC) of the hydrogel and is the weight of water in the equilibrated hydrogel, expressed as the percentage weight of the hydrogel.

PolyHEMA hydrogel has an equilibrium water content of around 40%, which because of the thermodynamically poor solvent power of water for polyHEMA is relatively unaffected by the number of cross-links in the initial dilution[160].

b) Hydrophilicity

This important property is relevant to other properties, such as permeability, mechanical strength and biocompatibility, which will all be discussed subsequently.

The hydrophilicity of a polymer is the result of the

presence of hydrophilic groups on the polymeric chains. It enables swelling of cross-linked polymers when they are equilibrated with water[145].

c) Permeability

Permeability is an important property in biomedical application of hydrogels for dialysis, ultra-filtration or adsorption of toxins and is determined by the physical and chemical nature of the water, whether free or bound[145].

d) Mechanical Properties

In its' dehydrated state, polyHEMA is hard and brittle, but when swollen in water, polyHEMA hydrogel is soft and exhibits rubber-like behaviour[160].

e) Biocompatibility

Biocompatibility is of two types - tissue and blood compatibility. Since this project is concerned with artificial liver support, the property necessary for the hydrogels is blood compatibility[145,159,162]. Materials useful for blood-contacting biomedical application should not cause:

i) thrombosis
ii) destruction of the cellular elements of the blood
iii) alteration of the plasma proteins
iv) destruction of enzymes
v) depletion of electrolytes
vi) adverse immune reactions
vii) toxic and allergic reactions

Biocompatibility of hydrogels is achieved due to the

presence of water in the structure. The biocompatibility of hydrogels is the most important property of hydrogels, from the point of view of biomedical application. They present a surface to blood, which does not cause disturbed physiological reactions such as blood clotting (135,162-164).

f) Surface Smoothness

The smoothness of a surface has been held to be important in the prevention of clotting, but even very smooth surfaces, such as glass are thrombogenic. Thus other properties are essential for overall biocompatibility (145).

4.2.(5)3. Biomedical Applications of Hydrogels

Hydrogels are one of the main group of materials considered for biomedical application[165]. They were first proposed by Wichterle and Lim for biomedical use, where hydrophilicity of the polymer was important, with regard to biocompatibility[154]. The division of their applications into separate areas in the biomedical field is presented in Fig 4.25, and has been extensively reviewed[145,160,161,165-170].

4.2.(5)4. Use of Hydrogels in This Project

The hydrogels used in this project are based mainly on the use of hydrogels of hydroxyethyl methacrylate, HEMA. These are designed and prepared by a chemist

Fig 4.25

Biomedical Uses of Hydrogels and Linear Hydrophilic Polymers

1.	Reconstructive Surgery	Plastic Surgery
		Replacement of ducts and canals
		Replacement of hard tissues
2.	Ophthalmic Applications	Soft contact lenses
		Scleral buckling agents
		Glaucoma drainage devices
		Artificial vitreous implants
3.	Artificial Kidney Membranes	Dialysis
		Ultrafiltration
4.	Coatings for Miscellaneous Biomedical Devices	Sutures
		IUD's
		Urinary catheters
		Artificial heart components
		Blood detoxicants
5.	Vehicles for the Release of Physiologically Active Compounds	Antibiotics
		Contraceptives
		Anticoagulants
		Anti-bacteria agents
		Anti-cancer drugs
		Antibodies
		Drug Antagonists
		Enzymes
6.	Pharmaceutical and Other Uses of Linear Synthetic Hydrophilic Polymers	Plasma substitutes
		Pharmaceutical applications
		Miscellaneous applications

(Dr. P.J. Skelly)[145] and a biochemist (Mr. U. S. Atwal and evaluated by the author (a biologist), using in-vitro techniques explained in Chapter 3. These adsorbents are synthesised as particulate hydrogels by a range of techniques including freeze-thaw techniques and variants on this technique, suspension polymerisation and inverse-suspension polymerisation. These methods will now be discussed in turn[171,172].

4.2.(5)4. (i) Freeze-Thaw Technique

The possibility of employing the principle of polymerisation on a crystalline matrix (used by Krach and Sanner and extended by Halden and Lee) was investigated by Dr. P. J. Skelly for the polymerisation of the hydrogel 2-hydroxyethyl methacrylate, commonly abbreviated to HEMA. This produced a macroporous hydrogel bead having strength, blood compatibility and adsorptive properties, appropriate to use in haemoperfusion studies.

This method involved the initial freezing or rapid cooling of the homogenous monomer-solvent mixture (of appropriate monomers and cross-linking agents in water and water-ethylene glycol mixtures, photo-polymerisation in the presence of, for example, benzoin or uranyl nitrate) to produce a network of solvent crystals between which, in the interstices is the monomer. On thawing and subsequent hydration, a highly porous (macroporous) hydrogel structure (polymer gel) is produced[145].

Variation in solvent : monomer ratio, the nature of the monomer and cross-linking agents and the cross-link density produces membranes having a range of equilibrium water contents. The properties of these membranes are different from their homogenous counterparts in respect of strength, opacity, water binding properties, and porosity (having mean pore diameters in the range of 0.2 - 2.0 microns).

4.2.(5)4.(ii) Variation on Freeze-Thaw Technique

Macroporous hydrogel beads prepared by Mr. U.S. Atwal used a technique involving the introduction of a stream of monomer solution (either dropwise or with a motorised syringe) into a stirred bath of non-solvent (e.g. heptane) cooled to -70°C by the addition of solid carbon dioxide. Variations in the rate of injection from 0.2 - 0.5 ml sec^{-1} caused a decrease in the mean diameter of the beads produced, from 3.0 mm to 0.1 mm. The frozen beads were either photopolymerised 'in-situ' or could be removed, stored in cold non-solvent and polymerised subsequently. In these beads, a uniform and inter-connected pore structure extends throughout the beads, and the internal surface area was several times greater than that of the superficial extended surface. Typical macroporous beads obtained at a fixed (50 : 50) monomer : solvent ratio (the solvent consisting of 75 : 25 water : ethylene glycol) have water contents in the region of 55%.

Variations in this ratio produce beads of different equilibrium water contents. Most of the water is held in the macropore, the remainder being strongly bound in the polymer matrix. The range of polymers synthesised by this technique, was extended and complemented by suspension polymerisation.

4.2.(5)4.(iii) Inverse Suspension Polymerisation

This technique uses either hexane (inverse suspension) or saturated brine solution (suspension polymerisation) as the continuous phase. Monomers that can be thus polymerised in particulate form, but which do not possess appropriate freezing behaviour for macroporous polymerisation were used. These include acrylamides and substituted acrylamides. Hydrogel beads having varying compositions and different adsorption characteristics were thus synthesised using a modification of a technique developed by Haymann et al and previously used by Goddard.

By suspension polymerisation (in saturated brine) the monomers were polymerised while maintained salted in organic phase dispersion, in the saturated brine, containing xanthum gum, and hydroxypropyl methyl cellulose as stabilisers.

4.2.(5)5. Macroporous Hydrogels

To create hydrogels of great permeability, either the water content of the gel can be increased to high levels to give large pores, or by using a high concentration of cross-linking agent, and a diluent in which the monomers are not highly soluble, one can create a porous structure of polymer nuclei (inter-connected with polymer chains) between which are discrete pores. The degree of porosity, depends on factors such as monomer to solvent (diluent) ratio and the percentage of cross-linking monomer[145]. Macroporous hydrogel beads contain many large discrete pores (an average pore diameter of 1300Å is not unusual) and these facilitate ion diffusion, so that high molecular weight ions can be more completely removed from solution. Such macroporous hydrogels synthesised and used in this project, extend the scope of ion exchange technique. The macroporous hydrogel beads had been used previously for exchange of large molecules such as bilirubin and bromosulphophthalein BSP in this laboratory, before the onset of this particular project. However, studies which involve smaller molecular weight substances had not been performed. This particular project evaluates the adsorptive capacity of the macroporous hydrogel beads for nitrogen-containing hepatic toxins, such as ammonia, amino acids and amines, in an attempt to use them as adsorbents for artificial liver support systems.

CHAPTER 5

REMOVAL OF AMMONIA BY POTENTIAL HAEMOPERFUSION ADSORBENTS

5.1 Introduction

The major priority of artificial liver support systems is to control the endogenous toxins elevated in liver failure, which lead to hepatic coma and ultimately death[38]. Ammonia is a key cerebral toxin in liver failure, but although it has been frequently stated that an adsorbent is required for the adsorption of ammonia, no solution to this problem has been achieved. Thus ammonia adsorption remains a desirable goal[173-183].

This chapter of the project aims to eliminate uncertainties associated with the removal of ammonia, by the comparative study of ammonia adsorption using a variety of substrates. It is hoped to provide, as a result, clinically useful adsorbents, suitable for use in the correction of hyperammonaemia in artificial liver support systems. The adsorbents employed in this study are charcoal, ion exchange resins (neutral polymeric resins) and novel hydrogel polymers. The performance of these adsorbents in adsorbing ammonia will now be discussed. Firstly the observations from the static adsorption studies for these adsorbents will be presented, followed by their dynamic adsorption studies.

The experimental procedures of these techniques, previously detailed in Chapter 3, briefly involve the interaction of ammonia at room temperature with selected adsorbents. Under static adsorption conditions, 5 ml model toxin

solution, ammonium chloride ($[NH_4Cl]_o \equiv 15 mg Nl^{-1}$) are shaken with 1g adsorbent using standard shake times of one hour and two hours. Dynamic adsorption studies involve perfusing up to 6 litres model toxin solution at a known flow rate over 5g adsorbent, for up to four days continuously.

5.2 Adsorption of Ammonia on Activated Charcoal : Static Adsorption Studies

Charcoal, an adsorbent with a broad adsorptive spectrum, is of continuous interest in liver support systems, and is extensively used in medical application for uraemia and drug intoxication[8,42,151,184,185]. However there is no recorded use in ammonia adsorption, as ammonia adsorption on charcoal is not a clinically practised technique. It has been stated, however that charcoal does not adsorb ammonia[8], but whether this means that charcoal adsorbs negligible quantities of ammmonia or none at all, remains uncertain. Because all charcoal haemoperfusion systems do not have the same clearance, it does not automatically mean that all charcoal haemoperfusion systems have low clearances or none at all[38]. With this in mind, charcoal was the first adsorbent chosen to be used in this study, as there was no quantitative information on the ability of charcoal (carbon) to adsorb ammonia in

the literature.

It is widely accepted, that most adsorbents used in artificial liver support systems need to be encapsulated with a 'biocompatible' membrane before use[8,22], for reasons already stated in Chapter 1 and detailed in Chapter 4. This project investigates the use of the hydrogel poly-2-hydroxyethyl-methacrylate as the possible coating material and activated charcoal is used as an adsorbent for developing the coating procedure, which is outlined in Chapter 4. A study of the influence of this coat (when applied as a thin coat to activated charcoal beads) on the adsorption of ammonia is performed, and will now be discussed.

Uncoated and coated activated charcoal beads of 14-25 mesh (0.6-1.2 mm diameter) were evaluated for their ability to adsorb ammonia, using the conventional static technique detailed in Chapter 3. By this technique 5 mls of a solution of ammonium chloride (NH_4Cl) containing 15 mg ammoniacal nitrogen per litre (15 mg NH_3-N/l) are shaken up with 1g pre-washed (see Chapter 2) activated charcoal for time periods between two and sixty minutes, and the supernatant liquid sample decanted off for analysis.

Under the conditions of examination described above, no ammonia adsorption was observed with the uncoated charcoal grade used. Although different grades of charcoals do differ in their adsorptive ability, they are not that

widely different. The observations made here, therefore, confirm the opinion that apparently exists, namely that charcoal is not an effective agent for ammonia removal in haemoperfusion.

Under the static adsorption conditions outlined overleaf, it is observed as shown in the histograms of Fig 5.1 that 2% polyHEMA coated charcoal beads prepared as described previously in Chapter 4 adsorb 0.15 mg NH_3-N per g beads in one hour from an ammonium chloride solution containing 15 mg NH_3-N/l. The polyHEMA coating is expressed as a percentage of the bead weight. The same figure demonstrates that 10% polyHEMA coated charcoal beads unexpectedly adsorb 2.4 mg NH_3-N/g beads. It was surprising that polyHEMA adsorbed ammonia and it was not expected that the greater percentage by weight of polyHEMA would affect the adsorption of ammonia so dramatically. Thus a fivefold increase by weight of polyHEMA (i.e. from 2% to 10% pHEMA) resulted in an increase in ammonia adsorption by sixteen times. This observation has great implications in the membrane properties of polyHEMA, (discussed in Chapter 4) which will be important for future work of this chapter. However, the adsorbed ammonia is not held strongly to polyHEMA as demonstrated in desorption studies, where the adsorption was shown to be reversible to the extent that the 2% polyHEMA coated charcoal released all its' adsorbed ammonia in one hour

Fig 5·1

ADSORPTION OF AMMONIA ON UNCOATED AND COATED CHARCOAL

CONDITIONS: $[NH_4Cl]_o \equiv 15$ mg Nl^{-1}; 1g adsorbent;
1 hr static adsorption study

Fig 5·2

ADSORPTION OF AMMONIA ON polyHEMA COATED ACTIVATED CHARCOAL

CONDITIONS: $[NH_4Cl]_o \equiv 15$ mg NH_3Nl^{-1}; 1g adsorbent;

o : 2% polyHEMA coated charcoal

• : 10% polyHEMA coated charcoal

Fig 5.1

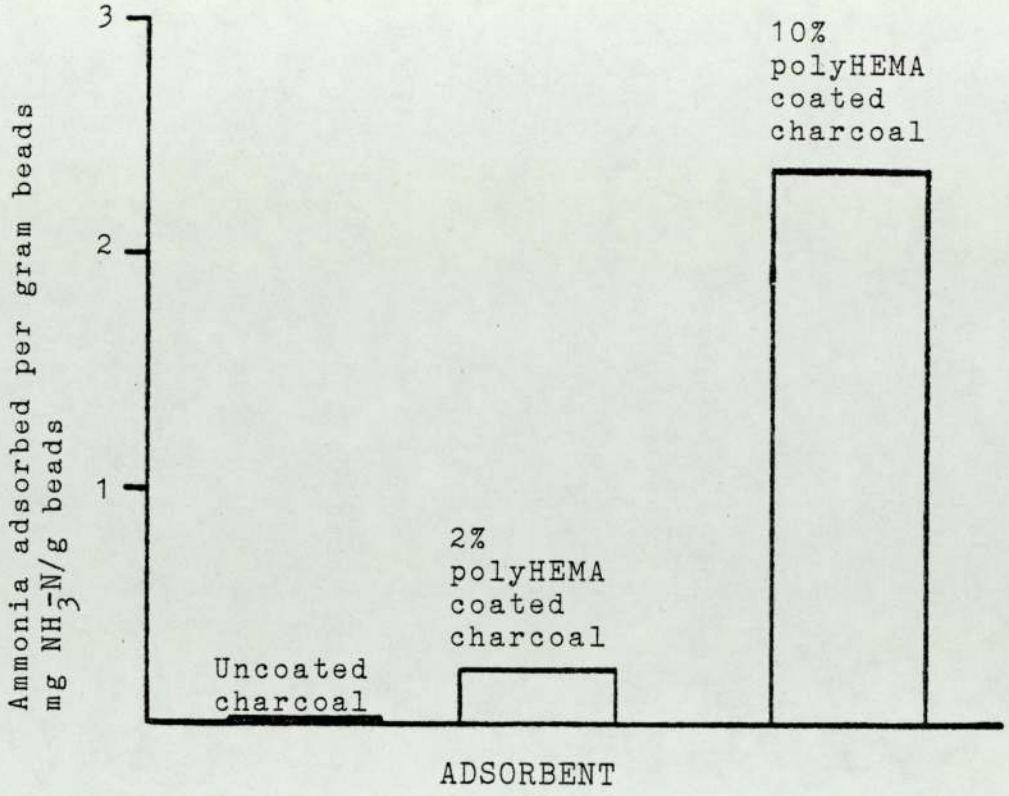
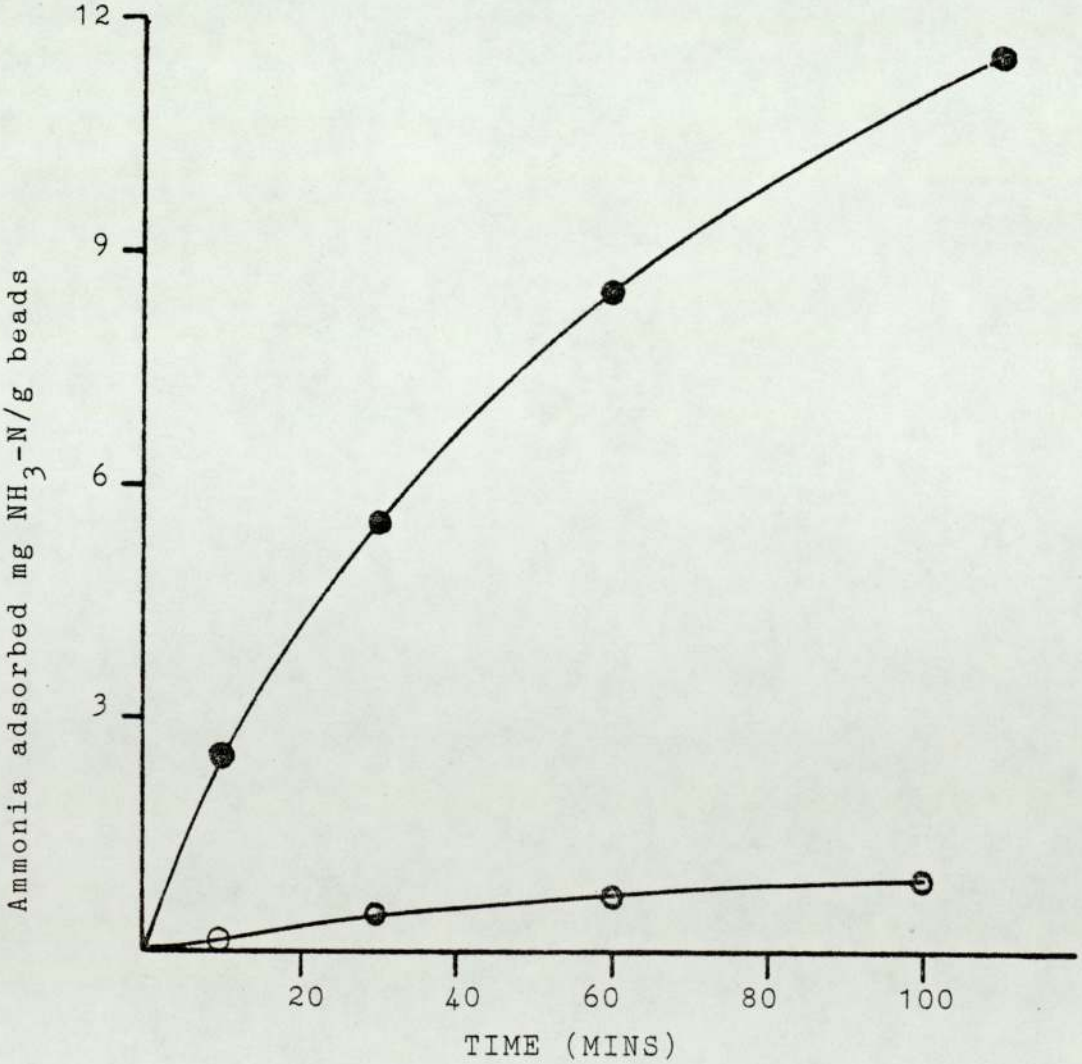

3
2
1
Ammonia adsorbed per gram beads
mg NH_3-N/g beads
10%
polyHEMA
coated
charcoal
2%
polyHEMA
coated
charcoal
Uncoated
charcoal
ADSORBENT

Fig 5.2


12
9
6
3
Ammonia adsorbed mg NH_3-N/g beads
20
40
60
80
100
TIME (MINS)

of gentle agitation with distilled water. Further static adsorption studies performed using coated charcoal beads, followed similar trends (Fig 5.2).

In this study employing polyHEMA coated activated charcoal a static adsorption time of one hour was adopted because the important observations at this stage were those concerning the effects of the polyHEMA coat on adsorption. This static adsorption time was retained for further experiments, since the coated beads were found to reach equilibrium adsorption in one hour. But to ensure that this was a reliable adsorption time, two hour static adsorption studies were also performed.

Due to the fact that a 5 ml sample taken from a 1 litre solution of NH_4Cl was used for the one hour static adsorption studies, the ammonia adsorbed by 2% polyHEMA coated charcoal can be corrected to 7.5×10^{-7} g NH_3-N (i.e. $1.5 \times 10^{-4} \times 5/1000$ or NH_3-N adsorbed $\times \frac{\text{volume sample}}{\text{volume stock-solution}}$). Similarly for the 10% polyHEMA coated charcoal beads, the corrected value for the amount of ammonia adsorbed is 1.2×10^{-5} g NH_3-N (i.e. $2.4 \times 10^{-3} \times 5/1000$).

Using these values and taking into account the weight of adsorbent used in haemoperfusion systems (200g) and the level of ammonia that would need to be removed in liver failure, the time required to reduce elevated hyperammonaemic levels in man can be calculated.

Normal blood ammonia levels are in the range of 20 - 70 μg NH_3-N/100 ml. Since the blood volume of man is 5l (5000 ml) this amounts to a total blood ammonia of 1000 - 3500 μg NH_3-N (or 1 - 3.5 mg NH_3-N). Elevated blood ammonia levels in liver failure have been said to reach 180 μg NH_3-N/100 ml. Similarly taking 5 litres as the total blood volume, the elevated level is equivalent to 9000 μg NH_3-N or 9 mg NH_3-N . In situations where levels are elevated from the lower limit (i.e. from 1 mg NH_3-N to 9 mg NH_3-N) a level of 8 mg NH_3-N would need to be removed. Where levels are elevated from the upper normal limit (i.e. from 3.5 mg NH_3-N to 9 mg NH_3-N) a level of 5.5 mg NH_3-N would need to be removed.

Further calculations show that with the use of 200g of 2% polyHEMA coated charcoal, a time of 37 - 54 hours would be required to reduce the ammonia levels to the normal range stated, assuming it was elevated to 9 mg NH_3-N. However, such perfusion times are not suitable for haemoperfusion systems. Similarly 200g of 10% polyHEMA coated charcoal beads can reduce elevated levels of ammonia to normal levels in 2-4 hours. This is a more suitable haemoperfusion time, and is similar to those used in haemoperfusion systems. Nevertheless, it is not very desirable to use polyHEMA coated charcoal as an adsorbent material for ammonia, since charcoal itself does not adsorb ammonia, although polyHEMA does. A wiser solution would

be to coat adsorbents capable of adsorbing ammonia, or to use adsorbents composed of the coating material - polyHEMA. Since polyHEMA based adsorbents are not commercially available, these would need to be purposely synthesised. With these points in mind, this study is continued by examining polymeric resins, and ion exchange resins for their ammonia adsorptive capacity.

5.3 Adsorption of Ammonia on Uncoated Polymeric Uncharged Resins : Static Adsorption Studies

To date there is no information in the literature on the ability of polymeric uncharged resins to adsorb ammonia in liver failure. This study uses macroreticular polymeric uncharged resins such as Amberlite XAD-2, XAD-4 and XAD-7 and observes their ability to adsorb ammonia. The structure and properties of these adsorbents have already been detailed in Chapter 3. From this study it is hoped that the use of such resins for ammonia adsorption will provide some useful information about the need for charges and functional groups on adsorbents, in ammonia adsorption.

The static adsorption technique outlined in Chapter 3 is used and the resins mentioned above are investigated for their ammonia adsorptive ability as follows:

A 5 ml solution of NH_4Cl containing 15 mg ammoniacal-nitrogen per litre (15 mg NH_3-N/l) is shaken with 1g of adsorbent for one hour and two hour periods, and the samples analysed for unadsorbed ammoniacal-nitrogen. Two static adsorption times (one hour and two hours) are adopted in these studies, to observe whether time has any significant effect on the equilibrium adsorptive capacity of the adsorbent material under observation.

Under the conditions stated it was found that in one hour adsorption studies, using the aromatic polystyrene resins, Amberlite XAD-2 and XAD-4, a quantity of 0.2 mg ammoniacal-nitrogen per gram beads (i.e. 0.2 mg NH_3-N/g beads) and 1.4 mg ammoniacal-nitrogen per gram beads (i.e. 1.4 mg NH_3-N/g beads) were adsorbed by these resins respectively from an ammonium chloride (NH_4Cl) solution containing 15 mg NH_3-N/litre. These results are shown in Table 5.1. Under similar conditions Amberlite XAD-2 and XAD-4 adsorbed 12.5 and 14.9 mg NH_3-N per g beads respectively in two hours. The adsorption of ammonia by these resins confirm the fact that there is an inverse relationship between surface area and pore size, with the smaller pore size adsorbent (in this case Amberlite XAD-4) having the greater surface area and thus a greater adsorptive capacity. A strikingly high adsorptive capacity achieved for two hour static adsorption studies suggests that equilibrium adsorption was not reached in one hour.

Amberlite XAD-7, an aliphatic resin of acrylic ester, however, did not adsorb ammonia in one hour or two hour static adsorption periods.

It may be possible that these resins actually adsorb more ammonia than the results show, but this capacity is not made apparent due to the simultaneous leaching of impurities from the adsorbents into the liquid sample. Attempts were made to reduce the impurities by prewashing (see Chapter 3). However, this problem continued especially with Amberlite XAD-7, in which case the leachable extracts were clearly detectable at the wavelength (660 nm) of the analytical system. Such impurities are certainly undesirable in artificial liver support systems. It may be that on desorption of adsorbed ammonia, the distilled water used, extracts ammonia (base extraction) together with perhaps residual preservatives (sodium chloride, sodium carbonate) and components of the resin. Because the precise nature of Amberlite XAD-7 has not been given by the manufacturers, it is difficult to state which components in the unknown R group of the resin is resonsible for the observations in this study.

The observations from the desorption studies performed for two hours using XAD-2 and 4 resins are presented in Table 5.1 and show that about 20% of the apparent adsorbed ammonia is later released. The ease of release suggests that ammonia is bound to the outer sur-

Table 5.1 Adsorption of Ammonia by Neutral Uncharged Polymeric Adsorbents

Conditions: $[NH_4Cl]_o \equiv 15$ mg Nl^{-1}; 1g adsorbent; 1 hr and 2 hr static adsorption studies

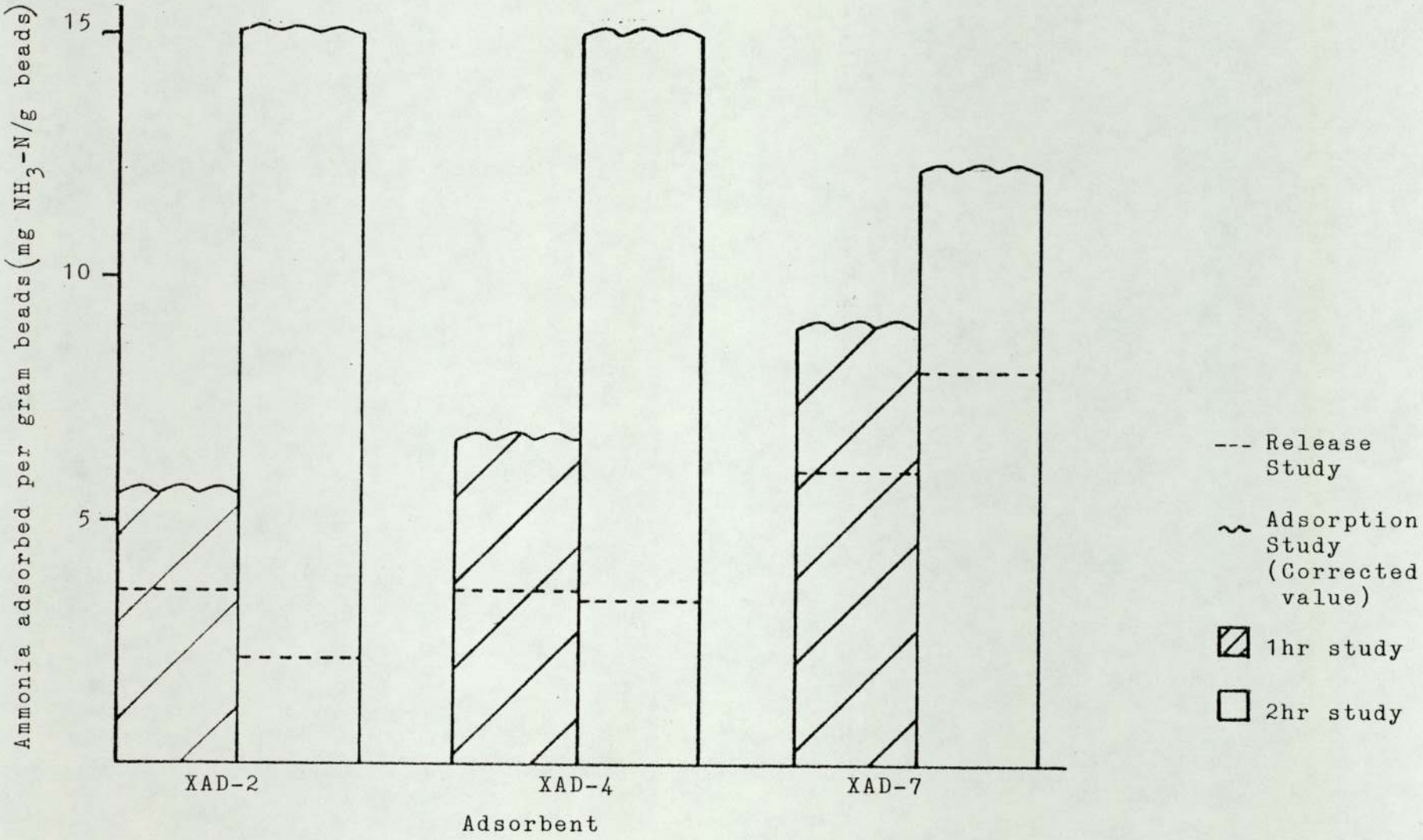
Study / Adsorbent	ONE HOUR STUDY			TWO HOUR STUDY		
	Adsorption Study		Desorption Study	Adsorption Study		Desorption study
	Ammonia Adsorbed mg NH_3-N/g beads		Ammonia Released mg NH_3-N/g beads	Ammonia Adsorbed mg NH_3-N/g beads		Ammonia Released mg NH_3-N/g beads
	Apparent	(Apparent + 1.5 release) Corrected		Apparent	(Apparent + 1.5 release) Corrected	
Amberlite XAD-2	0.2	5.6	3.6	12.5	15.0	2.2
Amberlite XAD-4	1.4	6.8	3.6	14.9	15.0	3.4
Amberlite XAD-7	0	9.0	6.0	0	12.2	8.1

face of the beads, with very few ammonium ions bound strongly to the inner surfaces. Thus the surface bound ammonia is easily desorbed.

The results for the investigation of polymeric uncharged Amberlite XAD resins are not very reliable, and the reproducibility is very low, due to the uncontrollable leaching effect observed with these resins. For this reason, the results for these adsorbents are therefore presented as histograms (Fig 5.3) with a 'wavy' horizontal line which indicates a degree of uncertainty of the results, and the fact that these values have been corrected, with the standard error of the mean being in the order of $\pm$ 1.5 mg NH_3-N. Results for desorption studies are presented as broken horizontal lines on the histograms. The polystyrene resins XAD-2 and XAD-4 have the tendency to produce slow leaching of impurities and do not reach equilibrium within one hour. However, there is a rapid increase in adsorption within two hours as indicated by the histogram (Fig 5.3). On the other hand the acrylic ester resin XAD-7 produces leaching of impurities very rapidly, to the extent that it appears that ammonia is not adsorbed at all. Corrected values are also used for this resin, and appear as a 'wavy' horizontal line on the histograms.

Fig 5.3 Adsorption of Ammonia by Neutral Uncharged Polymeric Adsorbents

Conditions: $[NH_4Cl]_o \equiv 15$ mg Nl^{-1}; 1g adsorbent; 1hr and 2hr static adsorption studies



5.4 Adsorption of Ammonia on Ion Exchange Resins (uncoated) :Static Adsorption Studies

It was felt that ion exchange resins would be useful adsorbent materials for observing the adsorption of ammonia. Since a large range of ion exchange resins is commercially available, comparisons can be made between the performance of the types selected. A study of this kind will provide information related to the effect the charge and functional groups of adsorbents have on their ability to adsorb ammonia.

One study employing a cation exchange resin for the treatment of experimental hyperammonaemia in dogs, had been performed at the onset of this project[64]. This technique had also been shown to be effective in the correction of hyperammonaemia in three patients. However, this method has not been tried by other workers and has not been properly evaluated[156].

From the range of ion exchange resins available, those used in this project included the basic (anionic) and acidic (cationic) types. These ion exchangers are examined for their ammonia adsorptive capacity. The static adsorption technique adopted is the same as that outlined in Chapter 3 and briefly involves shaking 5 mls of an NH_4Cl solution containing 15 mg NH_3-N/l with 1g adsorbent for periods of one hour and two hours, and analysing the supernatant liquid for ammoniacal nitrogen.

5.4.(1) Adsorption of Ammonia on a Basic (Anionic) Exchange Resin

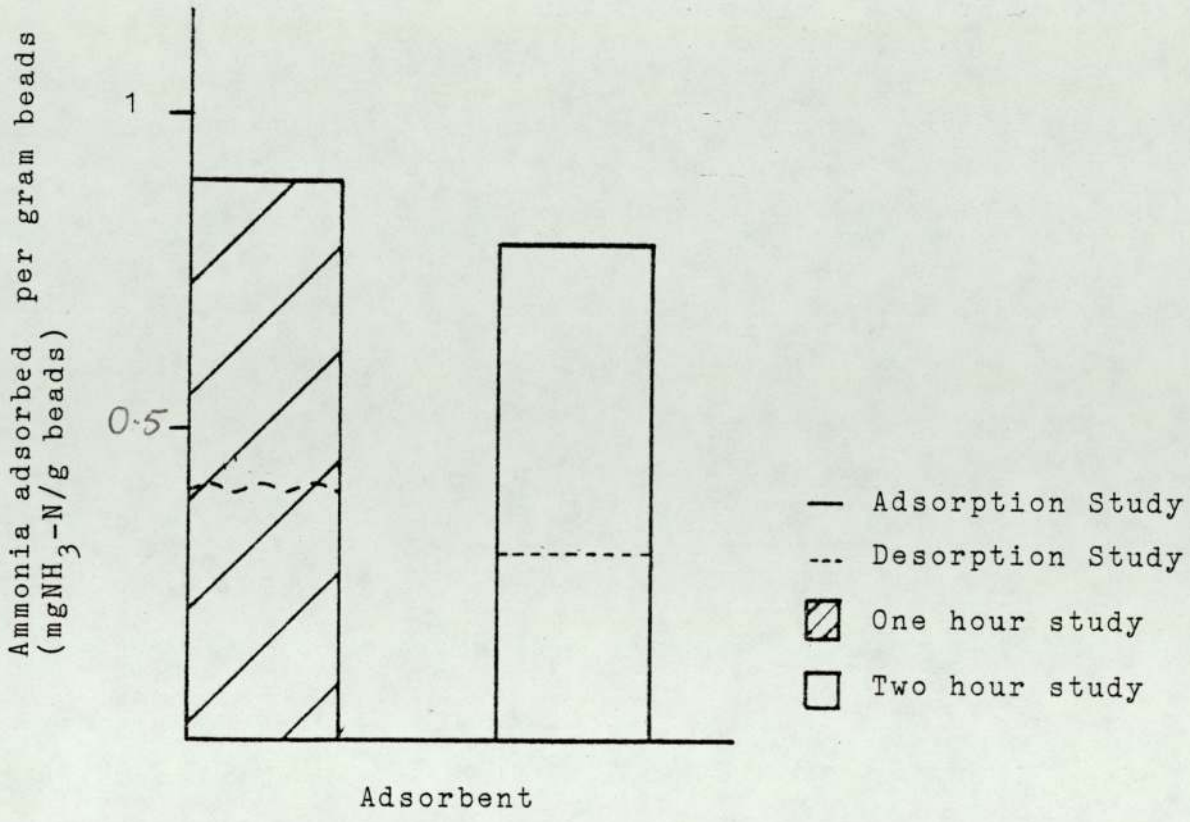


Fig 5.4 Adsorption of Ammonia by the Strongly Basic Ion Exchange Resin - Dowex 1-X4

Conditions: $[NH_4Cl]_o \equiv 15mg\ Nl^{-1}$; 1 hour and 2 hour static adsorption study; 1g adsorbent

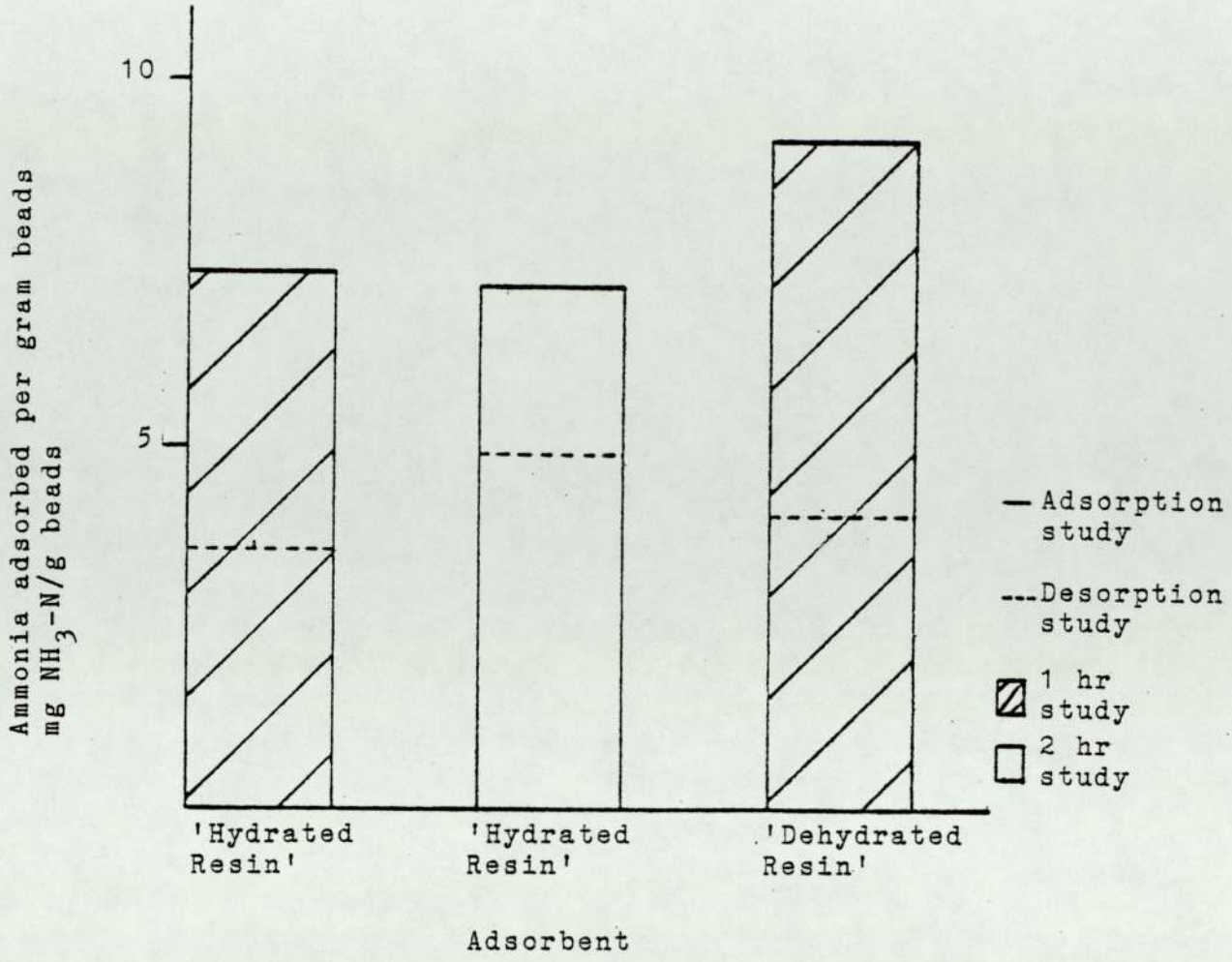
Under the conditions stated above, it was observed that 0.9 mg NH_3-N/g beads are adsorbed from an NH_4Cl solution containing 15 mg NH_3-N/l in one hour, and 0.8 mg NH_3-N/g beads in two hours. These results are shown in Fig 5.4. If the beads are not thoroughly washed

before use, as in the case of the one hour study, the release of adsorbed ammonia in subsequent desorption studies is apparently greater than that adsorbed. This is due to the leaching out of extracts of the resin structure, which is obviously an undesirable factor. The concentration of ammonia released in the one hour desorption study is therefore presented as a 'wavy' horizontal broken line and represents the best corrected result for the desorption study. Desorption studies performed for two hours show that this leaching tendency is effectively reduced by washing. In this case 0.3 mg NH_3-N/l is later released in two hour desorption studies - represented by a broken horizontal line in the histogram. However, in terms of adsorption capacity, this anionic exchange resin is not suitable for ammonia adsorption. Furthermore, the ease of desorption of the adsorbed ammonia (about 50%) is also undesirable, as is the leaching of components of the resin. Nevertheless, in contrast to the neutral uncharged polymeric resins, which also leach extracts, this anionic exchanger yields more reproducible results, with regard to the fact that equilibrium (although low) is reached in one hour.

5.4.(2) Adsorption of Ammonia on Weakly Acidic (Cationic) Exchange Resins

Using static adsorption conditions, the weakly acidic cation exchange resin Amberlite IRC-50(H^+) in the 'hydrated' state, was shaken for one hour and two hours, with 5 mls NH_4Cl solution, containing 15 mg NH_3-N/l. This adsorbent was shown to adsorb ammonia, such that 7.4 mg NH_3-N were adsorbed per gram beads in one hour and 7.2 mg NH_3-N/g beads in two hours. These results are presented in the form of a histogram in Fig 5.5. Subsequent one hour and two hour studies of this resin later release 3.6 and 4.9 mg NH_3-N/g beads respectively. Studies with the 'dehydrated' species of this resin,on the other hand, showed that a larger quantity of 9.2 mg NH_3-N/g beads were adsorbed in one hour and 3.9 mg NH_3-N/g beads released in desorption studies. Equilibrium adsorption for this resin is achieved within one hour and there is no significant difference in the adsorption capacity of the hydrated beads in one hour, compared to a two hour study.

The ammonia adsorptive capacity of this resin is adequate for reducing hyperammonaemic levels encountered in liver failure, but the observation that the adsorbed ammonia is easily released is undesirable. Nevertheless the results of this study will provide a basis for future discussion and comparison with the static adsorption studies of the hydrogels, in a subsequent section.



Conditions: $[NH_4Cl] \equiv 15mg\ Nl^{-1}$; 1 hr and 2 hr static adsorption study; 1g adsorbent

Fig 5.5 Adsorption of Ammonia by the Weakly Acidic Cation Exchange Resin, Amberlite 1RC-50(H^+)

Although dehydrated Amberlite IRC-50 resin beads adsorb a greater quantity of ammonia, the use of the dehydrated species of other adsorbents will be discontinued in further studies. This is because dry beads re-swell very rapidly when placed in aqueous solutions, thus causing severe strains which may lead to bead fracture or breakage.

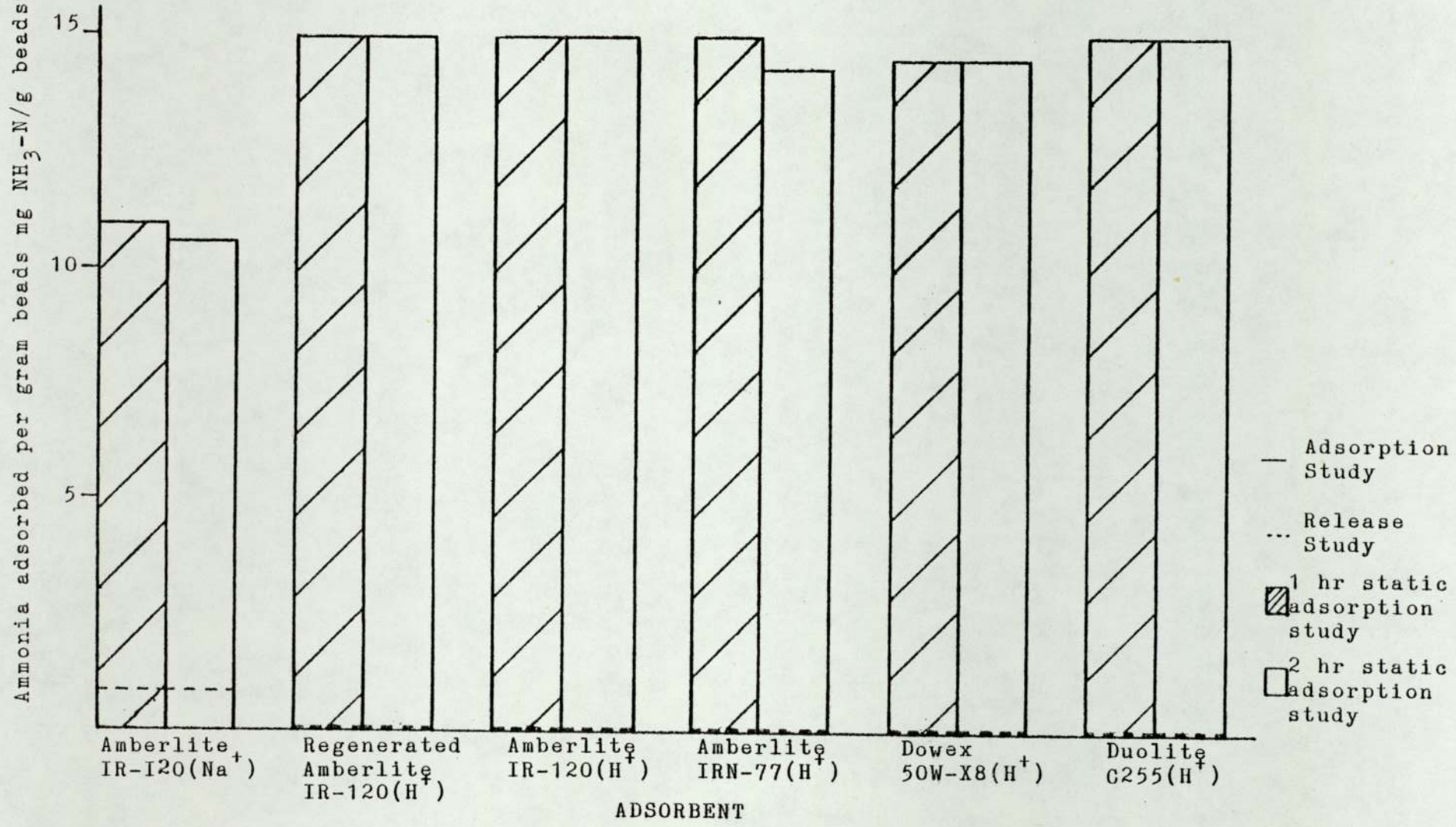
5.4.(3) Adsorption of Ammonia on Strongly Acidic (Cationic) Exchange Resins

Strongly acidic cationic exchange resins such as Amberlite IR-120 (Na^+ and H^+ form), Amberlite IRN-77(H^+), Dowex 50W-X8 (H^+) and Duolite C255 (H^+) were employed in this study. Amberlite IR-120 (Na^+) was also regenerated to the hydrogen form and used in this study. The regeneration procedure is outlined in Ref 152, 154. These resins all contain sulphonic acid functionality on a polystyrene divinylbenzene matrix. Their structure and properties have been detailed in Chapter 3.

Using the static adsorption technique outlined for the other adsorbents, it was observed that these ion exchange resins adsorb 14.0 to 15.0 mg NH_3-N/g beads from an NH_4Cl solution, containing 15 mg NH_3-N/l, in one hour and two hour static adsorption studies respectively. These results are shown in Fig 5.6 in histogram form.

Fig 5.6 Adsorption of Ammonia by Strongly Acidic Cation Exchange Resins

Conditions: $[NH_4Cl]_o \equiv 15mg\ Nl^{-1}$, 1 hr and 2 hr static adsorption study, 1g Adsorbent



It is evident from the results that the large ammonia adsorptive capacity is due to the acid function of the active group. The sodium form of Amberlite IR-120 (Na^+) exhibits a reduced ammonia adsorptive capacity with 11.0 mg NH_3-N/g beads being adsorbed, compared to an adsorption of 15 mg NH_3-N/g beads, for its' acid counterpart - Amberlite IR-120(H^+). From the desorption studies it is apparent that the strength of the adsorptive bonds of these resins for ammonia, is very powerful. During the one hour and two hour desorption studies the adsorbed ammonia remained bound, because of the acid function (H^+) of the resins. In the case where the sodium form of Amberlite IR-120(Na^+) resin is used, these bonds are only very slightly weaker and 0.9 mg NH_3-N/g beads is released after one hour and two hour desorption studies. These results are presented in Fig 5.6 with values for adsorbed ammonia represented by solid horizontal lines in the histograms and values for desorption studies as broken horizontal lines.

Equilibrium adsorption occurs very rapidly for these ion exchange resins. Previous studies involving Amberlite IR-120(Na) and Dowex 50W-X8(H^+) however did show that equilibrium adsorption is already reached within a ten minute contact time with 14.0 and 14.9 mg NH_3-N/g beads being adsorbed respectively by these resins. However,

to be able to compare the adsorptive capacity of these resins with other adsorbents, standard one hour and two hour static adsorption periods were maintained.

5.5 Adsorption of Ammonia on Coated Adsorbents : Static Adsorption Studies

The use of uncoated adsorbents in haemoperfusion systems is an accepted and widely used technique for the treatment of severe drug intoxication[22,53,59,61,62,186-191]. In this case the most critical aspect is to reduce the overdose in a relatively otherwise healthy patient. The use of uncoated adsorbents in haemoperfusion systems, however, is rarely practised in liver failure , because this leads to problems in biocompatibility and further complications in the state of the patient (as previously detailed in Chapter 4). It is important, therefore, to distinguish between cases of intoxication and liver failure in the practical sense. This thesis is concerned with artificial liver support for patients in liver failure.

It is known that the use of uncoated ion exchange resins as adsorbents also interferes with the electrolyte balance of the patient. Thus is was felt that the use of the neutral uncharged polymeric resins (which have negligible effects of this sort) as adsorbent materials

for ammonia, would be a valuable study in this project.

5.5.(1) Polymeric Uncharged Resins

Experiences with Amberlite XAD-2, 4 and 7 in this study have already shown that whilst uncoated XAD-2 and 4 may be efficient for ammonia adsorption, XAD-7 is unsuitable due to the 'leaching-out' of impurities observed and detailed in section 5.3. However, research at King's College Hospital, London[150,151] has centred on the use of this particular resin for clinical haemoperfusions, where bilirubin and bild acids (e.g. chenodeoxycholic acid) are successfully adsorbed. Before use, this resin is coated with the protein human serum albumin, abbreviated to HSA. The nature, structure and properties of this adsorbent material were discussed in Chapter 4. This project uses HSA coated Amberlite XAD-7 resin and observes its' ammonia adsorptive capacity over a one hour static adsorption period, using the same conditions outlined previously (i.e. 1g adsorbent, 5 ml NH_4Cl solution containing 15 mg NH_3-N/l). Experiences in this project with the biocompatible resin show that 10 mg NH_3-N/g beads are adsorbed after one hour static adsorption studies. The results are presented in Fig 5.7. One hour static adsorption studies were employed for this adsorbent as this was a realistic time for clinical situ-

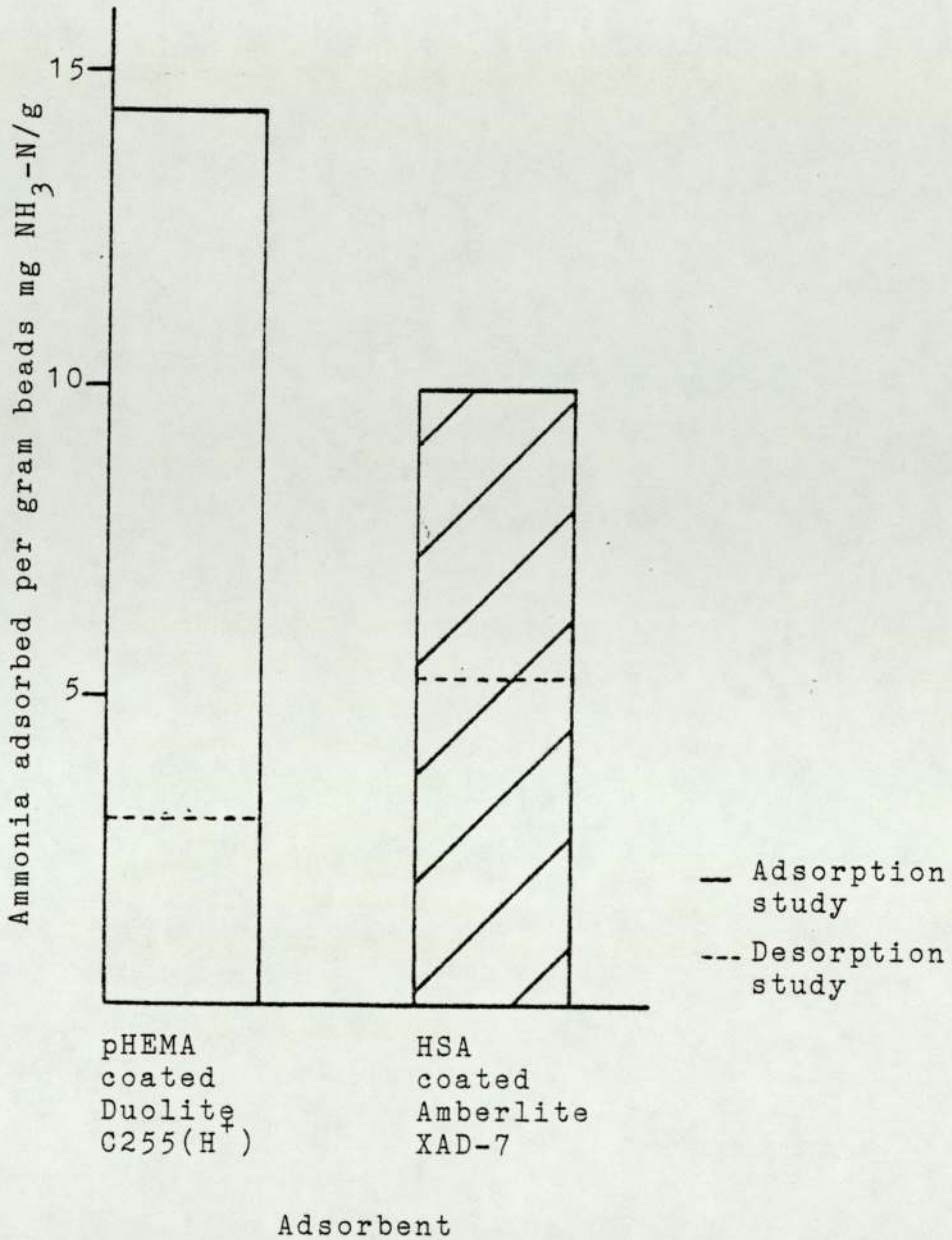


Fig 5.7 Adsorption of Ammonia on Biocompatible Coated Adsorbents

Conditions: $[NH_4Cl]_o \equiv 15 mgNl^{-1}$; 1 hr static adsorption study; 1g adsorbent

ations. Adsorption obtained with the coated Amberlite XAD-7 resin in this case is a property of the protein albumin encapsulation, since the uncoated species did not adsorb ammonia.

Amberlite XAD-7 resin has a high affinity for HSA which migrates into the inner pores of the macroporous resin, thus the great adsorptive capacity occurs because HSA adsorbs ammonia internally as well as externally, i.e. on the surface of the resin. However, the binding of ammonia to HSA can be said to be relatively weak because 5.3 mg NH_3-N/g beads (just over 50% of the adsorbed ammonia) is later released in one hour desorption studies.

5.5.(2) Ion Exchange Resins

The uncoated ion exchange resins, already used in static adsorption studies in this project, could never be used without a biocompatible coat in principle, and so in this study a biocompatible coat of poly 2-hydroxy-ethylmethacrylate or polyHEMA is applied to the resin before use. (The preparation and application of this coating material have been detailed in Chapter 4.2). However, the problem of biocompatibility is not of immediate interest at this stage, although it was considered. But, it is appropriate at this point to demonstrate whether the polyHEMA membrane behaves as predicted

on the basis of size and solubility of ammonia, as compared to that for oxygen, which had been previously evaluated on this hydrogel material by other workers in this laboratory[192-195].

Research concerning the transport of ammonia through polyHEMA membranes has not been recorded in the literature, and this work has not been performed by other workers. It has been demonstrated, however, from the earlier work on coated activated charcoal in this project, that polyHEMA does bind ammonia. Having thus studied the effect of polyHEMA on activated charcoal, (which is considered as a 'low-ammonia', or more correctly a 'non-ammonia' adsorbing material), a comparable study is performed with adsorbents having high ammonia adsorptive capacities. The use of the strongly acidic cation exchangers (which are very good at adsorbing ammonia) for encapsulation, will thus provide the most rigorous conditions under which the polyHEMA membrane properties can be demonstrated and evaluated.

The undesirable adsorption of electrolytes by these resins which apparently accompanies adsorption in haemoperfusion systems, can be easily corrected by the use of the Na, K, Ca, Mg forms of the resin, or by replenishing the electrolyte balance on perfusion[62,64,156]. For this reason, the ionic balance is not taken as a serious pro-

blem for immediate investigation in this project.

Because there is no relevance in coating the whole range of ion exchange resins used previously in this project and then investigating each one individually, only one cation exchange resin is chosen, for the application of the biocompatible polyHEMA coat. This resin (Duolite C255(H^+) is selected from the preliminary observations of the static adsorption studies, as being the 'cleanest' ion exchange resin, possessing a high ammonia adsorptive capacity. It is coated with polyHEMA by the technique previously outlined in Chapter 4.2 to produce a thin (1-3μm) uniform coat of 5% polyHEMA, and then used in one hour static adsorption studies following the same procedure and conditions used for uncoated adsorbents.

By comparison of the results obtained for the coated ion exchange resin (Duolite C255(H^+) with that of the HSA-coated polymeric uncharged resin Amberlite XAD-7, it is seen that polyHEMA coated Duolite C255(H^+) resin possesses a larger ammonia adsorptive capacity. These results are presented as histograms in Fig 5.7. This study demonstrates that ammonia adsorption by the ion exchange resin, is not hindered by the polyHEMA coat. However, the ease of release of the adsorbed ammonia, suggests weak non-specific binding of polyHEMA with ammonia.

5.6 Adsorption of Ammonia on Uncoated and Coated Ion Exchange Resins : Dynamic Adsorption Studies

Having observed the adsorptive capacity of uncoated and coated ion exchange resins for ammonia under static adsorption conditions, the rate of ammonia adsorption for these ion exchange resins was examined under dynamic adsorption conditions. To determine whether the transport properties of polyHEMA affect the ion exchange resin's ability to adsorb ammonia, similar dynamic adsorption studies were performed, using polyHEMA coated strongly acidic cation exchange resins. The procedure for the dynamic adsorption study is detailed in Chapter 3, but briefly involves placing the adsorbent into the perfusion cell and allowing the NH_4Cl solution containing 10 or 15 mg NH_3-N/l in the reservoir to fill the perfusion cell at its' own rate. The flow rate is then regulated when the perfusate begins to flow through the outlet tube, by means of a stop-cock at this exit. Perfusion is continued for the desired time, and analysis of liquid fractions are performed immediately for the determination of ammoniacal-nitrogen. The results obtained are expressed in graph-form, by plotting the midway point between two points, against sample numbers[196]. A smooth curve is then drawn as far as possible through the midpoints, to give an adsorption curve.

5.6.(1) Weakly Acidic (Cationic) Exchange Resin

Dynamic adsorption studies using Amberlite IRC-50 (H^+) were conducted at various flow rates to determine whether the flow rate of the perfusate had any effects on the ion exchange capacity or efficiency of the resin. The observations are presented in graph form in Figs 5.8 and 5.9, and show that flow rate does affect the capacity of the resin to adsorb ammonia to some extent. High flow rates cause higher leakage of the toxin being removed from solution and reduce the operating capacity. Thus, a slow flow rate was more efficient because saturation or equilibrium adsorption was achieved later than if a faster flow rate was used. Some quantitative illustration of this is provided by Fig 5.10, which shows the efficiency of ammonia removal at a given (30th) fraction number, as a function of flow rate. The initial state of the beads (i.e. hydrated or dehydrated) was found to have no significant effects on the performance of the resin. The cumulative graph of ammonia adsorbed by Amberlite IRC-50(H^+) resin at different flow rates is presented in Fig 5.11.

As a result of the dynamic studies in this section, (which provide a good basis for comparison with hydrogels) a flow rate of 2 mls/min is chosen as being the most suitable for subsequent studies. This flow rate is within the limitations of space velocities employed in this project,

Fig 5.8 ADSORPTION OF AMMONIA ON WEAKLY ACIDIC (CATIONIC) EXCHANGE RESIN - AMBERLITE IRC-50 (H^+) (HYDRATED BEADS)

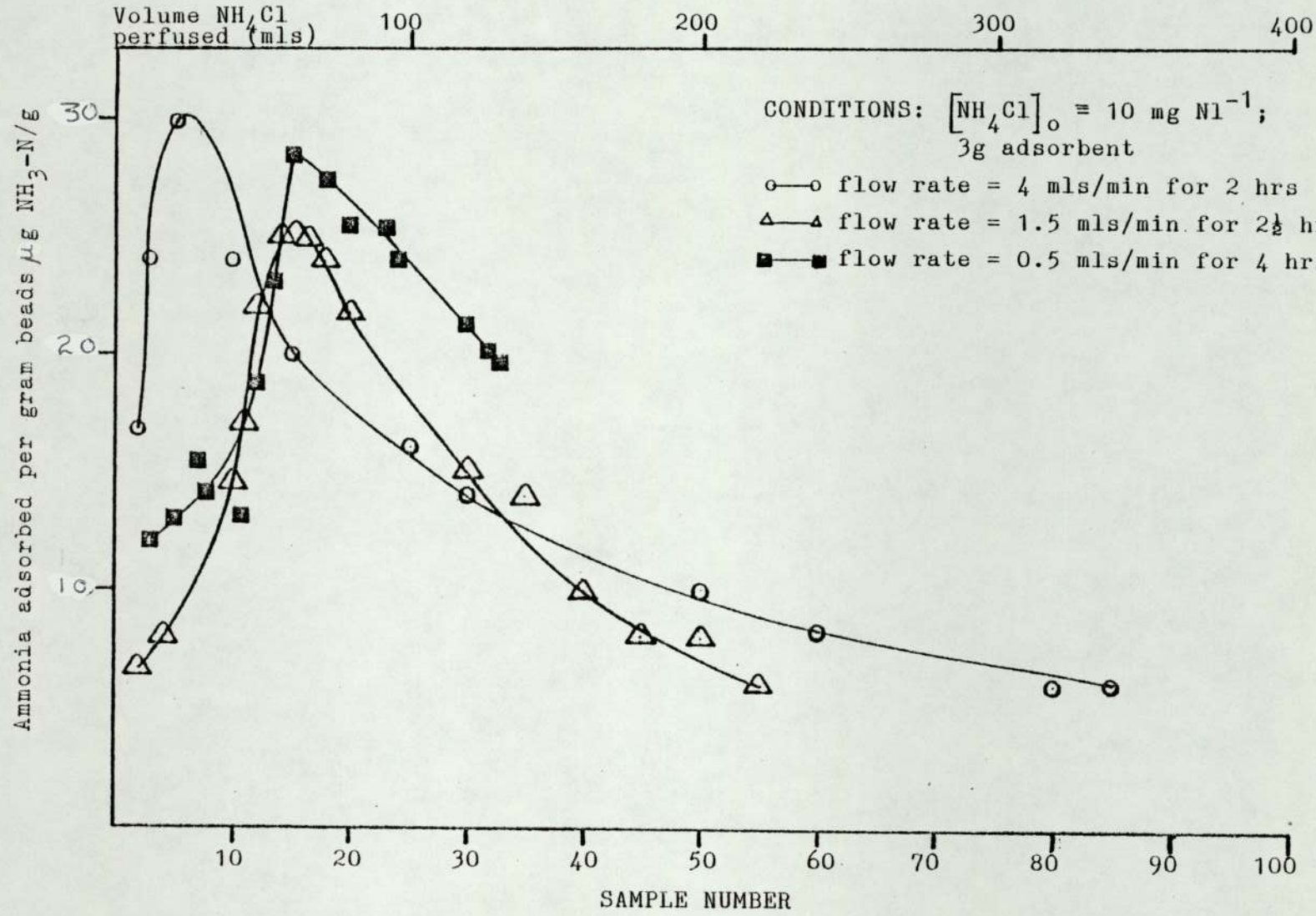


Fig 5.9 ADSORPTION OF AMMONIA ON WEAKLY ACIDIC (CATIONIC) EXCHANGE RESIN - AMBERLITE IRC-50 (H^+) (DEHYDRATED BEADS)

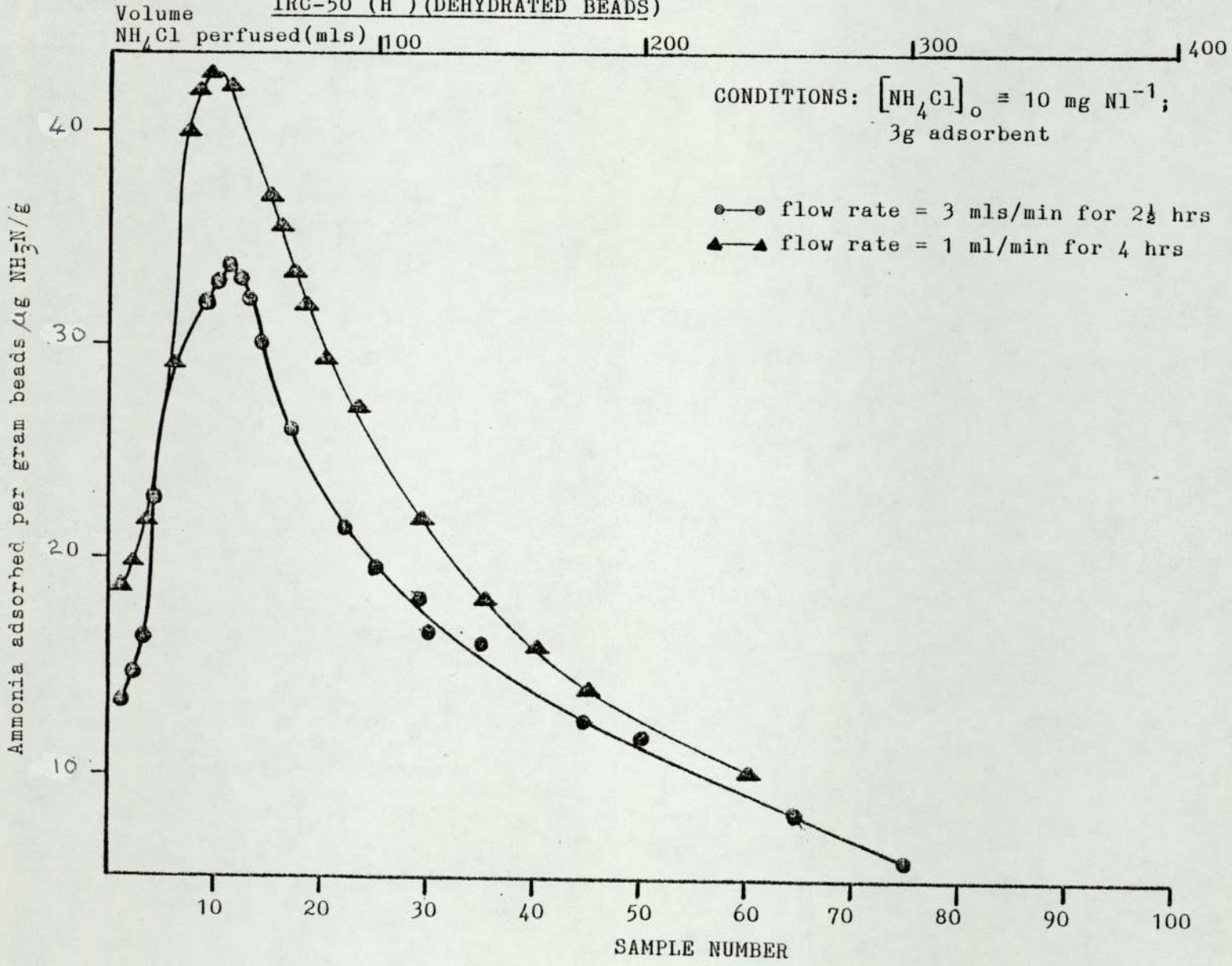


FIG 5.10 ADSORPTION OF AMMONIA ON THE WEAKLY ACIDIC CATION EXCHANGE RESIN - AMBERLITE IRC-50 (H^+),(AT THE 30th FRACTION NUMBER)

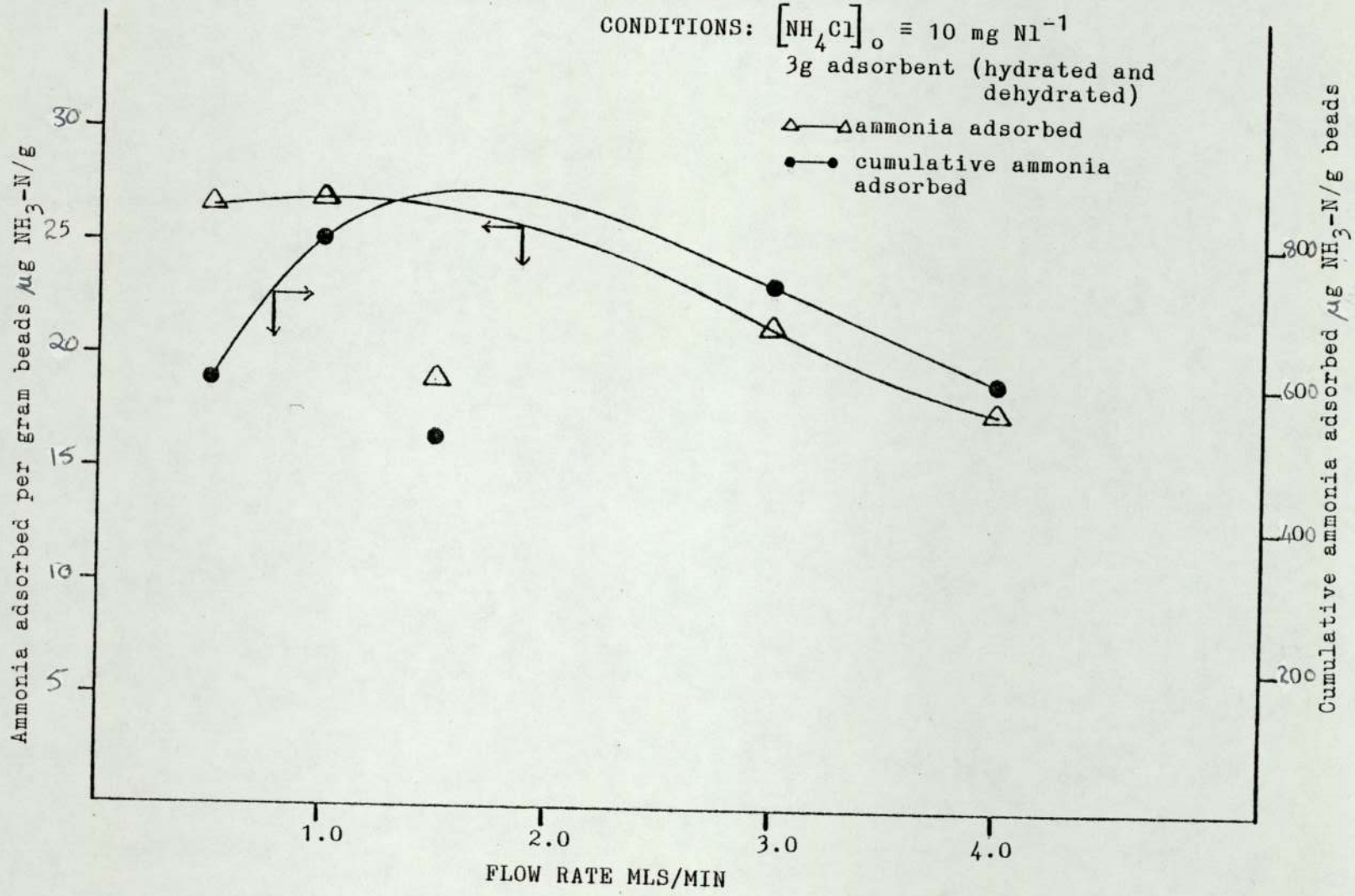
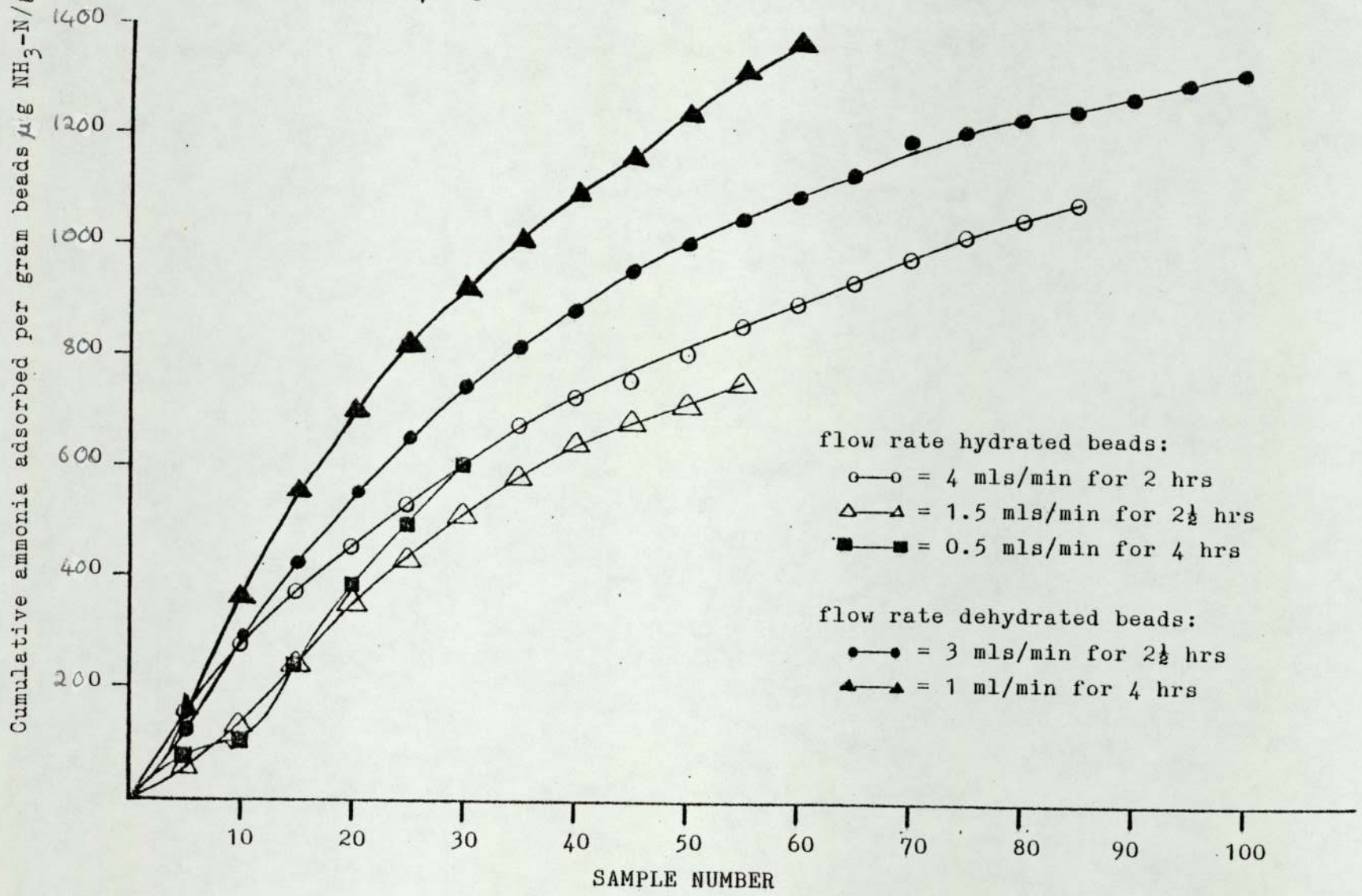


Fig 5.11 CUMULATIVE ADSORPTION OF AMMONIA ON THE WEAKLY ACIDIC (CATIONIC) EXCHANGE RESIN - AMBERLITE IRC-50 (H^+)

CONDITIONS: $[NH_4Cl]_o \equiv 10$ mg Nl^{-1}; 3g adsorbent;



and in this particular study gave representative results and did not produce extremes of behaviour.

5.6.(2) Strongly Acidic (Cationic) Exchange Resins

Dynamic adsorption studies performed using the strongly acidic ion exchangers show that their capacity and rate of ammonia adsorption are very high. Also their ability to adsorb ammonia continues for long periods (up to four days in some cases) as shown in Figs 5.12, 5.14 and 5.16, and thus saturation of the resin adsorptive sites is still not attained. The total amount of ammonia adsorbed is illustrated in the cumulative adsorption graphs for these adsorbents, also shown in Figs 5.12, 5.14 and 5.16. The long duration of adsorption can be said to be a property of the strong ionic binding of the ammonium ions to the fixed ionic groups of the resin structure and the availability of these sites. The graphs obtained for the different ion exchange resins examined e.g. Amberlite IR-120(Na^+), Dowex 50W-X8(H^+) and Duolite C255(H^+), all follow a similar pattern.

In order to study the feasibility of using polyHEMA as a biocompatible coating in conditions where its' ability to transport ammonia is important, studies with these strongly adsorbing resins in the coated state were performed. The coating technique is that described in

Chapter 4. The dynamic adsorption study demonstrated that the efficiency, rate and capacity of ammonia adsorption by these ion exchange resins are not hindered in any way by the polyHEMA coating. Thus, the graphs obtained for coated ion exchange resins are similar to that for the materials in their uncoated state. The results of that study are presented in Figs 5.13, 5.15 and 5.17, together with the cumulative ammonia adsorption by the resins, which demonstrate their infinite capacity to adsorb ammonia.

Ammonia adsorption by the coated resin is limited by the solubility of ammonia in water, membrane diffusion resistance and the transport of ammonia in the membrane to the resin. These factors, however, did not impede the adsorption of ammonia and steady state adsorption conditions were achieved within the dead-space volume observed in Chapter 3.

The above observations have important consequences for two distinct, but related areas. The first, for the design of particulate hydrogel adsorbents, the second for the design of membranes for semi-conductor-based ammonia sensors. The semi-conductor-based sensor study is detailed in Chapter 7, and that of particulate hydrogel adsorption in the following section.

Fig 5·14

ADSORPTION OF AMMONIA ON DOWEX 50W-X8 (H^+) RESIN

CONDITIONS: $[NH_4Cl]_o \equiv 15$ mg Nl^{-1}; 5g adsorbent; flow rate = 2 mls/min for $\simeq$ 4 days;

—— ammonia adsorbed; --- cumulative ammonia adsorbed

Fig 5·15

ADSORPTION OF AMMONIA ON POLYHEMA COATED DOWEX 50W-X8 (H^+) RESIN

CONDITIONS: $[NH_4Cl]_o \equiv 15$ mg Nl^{-1}; 5g adsorbent; flow rate = 2 mls/min for $\simeq$ 3 days;

—— ammonia adsorbed; --- cumulative ammonia adsorbed

Fig 5.14

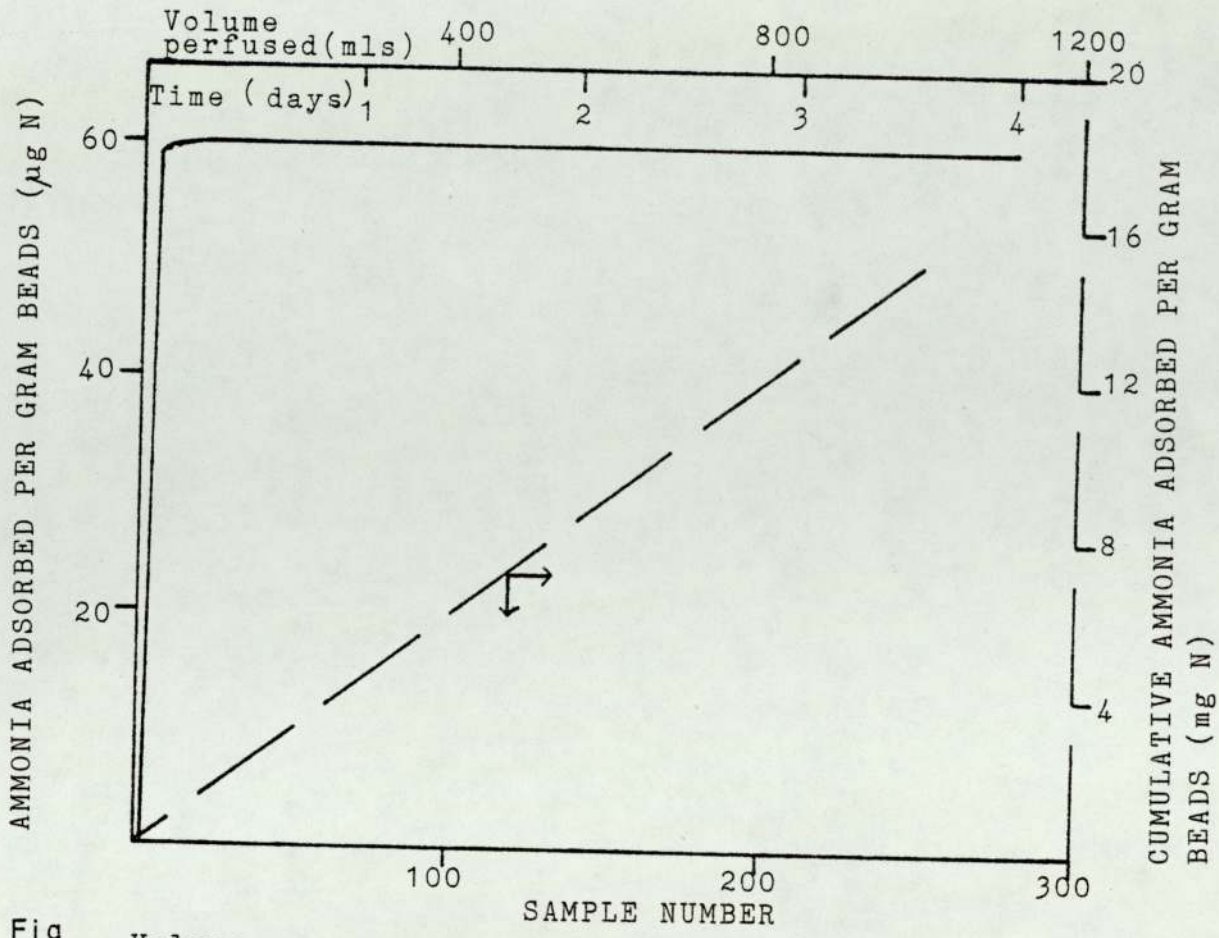


Fig 5.15

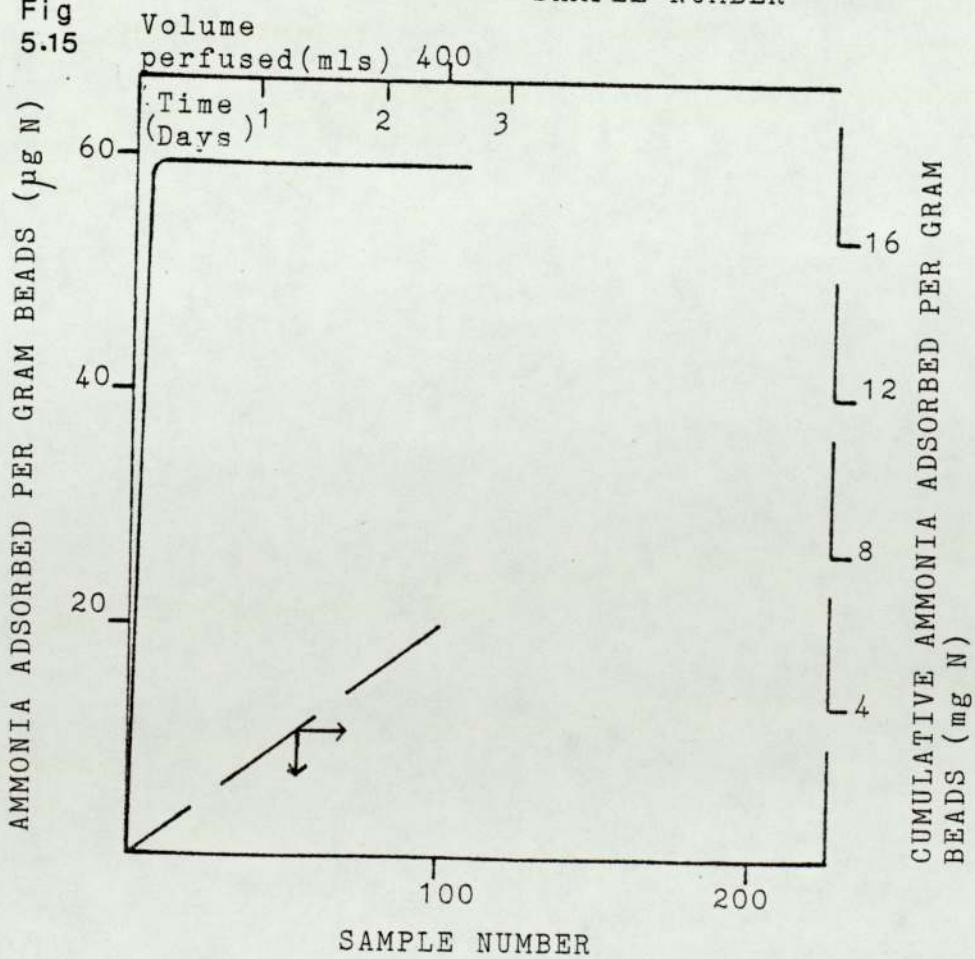


FIG 5·16

AMMONIA ADSORBED ON DUOLITE C255 (H^+) RESIN

CONDITIONS: $[NH_4Cl]_o \equiv 15$ mg Nl^{-1}; 5g adsorbent;
flow rate = 3 mls/min for $\simeq$ 4 days;

—— ammonia adsorbed; --- cumulative ammonia adsorbed

FIG 5·17

AMMONIA ADSORBED ON POLYHEMA COATED DUOLITE C255 (H^+) RESIN

CONDITIONS: $[NH_4Cl]_o \equiv 15$ mg Nl^{-1}; 5g adsorbent;
flow rate = 3 mls/min for $2\frac{1}{2}$ days;

—— ammonia adsorbed; --- cumulative ammonia adsorbed

Fig 5.16

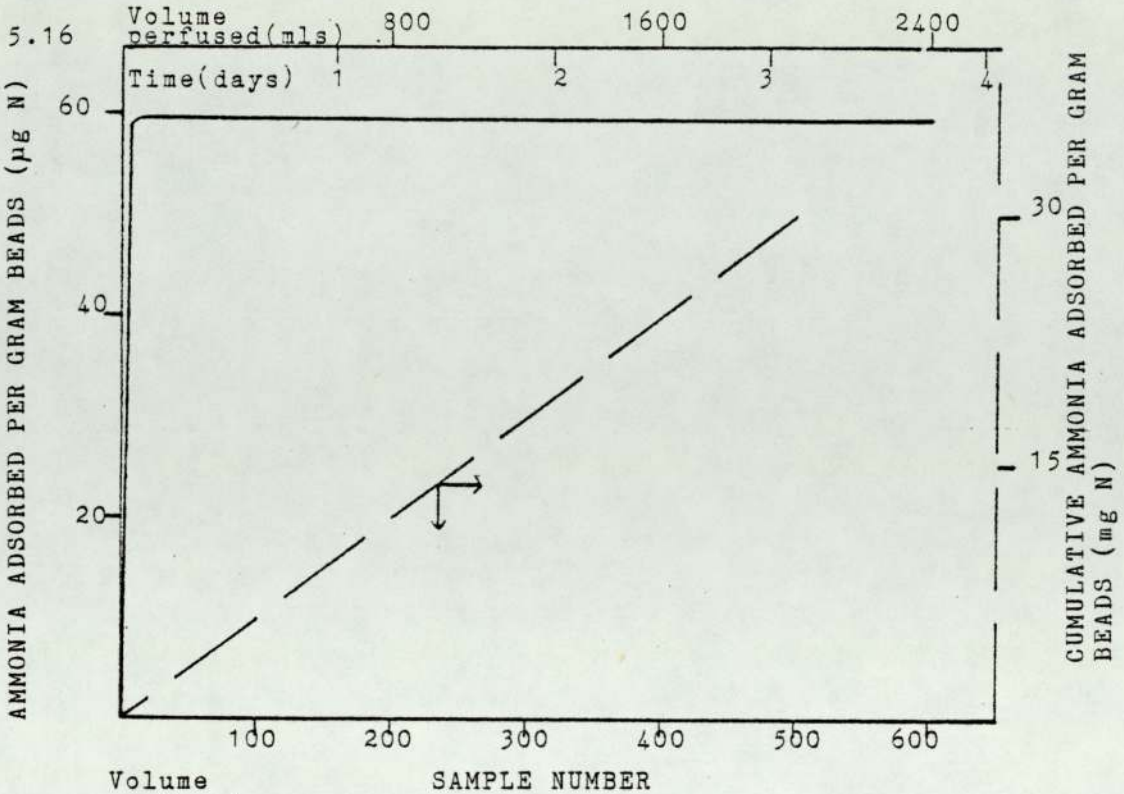
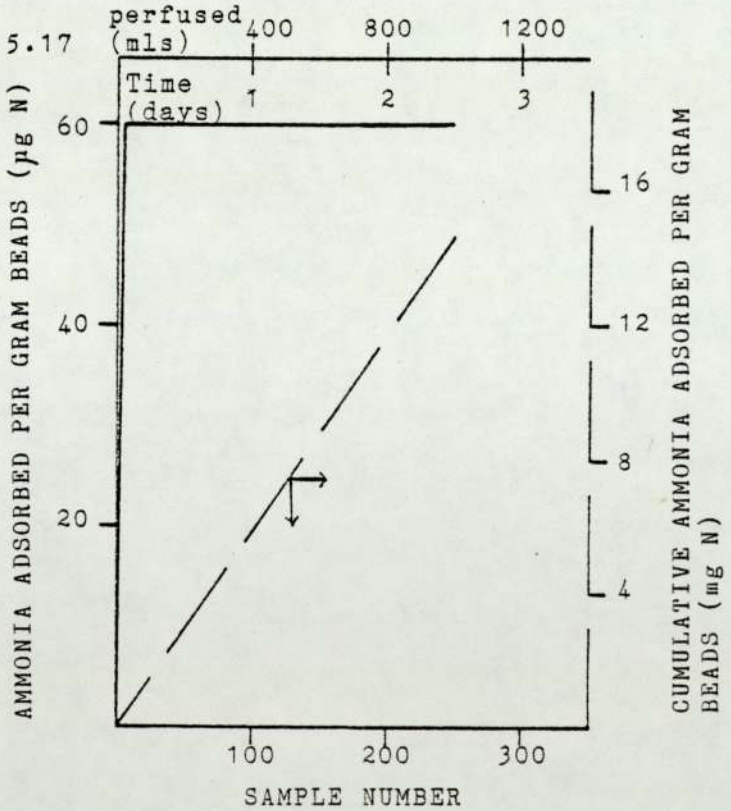


Fig 5.17



5.7 Adsorption of Ammonia on Particulate Hydrogels

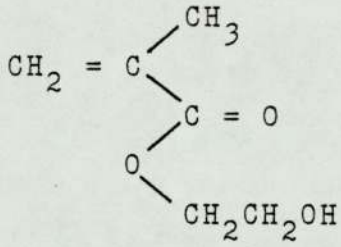
An 'off-the-shelf' artificial liver support system is not being sought in this project, as one major aim is to provide a new system based on novel adsorbents, using the observations from the study of ammonia adsorption on the commercially available adsorbents. By combining these observations it is felt that an adsorbent containing the hydrogel polyHEMA for biocompatibility and acidic functional groups for ammonia adsorption, would be efficient. Such polyHEMA based macroporous hydrogel adsorbents were synthesised in a separate project.

Available information concerning the clinical evaluation of these hydrogels as regards their biocompatibility shows that these hydrogels do meet the stringent biocompatibility requirements[145,197]. The spectrum of hydrogels investigated in this study is shown in Tables 5.2 — 5.4 in the appropriate sections. The monomers used to manufacture the synthetic hydrogels will now be presented.

Monomers Used to Manufacture Synthetic Hydrogels by Polymerisation

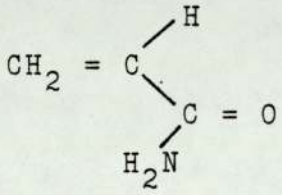
A wide variety of monomers have been used in making (non-ionic) hydrogels, by polymerisation of hydrophilic monomers. Some examples are given below:

i) Hydroxyethylmethacrylate



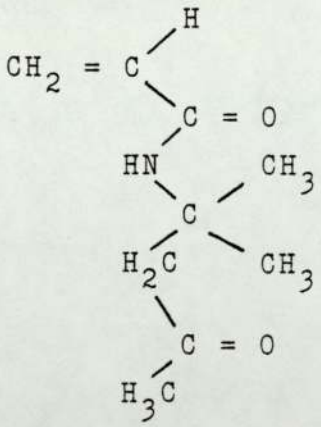
HEMA

ii) Acrylamide



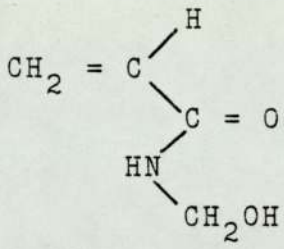
ACR

iii) Diacetone acrylamide (substitution of an amide hydrogen by a hydrophobic group)



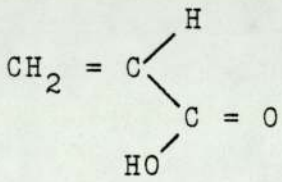
DAA

iv) N-Methylol acrylamide

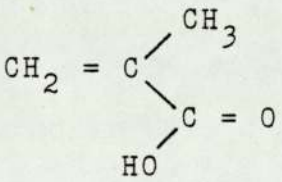


NMACR

In addition, acidic, cation-exchange ability is conferred on a hydrogel by copolymerisation with acidic monomers such as acrylic acid and methacrylic acid.

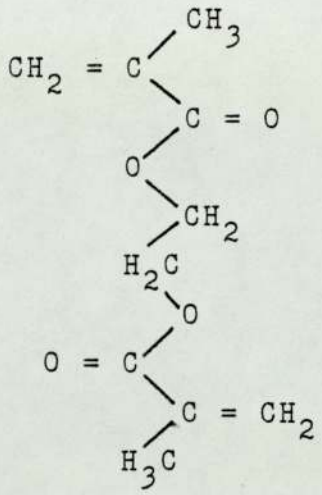


AA

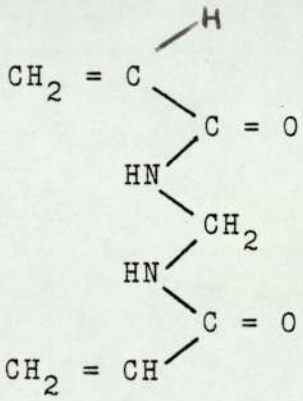


MAA

Also needed are cross-linking agents to give the structure the required integrity. There are a wide variety of these having different reactivities, hydrophilicities and other properties. Two of the most important are ethylene dimethacrylate (EDM) and NN' methylene bis-acrylamide (NN'MBA). These were employed in this study.



EDM



NN'MBA

As can be seen, these monomers have two vinyl groups -  of identical reactivity, thus cross-linked structures are produced on polymerisation.

5.7.(1) Static Adsorption Studies

In this study macroporous hydrogel beads, synthesised by the Freeze-thaw technique and variations on the Freeze-thaw technique (which are described in Chapter 4) are evaluated for their ability to adsorb ammonia. For these particular materials, an initial assessment is carried out using a variation on the static technique used with the other adsorbents. Briefly, under static adsorption conditions, 1g of the hydrogel adsorbent is shaken with 5 mls NH_4Cl solution containing 20 mg NH_3-N/l for a period of time (the time is arbitrary, and is stated with each study). The liquid sample is 'decanted-off' for analysis and a fresh NH_4Cl sample of the same volume and concentration as previously used, is shaken with the hydrogel beads. Similarly, this sample is analysed after the shaking period, and the procedure continued until three or four such samples are collected for analysis. The hydrogel bead types used in this study are shown in Table 5.2.

The evaluation of these adsorbents was performed when the beads were made available, and there are slight variations in the conditions (with respect to the initial concentration of the ammonia solution employed). The results of this study are presented in the form of a graph of cumulative ammonia adsorption, in relation to time in Fig 5.18, and demonstrate that particulates of HEMA and AA do

Table 5.2 Macroporous Hydrogel Beads Used in Static Adsorption Study

Code Number	Composition	Effective size(mm)	Equilibrium Water Content(%)	Synthetic Technique
F1	HEMA/EDM (90:10)	2.0-5.0	44.5	Freeze-thaw
F2	AA/EDM (100:8.5)*	0.1-3.0	75*	Freeze-thaw
F3	AA/EDM (100:8.5)	0.1-1.0	76	Freeze-thaw (Variation)
F4	HEMA/EDM (100:10)	0.5-1.0	56	Freeze-thaw (Variation)

*some possible doubt exists as to the precise identity of this specimen.

adsorb ammonia. In comparison, the ammonia adsorptive capacity of the HEMA particulates of system F1 is superior to that of the AA particulates of system F2.

Fig 5.18 ADSORPTION OF AMMONIA ON MACROPOROUS PARTICULATE HYDROGELS

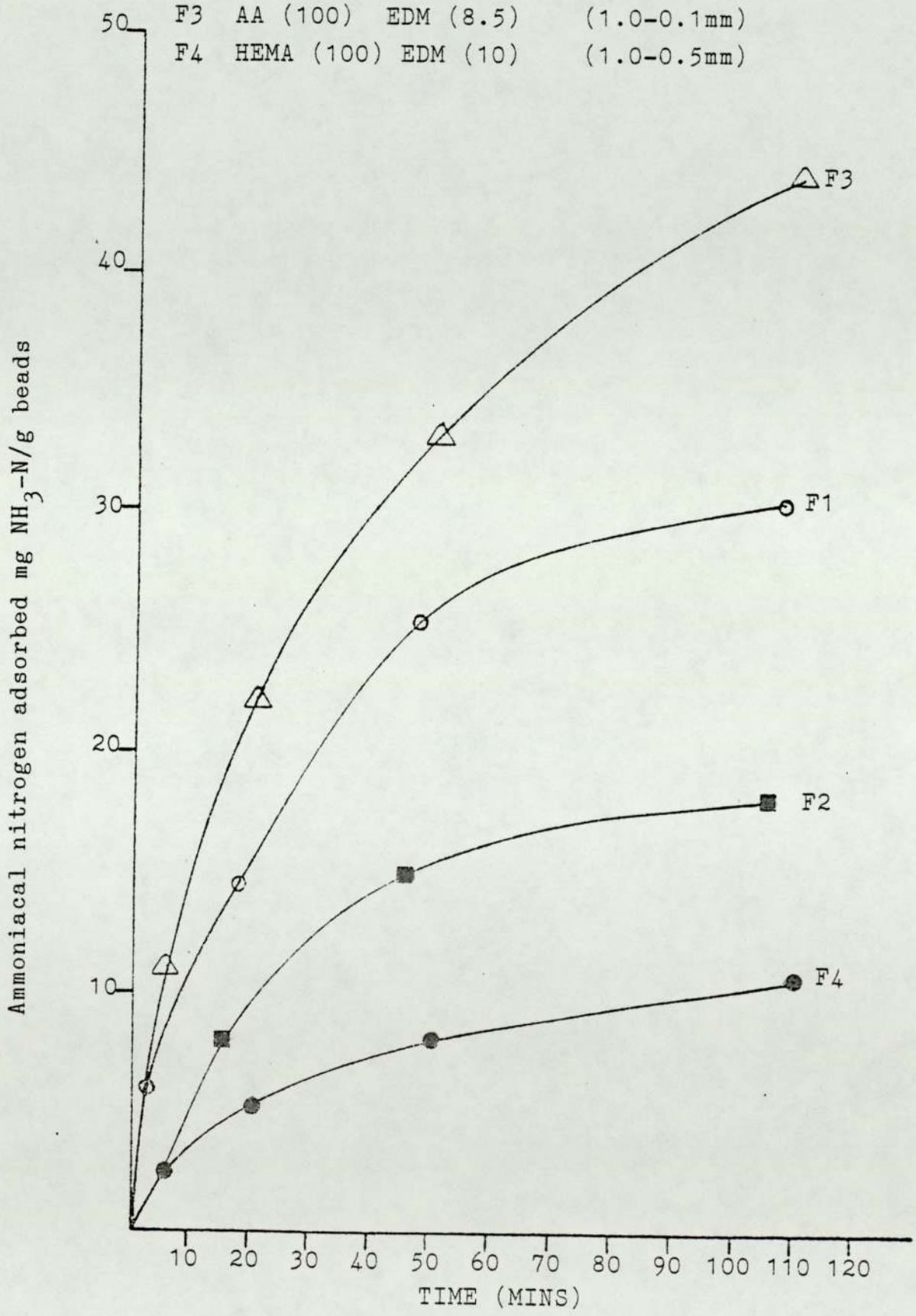
CONDITIONS: $[NH_4Cl]_o \equiv 20$ mg Nl^{-1}; 1g adsorbent

F1 HEMA (90) EDM (10) (5.0-2.0mm)

F2 AA (100) EDM (8.5) (3.0-0.1mm)

F3 AA (100) EDM (8.5) (1.0-0.1mm)

F4 HEMA (100) EDM (10) (1.0-0.5mm)



Particulates of acrylic acid (AA), possess an acidic cationic exchange ability. Such ion exchange ability has been observed to be particularly effective in attracting/ adsorbing ammonia in the study of the ion exchange resins. However, acrylic acid does not give rise to a very open macroporous pore structure and so there is little access to such ionic adsorption sites. Because the particulates of system F2 were of a broad size range 0.1 - 3.0 mm , ammonia adsorption in this case is due to the combination of the small particle size of some of the particulates (which confers a large surface area) and the availability of the acidic adsorptive sites, rather than the nature of the groups. Thus HEMA particulates of F1, although being a neutral and non-ionic hydrogel with a larger particle size and thus reduced surface area, adsorbs an appreciably larger quantity of ammonia than the acidic system F2, because of its' macroporous structure and chemical composition.

The fact that the surface area and particle size of an adsorbent is important in adsorption processes, is confirmed in the study of the acrylic acid system AA (100) . EDM (8·5) of F3. These particulates adsorb a quantity of ammonia far superior to that adsorbed by systems F1 and F2. The great adsorptive capacity of this particulate adsorbent in this case, is due to the small particle size of the beads. This confers, as stated previously, a large

surface area to the adsorbent, which in turn results in an increased adsorptive capacity of these particulates, (which are of the same composition as those of system F2). However, although a small particle size is important for increased adsorption, this is only so, provided that the surface area is great and easily available. This is confirmed by observations of the HEMA (100) EDM (10) system F4 of a small particle size and supposedly large surface area. Beads of this composition and a small surface area, i.e. system F1, were previously shown to possess a large ammonia adsorptive capacity. However, particulates of system F4 adsorbed very little ammonia, which suggests that in this case the available surface area was not easily accessible.

The ammonia adsorptive capacity of systems F1 and F3 is comparable to that observed with the weakly acidic cation exchange resin , Amberlite IRC-50. But the fact that the hydrogel systems are themselves biocompatible in nature, makes them more attractive adsorbents for possible use in artificial liver support systems.

In conjunction with these studies, macroporous hydrogel beads obtained from the spectrum of compounds synthesised by Freeze-thaw techniques, suspension polymerisation (in brine) and inverse suspension polymerisation (in hexane) were investigated for their ability to adsorb ammonia.

These particulates, shown in Table 5.3, were evaluated using the more conventional static adsorption methods used for the ion exchange resins. The technique is detailed in Chapter 3, but briefly, 1g of adsorbent is shaken with 5 ml NH_4Cl solution for one hour and the liquid samples are then analysed for unadsorbed ammonia. The observations of this study are presented in the form of histograms in Fig 5.19 and show the ammonia adsorbed per gram beads. (For comparison with the macroporous hydrogel systems, similar observations obtained with the clinically used HSA-XAD-7 adsorbent (i.e. Human Serum Albumin coated Amberlite XAD-7 resin) and polyHEMA coated activated charcoal, are also presented).

Investigations using these macroporous hydrogel particulates demonstrate that beads of the HEMA (40) AA (60) EDM (9·5) system S1, possess a large ammonia adsorptive capacity, comparable to that observed for the strongly acidic cation exchange resins. This adsorptive capacity is attributed to the presence of both HEMA and acrylic acid groups in the system which confers a different chemical structure and porosity to the particulates. The copolymerisation of the hydrogel HEMA with an acidic monomer such as acrylic acid confers an acidic cation exchange ability. This acidity, the small particle size of system S1 and also the porous surface of these particulates are all important factors for increasing the adsorption of

Table 5.3 Macroporous Hydrogel Beads Used in Static Adsorption Study

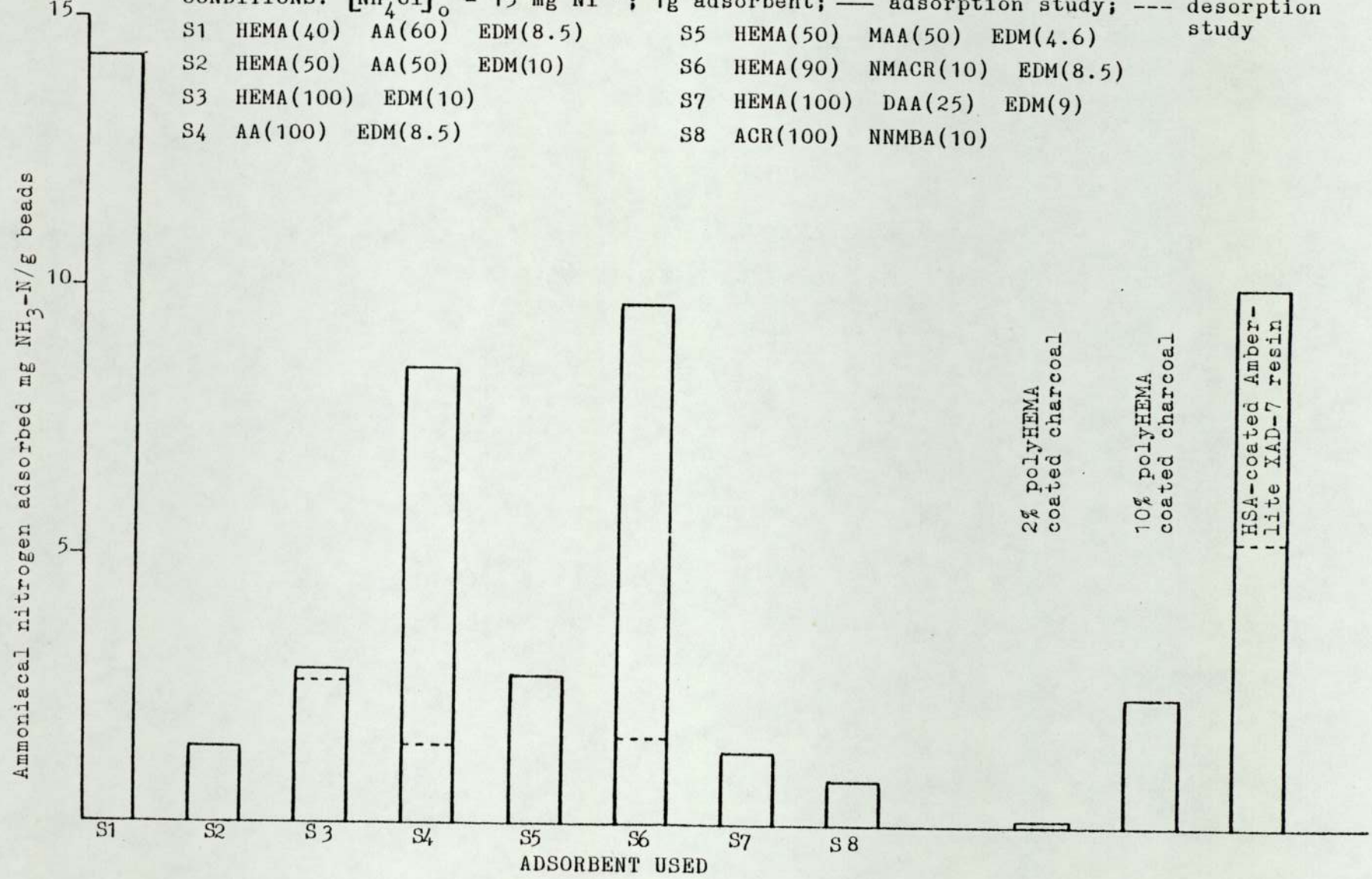
Code Number	Composition	Effective Size (mm)	Equilbrium Water Content E.W.C. (%)	Synthetic Technique
S1	HEMA/AA/EDM (40:60:8.5)	< 0.15	64	Suspension Polymerisation (brine)
S2	HEMA/AA/EDM (50:50:10)	1.0-4.0	57	Freeze-thaw
S3 (=F4, Table 5.2)	HEMA/EDM (100:10)	0.5-1.0	56	Suspension Polymerisation (brine)
S4 (=F2, Table 5.2)	AA/EDM (100:8.5)*	0.1-3.0	75*	Freeze-thaw
S5	HEMA/MAA/EDM (50:50:4.6)	0.025-0.125	58	Suspension Polymerisation (brine)
S6	HEMA/NMACR/EDM (90:10:8.5)	0.5-1.0	61	Suspension Polymerisation (brine)
S7	HEMA/DAA/EDM (100:25:9)	0.25-0.5	50	Suspension Polymerisation (brine)
S8	ACR/NN'MBA (100:10)	1.0-1.5	80	Inverse Suspension Polymerisation (hexane)

*some possible doubt exists as to the precise identity of this specimen

Fig 5.19 ADSORPTION OF AMMONIA ON MACROPOROUS PARTICULATE HYDROGELS AND COMPARISON MATERIALS

CONDITIONS: $[NH_4Cl]_o \equiv 15$ mg Nl^{-1}; 1g adsorbent; —— adsorption study; --- desorption study

S1 HEMA(40) AA(60) EDM(8.5)
S2 HEMA(50) AA(50) EDM(10)
S3 HEMA(100) EDM(10)
S4 AA(100) EDM(8.5)
S5 HEMA(50) MAA(50) EDM(4.6)
S6 HEMA(90) NMACR(10) EDM(8.5)
S7 HEMA(100) DAA(25) EDM(9)
S8 ACR(100) NNMBA(10)



ammonia.

The strength of binding of the adsorbed ammonia in the case of system S1 is investigated in desorption studies (which involve shaking the adsorbent with 5 ml distilled water in a manner similar to that employed in static adsorption studies). After a one hour desorption study, this system did not release its' adsorbed ammonia. Thus the HEMA:AA system binds very strongly to ammonia. The ammonia adsorption capacity was also investigated for the HEMA(50) AA(50) EDM(10) system S2, similar in composition to system S1. These systems are very similar in percentage of monomers employed, and so the reduced ammonia adsorptive capacity observed for S2, is due to its' larger particle size. Thus the surface area of these particles is greatly reduced, and so the available surface area for adsorption is similarly reduced.

Particulates composed of the hydrogel HEMA, e.g. HEMA(100) EDM(10) of system S3, possess a moderate ammonia adsorptive capacity, with 2.9 mg NH_3-N/g beads being adsorbed in one hour. This might suggest that polyHEMA on its' own is not a good adsorbent for ammonia, and that acrylic acid is necessary for ammonia adsorption. This is not so, as investigations have previously shown that HEMA adsorbs appreciable quantities of ammonia. The reduced adsorptive capacity of system S3 can thus be attributed to the lack of available adsorptive sites, and

the surface morphology of the adsorbent being only slightly porous. A porous surface within the structure conveys an effective surface area, and thus an effective ammonia adsorptive capacity. Desorption studies with this adsorbent demonstrated that the strength of binding of ammonia to the adsorptive sites is very weak. About 95% of the adsorbed ammonia was later released within a one hour desorption study.

The adsorption of ammonia by AA:EDM of system S4 reaches a high level of 8.5 mg NH_3-N/g beads being adsorbed in one hour. This adsorbed ammonia was confirmed to be very strongly bound to the acidic functionality (i.e. acrylic acid) of the particulates by one hour desorption studies, where only 1.42 mg NH_3-N/g beads were later released.

Particulates of the HEMA(50) MAA(50) EDM(4.6) system S5, containing acidic, cation exchange ability conferred by copolymerisation of the hydrogel HEMA with the acidic monomer MAA, methacrylic acid, were also employed in this study. Investigations using this adsorbent showed that its' capacity for adsorbing ammonia is low, with 2.8 mg NH_3-N/g beads being adsorbed in one hour. Subsequent desorption studies showed that all the adsorbed ammonia is later released. This release is also accompanied by the simultaneous leaching of impurities into the liquid

samples, which are capable of being detected by the analytical system.

Previous studies with cation ion exchange resins suggest that the acidic functionality of system S5 is capable of a greater ammonia adsorptive capacity than that observed. However, the low ammonia adsorption may be caused by an alteration in the chemical structures of methacrylic acid and HEMA on copolymerisation. Thus the nature and capacity of ammonia adsorption is interfered with.

Scanning electron micrographs of this particulate hydrogel (private communications)[198] show that the surface morphology is very smooth and is not a very open pore structure. Clearly, a porous surface favours an increase in the adsorptive capacity of an adsorbent.

Investigations with the basic system of HEMA(90) NMACR(10) EDM(8.5) system S6 demonstrate that the presence of the basic monomer N-methylolacrylamide in the composition of the hydrogel system confers a large ammonia adsorptive capacity. The observations also presented in Fig 5.19 show that of the ammonia adsorbed (i.e. 9.7 mg NH_3-N/g beads) 1.6 mg NH_3-N/g beads are released in a one hour desorption study. This large adsorptive capacity may be the property of the $-CH_2OH$ group of the N-methylolacrylamide structure, which attracts and binds ammonia. Of the systems studied, the monomers N-methylolacrylamide and HEMA are the only monomers used, which contain this

group ($-CH_2OH$) in their structure, and both systems possess effective ammonia adsorptive capacities. N-methylol-acrylamide is also very polar, and behaves similarly to HEMA. The large adsorptive capacity is also due to the small particle size of the particulates, which produces a large surface area. Scanning electron micrographs of the surface morphology of these particulates show a very porous surface (private communications)[198].

Another system investigated for its' ammonia adsorptive capacity is that of HEMA(100) DAA(25) EDM(9) system S7. Static adsorption studies with this basic hydrogel showed a very small ammonia adsorptive capacity (1.4 mg NH_3-N/g beads) with the subsequent release of the adsorbed ammonia, together with detectable leachable species from the hydrogel composition. These observations are shown in Fig 5.19. The low adsorptive capacity is due to the presence of diacetone acrylamide which contains a 'bulky' hydrophobic group in its' chemical structure. This may hinder ammonia adsorption by 'shielding' available adsorptive sites on the HEMA backbone.

The physical structure of the particulates, as observed from scanning electron micrographs of the surface morphology, (private communications)[198] show a very smooth surface and the pore structure is not very open.

The leaching phenomenon encountered in desorption

studies may be due to the presence of diacetone acrylamide (DAA) in the hydrogel. Previous studies with acetone showed that it extracts components from HEMA particulates. Thus, the presence of acetone in DAA may cause the extraction of certain chemical species to occur with the release of adsorbed ammonia in desorption studies.

Another particulate hydrogel system investigated, is that of the hydrogel acrylamide ACR(100) NN'MBA(10) system S8, synthesised by inverse suspension polymerisation in hexane, detailed in Chapter 4.2. In this system the cross-linking agent employed to give the structure the required integrity is NN'methylene bisacrylamide (NN'MBA). This monomer has two vinyl groups $CH_2=C\begin{matrix}R\\R\end{matrix}$ of identical reactivity to ethylene dimethacrylate (EDM), previously used as the cross-linking agent for the other systems studied. Observations with this system (S8) showed that these particulates do not possess a high ammonia adsorptive capacity. This may be due to the presence of amide groups HNC=O in the chemical structure of the monomers used, and the absence of the -OH group, found in methylolacrylamide and in HEMA, which adsorbs ammonia.

These novel macroporous hydrogel particulates were studied with the intention that they would provide the selective adsorbents desired for ammonia adsorption. A range of substances with different adsorptive capabil-

ities is desired by many workers in the field of liver support, to enable a more selective adsorption to take place in individual cases of liver failure. Thus, this would enable the adsorption of toxins to be more clearly tailored to suit the individual patient. In this study the toxin of concern has been ammonia.

The ammonia adsorption capacity of the range of hydrogel beads investigated is compared to that of the clinically successful adsorbent Human Serum Albumin:HSA-coated Amberlite XAD-7 resin (used for adsorption of bile acids) and to polyHEMA coated activated charcoal (though not exceptional) to see whether there are any similarities in ammonia adsorption. The clinical use of the coated polymeric uncharged resin is a recent addition to the biomedical field[150,199]. A recent charcoal system employed in in-vitro haemoperfusion circuits is that of the Biotec system[142]. Because of the continuous use of activated charcoal in the biomedical field, the observations of polyHEMA coated charcoal (although not of the Biotec system) is included in this section for comparison.

Comparing the ammonia adsorption for these systems, it is observed from the histograms presented in Fig 5.19 that the ammonia adsorption capacity of the 10% polyHEMA coated charcoal is very similar to that of the hydrogel systems of S3 and S5. The binding strength of

adsorbed ammonia is also relatively weak for these systems, as with polyHEMA coated charcoal system. However, in terms of useful adsorbents for the adsorption of relatively low ammonia levels, these hydrogel particulates are more attractive adsorbents than charcoal, as they themselves are biocompatible, unlike charcoal, which requires pre-treatment with a biocompatible hydrogel such as HEMA before use.

Observations with HSA coated Amberlite XAD-7 resin, (although not clinically used for the adsorption of ammonia) illustrate a capacity to adsorb appreciably large quantities of ammonia. Its' adsorption capacity can be compared to that of the hydrogel systems S1, S4 and S6, all of which are exceptionally good ammonia adsorbers. The strength of ammonia binding of the polymeric resin, however, is weaker than that observed for the hydrogel systems. Because HSA-coated Amberlite XAD-7 resin is already successful clinically, its' potential use as an adsorbent for ammonia in artificial liver support systems is suggested. In the case of the hydrogel systems, experimental ex-vivo haemoperfusion trials and, indeed, the haemoperfusion of suitable hyperammonaemic animal models, are essential before the clinical use of the novel hydrogel adsorbents in liver failure.

Comparing the performance of the spectrum of macroporous hydrogel particulates employed in this study, with

that for the ion exchange resins, it is apparent that the ammonia adsorptive capacity of the acidic system S2 (HEMA/AA/EDM), the basic system S7 (HEMA/DAA/EDM) and S8 (ACR/NN'MBA) is similar to that encountered with the strongly basic anion exchange resin, Dowex 1-X4 (Cl^-). However, for application in artificial liver support systems, the preferred adsorbent for use is the hydrogel system because of the moderate efficiency and biocompatibility of these particulates.

The ammonia adsorptive capacity of systems S3 (HEMA/EDM) and S5 (HEMA/MAA/EDM) on the other hand is greater (2-3 fold) than the previously mentioned systems and comparable to the capacity of the 10% polyHEMA coated activated charcoal. These hydrogels could be effectively used for reducing moderate hyperammonaemic levels in artificial liver support systems. Likewise, hydrogels of systems S4 (AA/EDM) and S6 (HEMA/NMACR/EDM) could also be employed in artificial liver support systems. The capacity of such beads for ammonia is very effective and comparable to that demonstrated by the weakly acidic cation exchange resin, Amberlite IRC-50(H^+). These hydrogel systems are therefore very attractive adsorbents for reducing highly elevated ammonia levels in liver failure.

5.7.(2) Dynamic Adsorption Studies

In order to investigate the ammonia adsorptive capacity and the rate and efficiency of ammonia adsorption for the particulate hydrogels, dynamic adsorption studies are performed. The particulates used in this study are shown in Table 5.4. As stated in Chapter 3, these studies are performed using a scaled-down perfusion cell, ensuring that correct space velocities are retained and all conditions closely mimic that of typical haemoperfusion systems (as far as possible). The particulates used in this study are those selected from the spectrum available for showing efficient (high) ammonia adsorption under static adsorption conditions.

The dynamic adsorption technique used is that detailed in Chapter 3, and briefly involves perfusing a known weight of adsorbent with a solution of NH_4Cl of known concentration, at a selected flow rate, regulated by means of a stop-cock. The observations from these dynamic adsorption studies are then converted to a haemoperfusion system, where the weight of adsorbent normally used is approximately 200g. Taking the blood volume of man as 5 litres, and assuming that normal blood ammonia levels are in the range of 1.0-3.5 mg NH_3-N and in elevated conditions these levels rise to 9.0 mg NH_3-N, the level of ammonia required to be removed in liver failure would be in the range of 5.5-8.0 mg NH_3-N. From the dynamic adsorption studies, the

Table 5.4 Macroporous Hydrogel Beads Used in Dynamic Adsorption Study

Code Number	Composition	Effective Size (mm)	Equilibrium Water Content EWC (%)	Synthetic Technique
D1 (=F1, Table 5.2)	HEMA/EDM (90:10)	2.0-5.0	44.5	Freeze-thaw
D2	HEMA/AA/EDM (75:25:10)	2.0-5.0	49.0	Freeze-thaw
D3 (=S1, Table 5.3	HEMA/AA/EDM (40:60:8.5)	< 0.15	64.0	Suspension Poly-merisation (brine)
D4 (=S6, Table 5.3)	HEMA/NMACR/EDM (90:10:8.5)	0.5-1.0	61.0	Suspension Poly-merisation (brine)

time required to remove this level of ammonia, using a known weight of the novel macroporous particulate hydrogel adsorbents, can be calculated. This calculated time is illustrated by the point X on the cumulative adsorption graphs of the dynamic studies.

Dynamic adsorption studies performed using macroporous hydrogel particulates of systems D1, D2, D3 and D4 demonstrate that effective ammonia adsorptive capacities are achieved for systems D1, D2 and D3. Systems D1 and D2 behave very similarly in their adsorption of ammonia, but system D2 has a slightly greater capacity for ammonia.

Investigations with system D1 showed that within thirty minutes of perfusion, a maximum of 12 μg NH_3-N were being adsorbed per gram beads. In comparison, a thirty minute static adsorption study using these particulates produced an adsorption of 11 mg NH_3-N/g beads. However, such a direct comparison of ammonia adsorption with time cannot be made for both systems. By scaling down the weight of particulates used and the volume of solution perfused in the dynamic adsorption system, to that encountered in the static adsorption system, the equivalent contact time of the toxin solution with the adsorbent, for the dynamic study, can be calculated. Calculations show that this is equivalent to allowing 5 ml toxin solution to be in contact with 1g beads, for about eight minutes in the dynamic adsorption system.

The total ammonia adsorbed within three and a half hours of perfusion for system D1 was about 550 μg NH_3-N/g beads, as shown in the cumulative curve of Fig 5.20. The formation of a plateau at this stage on the curve demonstrates that the time required for the adsorption of ammonia on this hydrogel (system D1) to reach equilibrium (or for this adsorbent to be saturated) is approaching. The large particle size, small surface area and the availability of adsorptive sites on the adsorbent being at a minimum probably all contribute to equilibrium adsorption being almost achieved at three and a half hours in this rate experiment.

In the case of liver failure, ammonia levels are elevated such that levels in the range of 5.5-8.0 mg NH_3-N would need to be removed in a typical haemoperfusion system, in order to arrive at normal physiological levels. From the study of the hydrogel system D1, it is calculated that this range of elevated ammonia could be reduced by perfusing 200g of such beads at a flow rate of 200 mls/minute for about sixty - ninety minutes, an acceptable clinical perfusion time. This calculated time range is illustrated as points X-X on the cumulative adsorption curve. Similarly, for particulates of systems D2 it is calculated that in order to remove an elevated ammonia level of 8 mg NH_3-N, 200g of these beads would need to be perfused at 200 mls/minute for about one hour. This

FIG 5·20

ADSORPTION OF AMMONIA ON HEMA/EDM (90:10) PARTICULATE HYDROGEL (DT)

CONDITIONS: $[NH_4Cl]_o \equiv 10$ mg Nl^{-1}; 3g adsorbent;
flow rate = 2 mls/min for 3½ hours;
—— ammonia adsorbed; --- cumulative ammonia adsorbed

FIG 5·21

ADSORPTION OF AMMONIA ON HEMA/AA/EDM (75:25:10) PARTICULATE HYDROGELS (D2)

CONDITIONS: $[NH_4Cl]_o \equiv 10$ mg Nl^{-1}; 3g adsorbent;
flow rate = 2 mls/min for 3½ hours; 4mls/sample
—— ammonia adsorbed; --- cumulative ammonia adsorbed

Fig 5.20

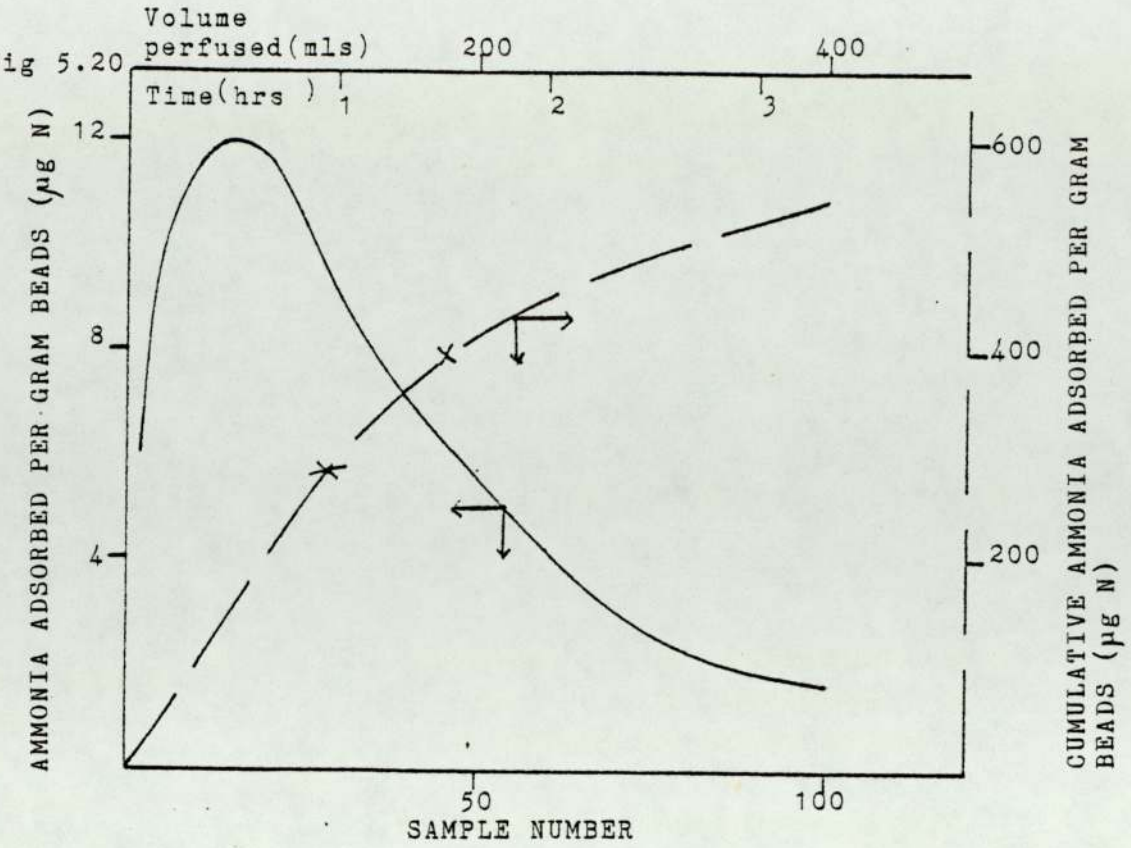
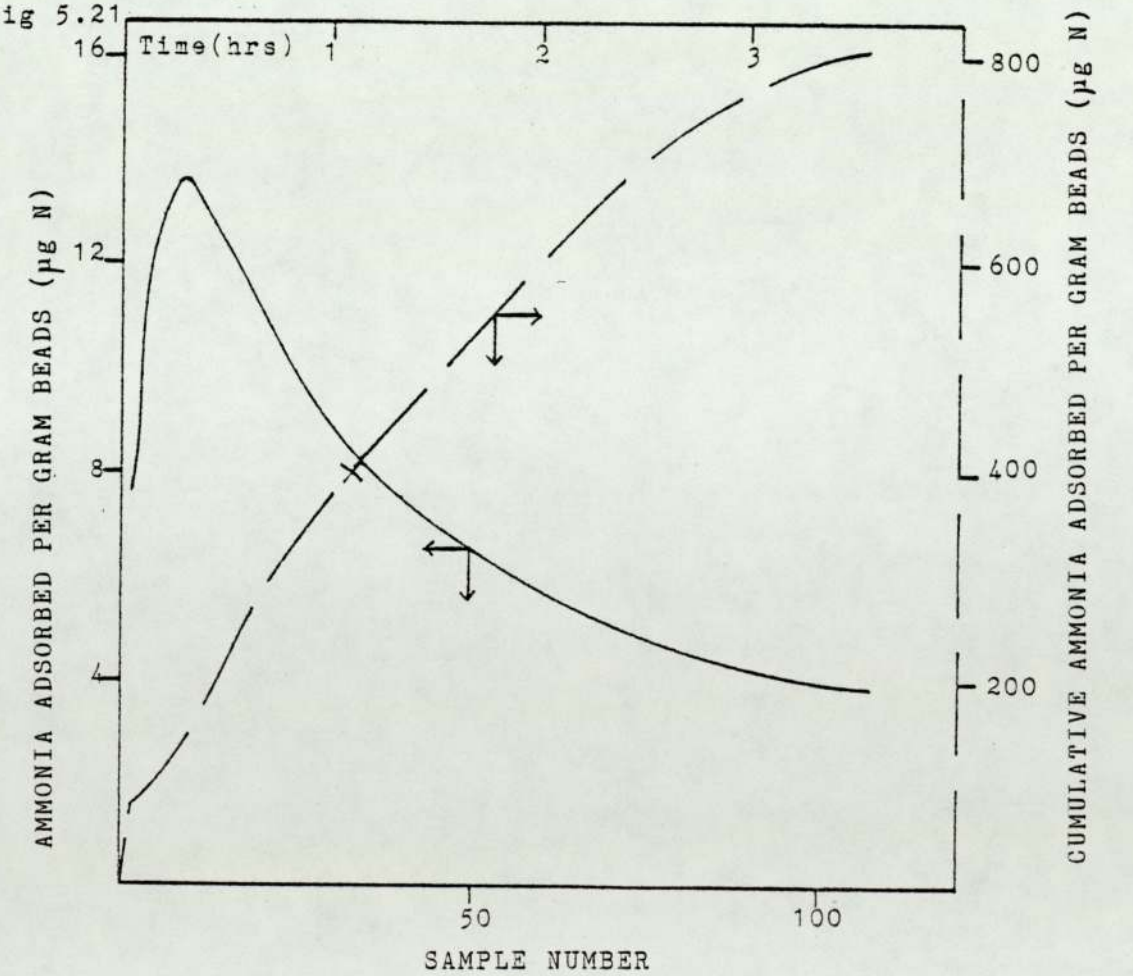


Fig 5.21



perfusion time illustrated by the point X on the cumulative adsorption curve of Fig 5.21 is in accordance with typical haemoperfusion systems.

In addition to this study, the adsorption of ammonia was investigated using another acidic macroporous hydrogel bead type, that of system D3, (which is similar in composition to system D2) and also the basic system D4 containing N-methylolacrylamide (NMACR). Ammonia is adsorbed efficiently by system D3, which from the cumulative ammonia adsorption curve, Fig 5.22, appears to be an infinite capacity. From the performance of these macroporous hydrogel beads, it is calculated that a typical haemoperfusion system employing 100g of such beads would remove 8 mg NH_3-N at a flow rate of 300 mls/min within five minutes of perfusion.

In comparison to this acidic system, investigations of the basic system D4 of HEMA(90) NMACR(10) EDM(2.5) showed that ammonia is adsorbed in initial stages of perfusion, but adsorption declines thereafter as equilibrium is achieved very rapidly. At this stage negligible quantities of ammonia are adsorbed, because the adsorbent is virtually saturated. These observations are presented in Fig 5.23. However, when applying the performance observed with these basic particulates to a typical haemoperfusion system it is calculated that 400g of this adsorbent, perfused at 300 mls/min for two hours, would be adequate to remove elevated ammonia levels (of 8mg

FIG 5·22

ADSORPTION OF AMMONIA ON HEMA/AA/EDM (40:60:8.5) PARTICULATE HYDROGEL D3

CONDITIONS: $[NH_4Cl]_o \equiv 15$ mg Nl^{-1}; 5g adsorbent; flow rate = 3 mls/min for 18 hours;

—— ammonia adsorbed; --- cumulative ammonia adsorbed

FIG 5·23

AMMONIA ADSORBED ON HEMA/NMACR/EDM (90:10:8.5) PARTICULATE HYDROGEL (D4)

CONDITIONS: $[NH_4Cl]_o \equiv 15$ mg Nl^{-1}; 5g adsorbent; flow rate = 3 mls/min for 12 hours;

—— ammonia adsorbed; --- cumulative ammonia adsorbed

Fig 5.22

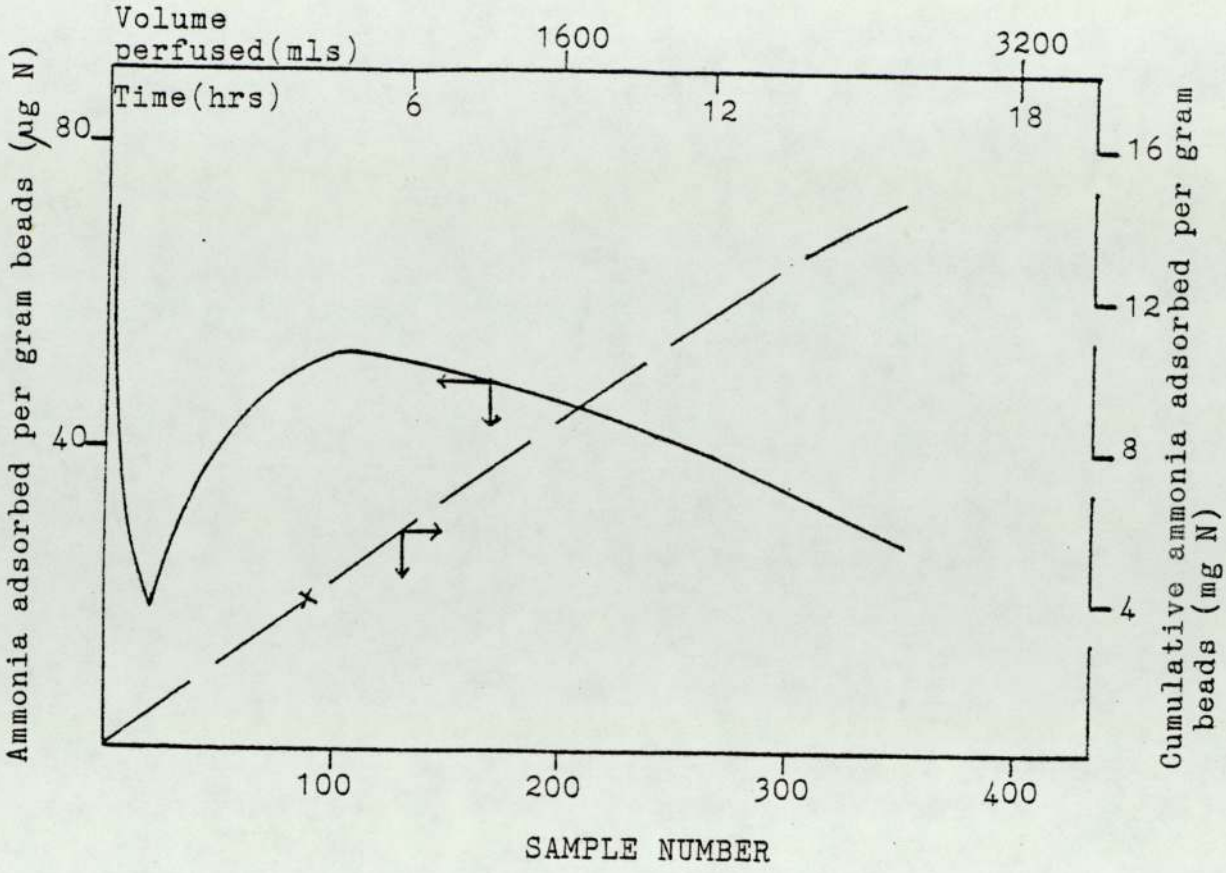
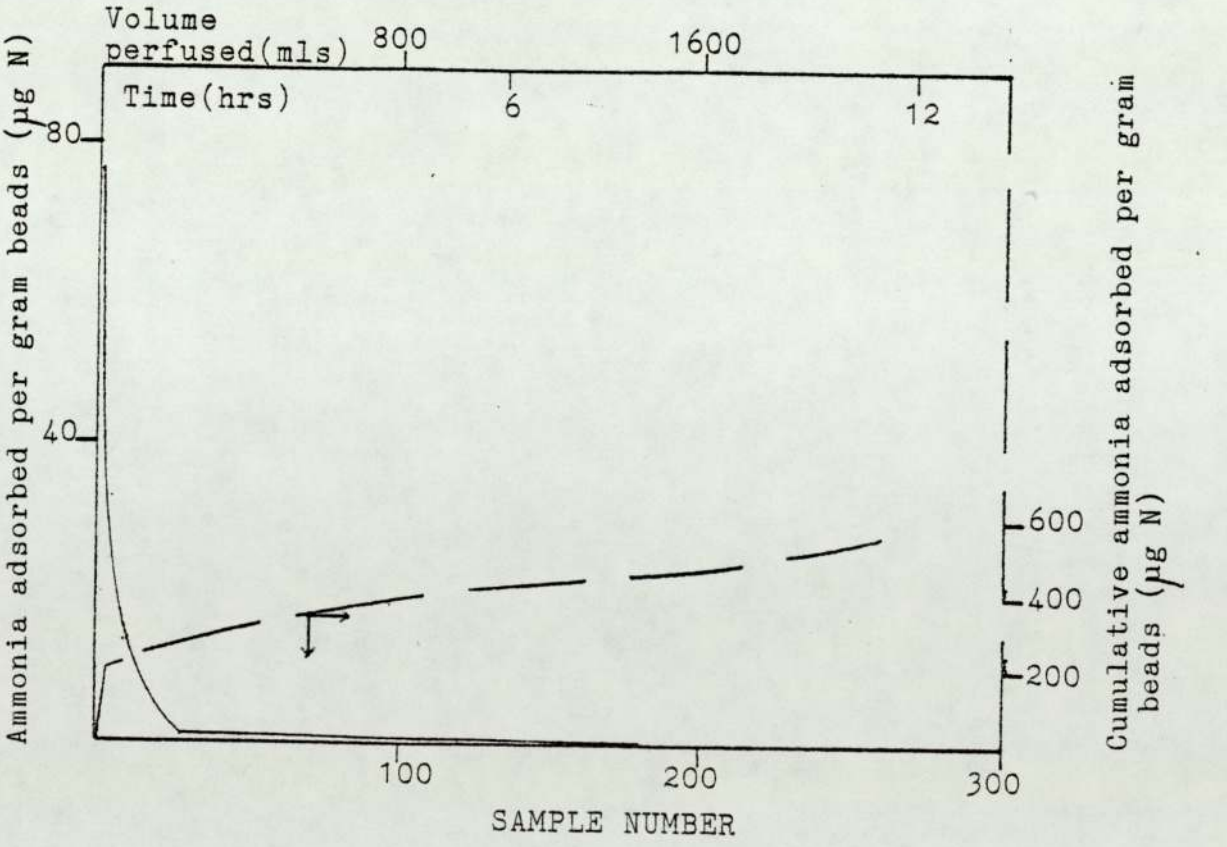


Fig 5.23



NH_3-N) encountered in liver failure. The time required for this level of ammonia to be adsorbed is illustrated by the point X on the cumulative adsorption curve. However, because this adsorbent reaches equilibrium adsorption very rapidly, it would not be suitable for application in haemoperfusion systems.

The adsorption of ammonia under dynamic adsorption conditions, gives an indication of the rate of adsorption, which is different in principle to that of static adsorption conditions. Under static adsorption conditions the basic particulates of system D4 (S6 - Fig 5.19) displayed a high ammonia adsorptive capacity in one hour. However, as demonstrated by the results, a high adsorptive capacity under static conditions does not necessarily imply an effective adsorption capacity in dynamic adsorption studies.

Scanning electron micrographs of these particulates (private communications) show a porous surface morphology (which suggests a high adsorptive capacity) and a large open pore structure. However, in spite of this combination the basic nature of the N-methylolacrylamide polymer species of these particulates and the absence of groups with great specific ammonia binding ability, produce relatively little adsorption. The fairly marked initial adsorption was due to the high surface area of the particulates.

The strength of binding of the adsorbed ammonia is

an important factor in the continuous dynamic adsorption process. Desorption studies have previously shown that this hydrogel system, D4, releases about 16% of its' adsorbed ammonia in a one hour study (S6 Fig 5.19). Simultaneous adsorption and release of ammonia probably occurs throughout the dynamic perfusion of the model solution over this hydrogel. The observations of this study suggest that the release of adsorbed ammonia is exaggerated in the dynamic system, such that ammonia appears not to be adsorbed.

The adsorption of ammonia by this basic hydrogel is very similar to that by the basic ion exchange resin, Dowex 1-X4, where adsorption under static adsorption studies was very low.

5.8 Discussion

The continuing bleak prognosis for patients with fulminant hepatic failure has increasingly stimulated investigations into the feasibility of developing some sort of artificial device which could perform the essential life preserving functions of the liver. For the most part, these studies have focussed on methods for carrying out the excretory functions of the liver.[55] However, the development of such artificial hepatic support systems has proceeded empirically in the face of substantial

ignorance of the toxins to be removed, or of the metabolic abnormalities to be corrected.

A number of different approaches to the development of such systems have been proposed (as previously described in Chapter 1) and several have been brought into clinical use after only a minimal amount of in-vitro and animal testing. Because of the complexity of the abnormal metabolic state which these devices are being called upon to alter and the lack of precise knowledge in the past of the nature of the toxins to be removed, a successful outcome was prevented even if the experimental device was by chance to remove the appropriate metabolites[55].

Ammonia is known to be an important cerebral toxin in liver failure, and its' adsorption remains a desired goal in artificial liver support systems[8]. The adsorption of ammonia has been of particular interest in this chapter and a range of commercially available adsorbents such as activated charcoal, polymeric neutral resins and basic and acidic ion exchange resins were employed as potential adsorbents. Observations from these studies were later used as guidelines in the selection of purposely synthesised macroporous hydrogel particulates (designed and synthesised in a separate project)[198] for the adsorption of ammonia.

Before discussing adsorption by the various adsorbent

materials used, some general points as to the factors affecting adsorption will be made. Adsorption depends on factors such as particle size, surface area, porosity, moisture content and surface charge of the adsorbent. Generally speaking, functional groups were found to be important in the adsorption process, in this project. However, it was found that ammonia could be adsorbed on the polymeric neutral Amberlite resins, which contain no ion exchange functionality. A simple bead of the Amberlite resin consists of an agglomeration of a large number of very small microspheres. In the absence of ionically functional sites, such polymeric adsorbents derive their adsorptive properties from the combination of macroreticular porosity, pore size distribution and high surface area[148]. Since ammonia is a small molecule and is very soluble in water, it readily penetrates the porous structure of the polymeric resin, which is of an open-cell variety. This demonstrates the non-specific affinity of the resin for ammonia and ammonia binds to the solid polymer surface by both weak van der Waals' forces and hydrogen bonding.

Adsorption of ammonia on the basic ion exchange resin Dowex 1-X4 (Cl^-) (Fig 5.4) occurs via weak polar interactions between ammonia and polar groups in the resin structure. Such binding was shown to be relatively weak in desorption studies. On the other hand, adsorption of ammonia on the acidic ion exchange resins occurs by chemical-

ly more specific electrostatic forces. Since the stationary phase of the acidic ion exchange resin is negatively charged, ion exchange takes place between ammonia (as ammonium ions) and the positively charged mobile phase. Thus, ammonia is ionically bound to the resin.

The commercially based adsorbents employed were of various particle sizes, such that the polymeric neutral resins ranged in particle size from 0.25 - 1.22 mm. On the other hand, the strongly acidic and basic ion exchange resins covered a much smaller size margin of 0.45 - 0.62 mm. However, the weakly acidic ion exchange resin had an even smaller particle size range of 0.33 - 0.5 mm. A small particle size is known to enhance adsorption, since a larger surface area is presented for adsorptive processes. Of the other factors mentioned, porosity was very important for adsorption in the case of the polymeric neutral resins and surface charge was the important factor in the case of the acidic ion exchange resins. Adsorption of ammonia on the weakly acidic ion exchange resin Amberlite IRC-50 (H^+) was less efficient than that on the strongly acidic ion exchange resins, since the weak carboxylic acid group of the former is less strongly ionized than the sulphonic acid group of the latter.

It is appreciated that adsorption of ammonia on such bioincompatible adsorbents as those described previously (200), would never be undertaken in haemoperfusion systems for artificial liver support, without the application of a

biocompatible encapsulation membrane. Some of the factors associated with the behaviour of one type of biocompatible membranes have been of importance in the experimental results presented in this chapter. The hydrogel, polyHEMA was chosen for use in this project as a biocompatible coating material and was used specifically for encapsulating activated charcoal, and ion exchange resins, because of previous experiences with polyHEMA in this laboratory.

The factors affecting the transport of molecules (e.g. O_2) across synthetic polymers (including hydrogels and hydrophobic polymers) and also factors affecting the permselectivity of polymer membranes to molecules have been examined in previous projects[192-195]. In addition the effect of water binding properties of hydrophilic (hydrogel) polymers on transport properties has been studied. As a result, HEMA was found to possess a moderate degree of oxygen permeability properties for contact lens application.

In the light of the previous findings, it was proposed that because ammonia is broadly similar to oxygen on the basis of size and solubility, its' permeability and transport by HEMA may also be similar to that observed for oxygen. The average pore radius given for polyHEMA hydrogel is $4A^{\circ}$ and indicates that ammonia being a small mole-

cule should penetrate the hydrogel network, as found for oxygen. The transport of molecules through hydrogel membranes is governed by the water content of the hydrogel. The equilibrium water content of HEMA is about 40%. It is this water in the hydrophilic polymer which governs the transport properties of the hydrogel[197].

A particular point of interest in the evaluation of polyHEMA coated activated charcoal was that polyHEMA initially used as a transport membrane for ammonia, actually adsorbed ammonia as shown in Figs 5.1 and 5.2. It is suggested that the adsorption of ammonia on polyHEMA takes place because of the polar nature of polyHEMA which attracts ammonia. Binding then occurs via weak polar interactions of ammonia and polar sites on the polyHEMA backbone.

Another interesting observation was that ammonia adsorption on polyHEMA coated activated charcoal was significantly increased by the application of thicker polyHEMA coatings. Such coating solutions were obtained by the evaporation of a 10% polyHEMA solution (wt/vol). Thus, as shown in Figs 5.1 and 5.2, 10% polyHEMA coated activated charcoal adsorbed more ammonia than did the 2% polyHEMA coated activated charcoal. However, with the use of coated activated charcoal, in order to achieve the level of ammonia adsorption necessary for an artificial liver support system, a fairly thick polyHEMA coating would be required. Since activated charcoal was merely used as a

support material in this case, and not as an adsorbent, the application of thick polyHEMA coatings was pointless. A more relevant approach was to synthesise hydrogel particulates based on HEMA, and use them as adsorbents. This idea was then persued and will be discussed later.

To further the microencapsulation study, polyHEMA coatings were applied to the strongly acidic cation exchange resin, Duolite C255 (H^+), which was shown to adsorb ammonia (Fig 5.6). The thickness of the coating applied was in the calculated range of 1-3 μm and contained about 5% polyHEMA by weight. Although a better coverage of the adsorbent may be achieved with thicker coatings, the thickness of the dense coating would eventually hinder adsorption, since available adsorption sites would not be easily assessible. Such coatings of the type applied did not affect the ability of Duolite C255 (H^+) resin to adsorb ammonia under static adsorption conditions (Fig 5.7). In addition, a range of cation exchange resins was encapsulated with polyHEMA and ammonia adsorption investigated under dynamic adsorption conditions. The capacity and rate of ammonia adsorption were not impeded by polyHEMA coatings, as shown in Figs 5.12-5.17, and ammonia adsorption on the coated resins was similar to that observed on their uncoated counterpart. However, the ammonia adsorption capacity of the ion exchange resins was much greater than would be needed for the biomedial appli-

cation in artificial liver support systems. It is apparent on considering the performance of the ion exchange resins under dynamic conditions, (Figs 5.12-5.17) that 1g of polyHEMA coated ion exchange resin adsorbed ammonia in the order of 3 mg NH_3 in nine hours. Typical haemoperfusion systems[16,173,201] employ 100-200g of adsorbent material and elevated ammonia levels are in the order of 180 µg NH_3/100 ml. It is therefore clear that ion exchange resins would be too powerful for this application. Furthermore, the sulphonic acid functional groups of the strongly acidic ion exchange resins (although desirable), adsorbs ammonia at the expense of essential inorganic cations[36].

The search for suitable adsorbent materials with suitable properties has proved difficult due to the adverse effects on blood platelets and other blood constituents [175-178,182,183,200]. The requirements for purposely designed porous membranes for adsorbents is still a desired goal in artificial liver support systems. Research workers at King's College, London, have been successful in the clinical adsorption of bile acids and bilirubin on Amberlite XAD-7 resin, rendered biocompatible by the encapsulation with Human Serum Albumin (HSA)[150].

Static adsorption studies employing polymeric neutral resins of the Amberlite type, showed that the adsorption of ammonia on Amberlite XAD-2 and XAD-4 resins was variable

and inconsistent, and equilibrium adsorption was achieved slowly, whilst Amberlite XAD-7 did not adsorb ammonia. Further investigations were not carried out, as in addition to this, these resins had the tendency to leach residual monomeric compounds into the perfusate, which presented difficulties. Although the application of biocompatible coatings (such as polyHEMA used for activated charcoal and ion exchange resins) would control the leaching to some extent, the encapulsation of the neutral resins with polyHEMA was not justified by their ability to adsorb ammonia.

HSA coated Amberlite XAD-7 resin was employed in static adsorption studies and it was somewhat surprising to note that this system adsorbed ammonia (results presented in Fig 5.19). This implied that adsorption was a property of the protein coat. Such an adsorption system is a typical example of the use of a base material, in this case, the polymeric neutral resin, Amberlite XAD-7, as a support system for the application of a biocompatible membrane, which is also capable of adsorption. It is suggested that binding of ammonia to the protein albumin occurs via strong protein forces, weak van der Waals' forces and general polar interactions. These studies lead to the assumption that HSA coated Amberlite XAD-7 resin could be used for the simultaneous removal of ammonia in liver failure.

The information obtained for ammonia adsorption studies on various adsorbent materials can be conveniently summarised at this point. Activated charcoal does not adsorb ammonia, whereas polyHEMA coated activated charcoal does, due to the interaction of ammonia with polyHEMA. The neutral polymeric resins, e.g. Amberlite XAD-2 and XAD-4 adsorb ammonia, but adsorption is very variable. However, Amberlite XAD-7 resin does not adsorb ammonia (Table 5.1, Fig 5.3). On the whole, the opinion arrived at from this study is that uncoated or polyHEMA coated activated charcoal and the polymeric neutral resins, either uncoated or HSA coated, would have a very limited role in the treatment of hyperammonaemia in hepatic coma and by themselves would do little to alter the elevated ammonia levels encountered. Ion exchange resins, on the other hand, are not suitable, either coated or uncoated, because of their 'infinite' capacity for ammonia.

The interesting concept of using polyHEMA as a particulate adsorbent arose from these early results. In the light of such findings it was felt that the incorporation of weak acidic groups (such as the carboxylic acid groups of the weakly acidic ion exchange resins) into the particulate structure, would provide a more logical adsorbent system than would the use of polyHEMA coated ion exchange resins. The use of coated resins would involve extensive variations

in the properties of the encapsulating membrane to enable the resins to selectively remove lower levels of ammonia.

Hydrogel particulates based on HEMA are biocompatible and also encompass important factors, such as strength, regularity of shape and particle size, polarity and ion exchange ability of the commercially available cationic exchange resins. Such hydrogel adsorbents could also be synthesised to fit the necessary requirements for the adsorption of ammonia in artificial liver support systems[197].

Generally, the internal structure of adsorbents is important in governing the surface area properties. Therefore, the particulate hydrogels were synthesised (during the course of the project by Dr. P. J. Skelly and Mr. U. S. Atwal) as macroporous adsorbents, possessing large discrete pores in their structure (private communications)[198] to aid adsorption. A range of these biocompatible macroporous hydrogel particulates based on HEMA and varying in composition, particle size, porosity and water content, (equilibrium water content, EWC = 45-80%) was evaluated by the author.

The ability of the hydrogels to adsorb ammonia is demonstrated in Figs 5.18-5.23. The overall conclusions from these studies were that the incorporation of acidic monomers, such as acrylic acid in the polymer structure imparted an ion exchange ability to the hydrogel particulates. This then enhanced ammonia adsorption due to the chemical specificity of such groups and the interaction

of HEMA (Figs 5.21-5.22). It could, therefore, be suggested that the binding of ammonia (as ammonium ions) to the acidic residues takes place by specific ionic bonding. The attraction to polar groups of the monomers in the particulate composition also play a role. Weaker hydrogen bonding mechanisms, and weak van der Waals' forces may also be important. Interfacial tension may also play a part in adsorption efficiency. Certain other factors which include the porosity of the particulates and a small particle size also facilitate adsorption, but the water content of the particulates did not appear to affect the adsorption of ammonia.

It is suggested that the transport of ammonia through water swollen polymer particulates is very similar to that of oxygen, since ammonia and oxygen are broadly similar as regards their size and solubility (as previously stated). Thus, ammonia is readily transported through the water matrix in the pores of the particulate to available adsorption sites.

Hydrogel particulates based on polyHEMA and containing basic monomers such as diacetone acrylamide and N-methylolacrylamide also adsorbed ammonia (Fig 5.19). Attraction to such particulates is due to the attraction of ammonia to the polar sites on the polyHEMA backbone. Thus binding occurs as a result of weak polar interactions. Basic particulates however, do not have a large capacity

for ammonia (Fig 5.23) and equilibrium adsorption is achieved relatively early. Such particulates would be useful for the adsorption of relatively low ammonia concentrations in artificial liver support systems. They may, however, be better suited for the adsorption of acidic chemical species (such as bile acids)[198] since they possess basic attraction sites.

In summary, therefore, it can be said that the use of synthetic macroporous hydrogel beads would provide ways of producing purposely designed adsorbents for use in acute liver failure. By controlling the structure and composition of these particulate hydrogels, they can be tailored to suit the needs of individual patients for use in artificial liver support systems. Functional groups can be introduced effectively into the polymer/copolymer structure, so that the adsorbent can be specific for ammonia or can adsorb various amounts of ammonia, depending on the individual's requirements.

The novel macroporous hydrogel particulates satisfy the general criteria relevant to the choice of adsorbents for haemoperfusion techniques[145]. They are manufactured as strong attrition resistant hydrogels of physical and chemical stability. They are non-toxic, are capable of being sterilized and exhibit no deleterious effects on the blood, thus they are also biocompatible. They are comparatively inexpensive and their mode of synthesis is

less complicated than that of the commercially available adsorbents used in this project. Their capacity to adsorb ammonia (under dynamic adsorption conditions) would allow the quantity of adsorbent, if employed in typical haemoperfusion systems, to be minimized to practical amounts.

It has been calculated that in order to adsorb the levels of ammonia encountered in liver failure (e.g. 5.5-8.0 mg NH_3-N from the total blood volume of man) the use of 200g of hydrogel particulates composed of either HEMA (e.g. system D1) or HEMA and acrylic acid (e.g. system D2) would remove 8 mg NH_3-N within one and a half hours at a perfusion rate of 200 mls/min. This time is denoted by 'x' in the dynamic adsorption curves of Figs 5.20 and 5.21. By increasing the acrylic acid content of particulates (e.g. system D3) ammonia adsorption can be enhanced and the use of 100g of such hydrogel particulates perfused at 300 mls/min would remove similar elevated ammonia levels in one and a quarter hours (Fig 5.22).

In terms of ammonia adsorption capacity, these hydrogel particulates may be potentially valuable as an essential excretory component in artificial liver support systems for the direct removal of ammonia from blood of hepatic encephalopathic patients. Obviously, it is appreciated that before the novel macroporous hydrogel particulates can be used for humans, rigorous testing on biological samples and animals would be required and is the next logical step for future work in this area.

CHAPTER 6

REMOVAL OF AMINO ACIDS AND AMINES BY POTENTIAL HAEMOPERFUSION ADSORBENTS

6.1 Introduction

All amino acid levels are deranged in liver failure, as detailed in Chapter 4.1, but these pathological levels differ from one individual to another, and with the varying aetiology and severity of the liver disease[76,202] In many cases, whilst some amino acids (e.g. methionine and aromatic amino acids such as phenylalanine, tyrosine) are greatly increased, others (e.g. branched-chain amino acids, such as valine, leucine, isoleucine) are decreased.[203] A range of amino acids shown in Table 6.1 (where they are classified on the basis of the polarity of their R-groups) have been employed in this study in an attempt to selectively adsorb them on various commercially based adsorbents. The observations from this investigation are used as guidelines for the design and development of novel macroporous hydrogel particulates, and their eventual use as adsorbents for the adsorption of amino acids.

Conventional one hour and two hour static adsorption studies and dynamic adsorption studies, previously used for the adsorption of ammonia (Chapter 5) are also employed here, using similar conditions. The concentration of amino acid used is expressed as a function of the amino acid-nitrogen contained in the experimental solution. All amino acid solutions employed, initially contain 15 mg Nl^{-1}. The analytical system used is the

Table 6.1 Amino Acids Used in Study

a) Amino acid with non-polar (hydrophobic) R-groups

Alanine (Ala), Valine (Val), Isoleucine (Ile), Phenylalanine (Phe), Methionine (Met)

b) Amino acid with uncharged polar R-groups

Glycine (Gly), Cysteine (Cys), Tyrosine (Tyr)

c) Amino acid with positively charged polar R-groups (basic)

Histidine (His)

Technicon Auto-Analyser, and the nitrogen in the samples was measured using the same analytical method as that employed for ammoniacal-nitrogen. The amino acid levels in the samples, however, are compared to standard solutions of the amino acid in question, which contain 15 mg Nl^{-1}. Similarly, the adsorption of false neurotransmitter amines (such as Octopamine (OTA), Phenylethanolamine (PEA) and Ethanolamine (ETA)) on various commercially based adsorbents and novel macroporous hydrogel particulates,are studied. To avoid confusion with the amino acids, the abbreviated forms of the false neurotransmitters are expressed with capital letters.

(The structure, properties and function of the false neurotransmitters are detailed in Chapter 4.1).

The adsorption of amino acids and false neurotransmitter amines on various adsorbent materials will now be considered. Some experiments with urea are also included. As in the case of amino acids, the concentration of urea and amines employed in this study is expressed as a nitrogen concentration, and an initial sample concentration of 15 mg Nl^{-1} is adopted throughout. Again, the analytical method used is the same as that previously used for ammonia (Chapter 5).

6.2 Adsorption of Amino Acids and Amines on a Range of Adsorbents : Static Adsorption Studies

The adsorption of amino acids was initially investigated using activated charcoal as the adsorbent material. 1g of this adsorbent was shaken (in the conventional manner for static adsorption studies, detailed in Chapter 3) with 5 ml amino acid solution containing 15 mg nitrogen per litre (15 mg Nl^{-1}) for one hour and two hour periods. The sample liquor was then decanted off for analysis of unadsorbed nitrogen. Desorption studies were then performed under similar conditions, using 5 ml distilled water, and samples analysed for the subsequent release of adsorbed amino acid. A summary of results obtained with

charcoal, neutral resins, basic ion exchange and weakly acidic ion exchange resins is shown in Table 6.2. A brief report of the preliminary findings will now be presented.

Although activated charcoal is known to possess a broad adsorptive spectrum, not all amino acids studied were adsorbed by charcoal, a non-selective and non-specific adsorbent. This confirms the view held by other workers[117], namely that activated charcoal is capable of removing amino acids, particularly methionine, phenylalanine, tyrosine (and histidine in this study), and, to a lesser extent, other amino acids.

Similar adsorption studies with Amberlite XAD-2 and XAD-4 resins (polymeric uncharged resins of polystyrene) demonstrated that their chemical structure does not produce any systematic effects on the adsorption of the various amino acids studied. There was a fair degree of scatter in the results, particularly due to impurities on the part of the resins, and in general there was no significant uniform trend in the adsorption of the amino acids. In fact, these observations are comparable to those obtained with activated charcoal.

In accordance with the fact that a greater surface area/small pore size combination results in an increase in adsorption, the adsorption of amino acids by Amberlite XAD-4 resin was slightly greater than that by Amberlite

Table 6.2 General Trends in Model Toxins

Model Toxin / Adsorbent	AMINO ACIDS ($mgNl^{-1}$)		AMINES ($mgNl^{-1}$)		UREA ($mgNl^{-1}$)	
	Adsorbed	Released	Adsorbed	Released	Adsorbed	Released
Activated Charcoal	3.6 ± 2.0 (10)	3.5 ± 1.8 (10)	8.0 ± 3.9 (2)	4.6 ± 0.9 (2)	0 (2)	0 (2)
Amberlite XAD-2	3.4 ± 3.5 (16)	2.5 ± 1.6 (16)	6.8 ± 5.2 (6)	3.7 ± 1.8 (6)	10.5 ± 6.4 (2)	4.6 ± 2.3 (2)
Amberlite XAD-4	3.8 ± 3.0 (18)	2.3 ± 1.2 (18)	6.9 ± 4.4 (6)	3.0 ± 2.0 (6)	2.2 ± 2.7 (2)	2.2 ± 2.7 (2)
Dowex 1-X4 (Cl^-)	7.0 ± 3.7 (18)	2.9 ± 2.0 (18)	5.3 ± 2.7 (6)	4.9 ± 2.6 (6)	6.3 ± 2.4 (2)	6.3 ± 2.4 (2)
Amberlite IRC-50(H^+)	6.4 ± 4.5 (15)	2.5 ± 2.4 (15)	7.2 ± 3.0 (6)	2.7 ± 1.0 (6)	3.0 (1)	3.0 (1)

XAD-2. On the whole, charcoal and the XAD resins possess a higher adsorptive capacity for the amines than the amino acids, but again, there was a considerable degree of scatter in the results. The results presented in Table 6.2 show the mean concentration of amino acids, amines and urea adsorbed in static adsorption studies, and also the mean value released in desorption studies. Each result is a mean value $\pm$ S.E.M. The number of studies are shown in brackets.

The adsorption of urea by the XAD-2 and XAD-4 resins is fairly similar to that observed for ammonia (Chapter 5, Fig 5.3). (Urea was not adsorbed by charcoal and Amberlite XAD-7 resin). In conclusion, charcoal and the XAD resins show relatively little affinity for amino acids, but are better for adsorbing amines. The XAD resins, because of their strength, however, present a more attractive option. Clearly, there is some question of impurity, as these resins are liable to leach impurities to the samples. Also, the effect of haemoperfusion on the formed elements of the blood and particularly the platelets is far more serious during haemoperfusion with Amberlite resin than with charcoal[16].

The clinically employed Amberlite resin system, albumin coated Amberlite XAD-7 resin, however, does overcome this problem by the protein coat which also serves a biocompatible role. The observations for this

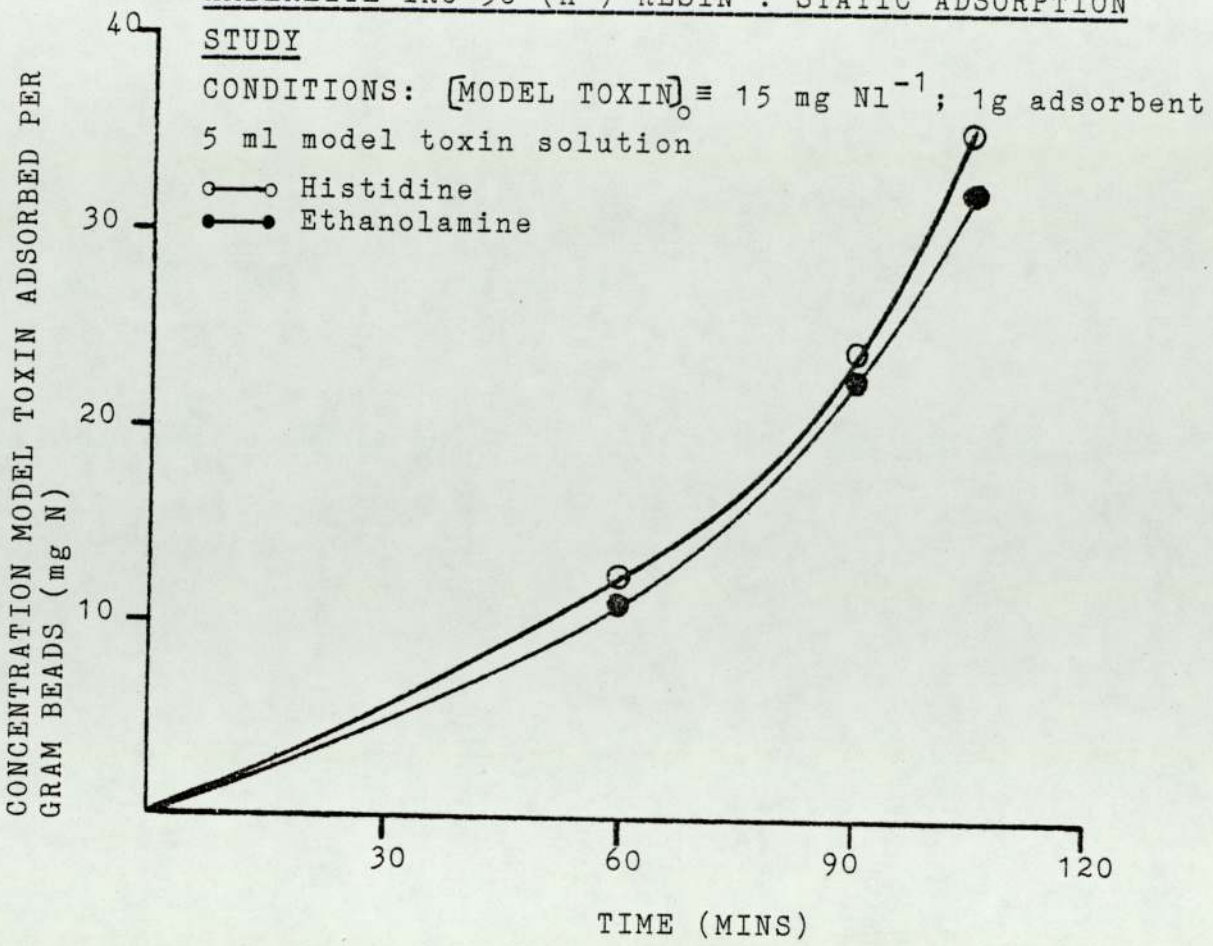
coated resin will be discussed in a subsequent section.

The adsorption of model toxins was also investigated using the strongly basic anionic exchanger Dowex 1-X4(Cl^-), under static adsorption conditions. The observations of this study are presented in Table 6.2, and demonstrate that this ion exchanger possesses a higher average adsorption capacity for the amino acids than for the amines and urea. The desorption studies also show a stronger binding of the amino acids to this basic resin.

The weakly acidic cationic exchange resin, Amberlite IRC-50 (H^+), on the other hand, adsorbs relatively similar concentrations of amino acids and amines, with the amines being adsorbed only slightly better. However, there was some degree of scatter in the results and the amino acid histidine and the false neurotransmitter ethanolamine were being adsorbed with the adsorbent showing a slightly better than average affinity for them (e.g. 15 mg Nl^{-1} and 11 mg Nl^{-1} adsorbed respectively).

A separate adsorption study, employing histidine and ethanolamine, was carried out, using a variation on the static adsorption study. This particular study, performed on a cumulative time basis, involved shaking the adsorbent with 5 ml model toxin solution (containing 15 mg Nl^{-1}) for a period of time, and then analysing the liquid sample. A fresh model toxin sample (of the same

Fig 6.1 ADSORPTION OF HISTIDINE AND ETHANOLAMINE ON AMBERLITE IRC-50 (H^+) RESIN : STATIC ADSORPTION STUDY



volume and concentration) was then added, and the procedure continued until three such samples were collected. The observations of this study, presented in Fig 6.1, show that the adsorption of histidine and of ethanolamine on Amberlite IRC-50 (H^+) follows a similar pattern.

6.3 Adsorption of Amino Acids and Amines on Strongly Acidic (Cationic) Exchange Resins : Static Adsorption Studies

The strongly acidic cation exchange resins were also investigated for their adsorption of selected model toxins under static adsorption conditions. (The structure and properties of the cation exchangers used are detailed in Chapter 4.2). The model toxins, i.e. amino acids, false neurotransmitter amines and urea, were adsorbed very efficiently on the ion exchange resins, as shown in Figs 6.2 - 6.4. The sodium form (Na^+) of Amberlite IR-120 resin, as expected, demonstrated a reduced adsorption capacity for the model toxins studied, when compared to its' acidic (H^+) counterpart. The ability of the other cation exchange resins to adsorb amino acids and amines were, in most cases, very similar to each other, but Dowex 50W-X8 (H^+) resin adsorbed smaller amounts of some of the model toxins studied, as shown by the histograms. Similar results were obtained for two hour static adsorption studies, but only those for one hour studies are presented here.

Fig 6.2 ADSORPTION OF AMINO ACIDS BY STRONGLY ACIDIC CATION EXCHANGE RESINS: STATIC ADSORPTION STUDY (1 HOUR)

CONDITIONS: $[\text{Amino acid}]_o \equiv 15$ mg Nl^{-1}; 1g adsorbent, 5ml amino acid solution

□ Amberlite IR-120 (Na^+); ▨ Amberlite IR-120 (H^+); ▤ Amberlite IRN-77(H^+)

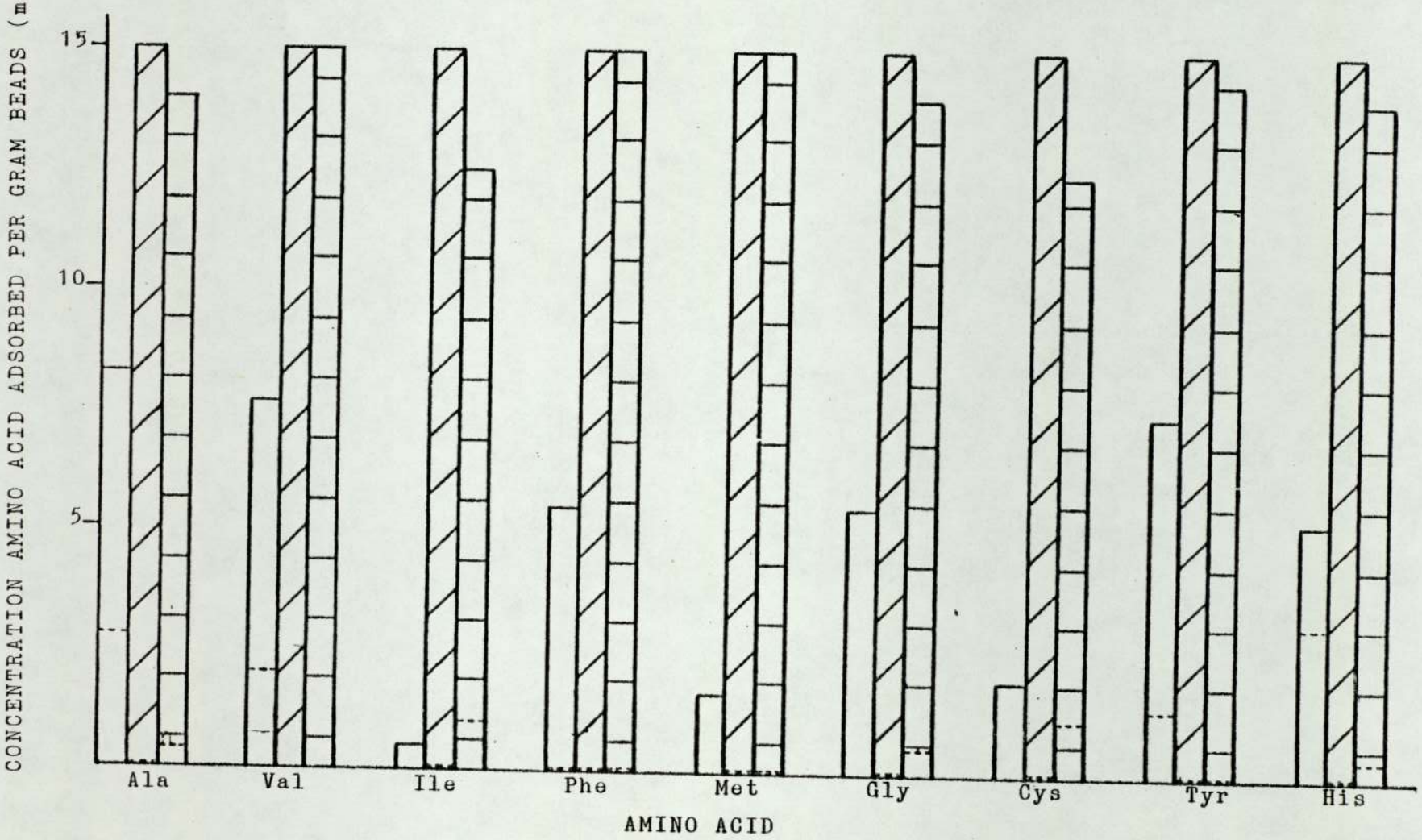


Fig 6.3 ADSORPTION OF AMINO ACIDS BY STRONGLY ACIDIC CATION EXCHANGE RESINS: STATIC ADSORPTION STUDY (1 HOUR)

CONDITIONS: $[\text{amino acid}]_o \equiv 15$ mg Nl^{-1}; 1g adsorbent; 5 ml amino acid solution

□ DOWEX 50W-X8; ▨ DUOLITE C255(H^+): ▤ POLYHEMA COATED DUOLITE C255(H^+)

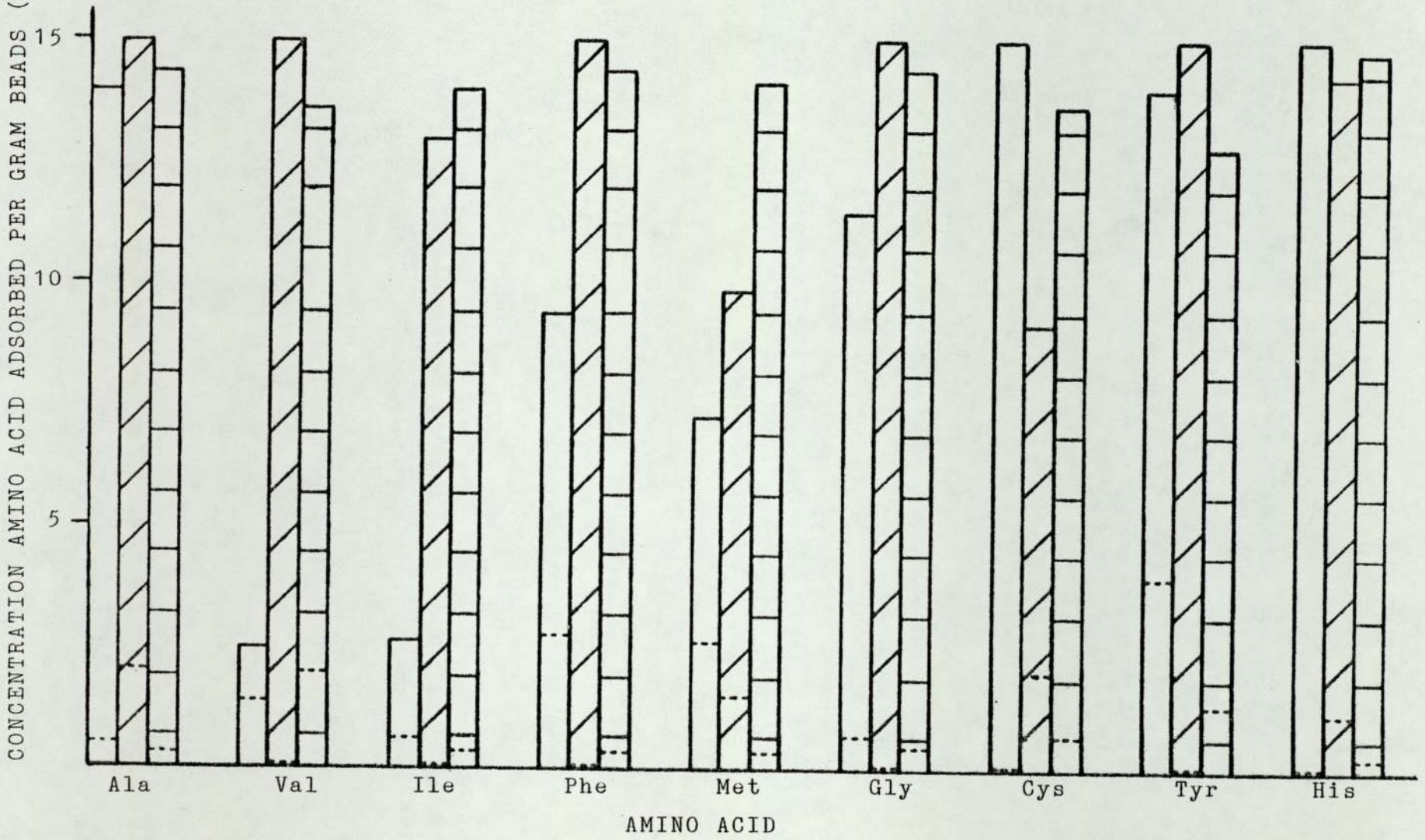
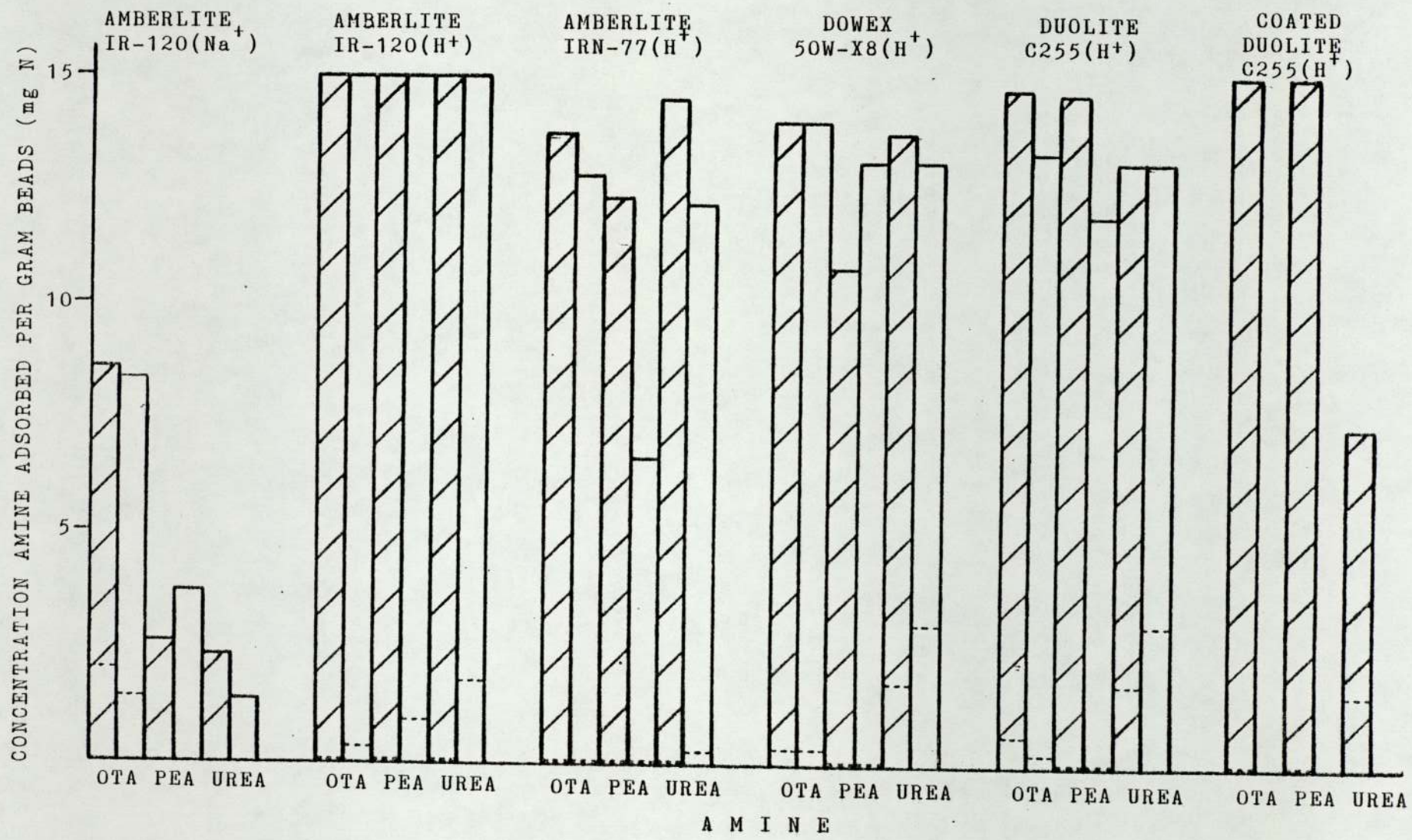


Fig 6.4 ADSORPTION OF AMINES ON STRONGLY ACIDIC CATION EXCHANGE RESINS: STATIC ADSORPTION STUDY

CONDITIONS: $[AMINE]_o \equiv 15$ mg Nl^{-1}; 1g adsorbent; 5 ml amine solution

▨ 1 hour study; □ 2 hour study —— adsorption study; --- desorption study



It is appreciated that most adsorbents require a biocompatible coating of some kind before their use in clinical haemoperfusion systems, for reasons previously detailed in Chapter 4.2. This project uses the hydrogel polyHEMA as the coating material for Duolite C255 (H^+) resin. This resin was selected for coating because it is clean (i.e. it does not produce a 'colour throw' into samples) and has a great affinity, when uncoated, for the model toxins studied. The effect of the polyHEMA coating on the adsorption of model toxins by this resin was investigated in a one hour static adsorption study. The results obtained show that the model toxins, e.g. amino acids (presented in Fig 6.3), false neurotransmitter amines and urea (presented in Fig 6.4) were adsorbed, with adsorption being very similar to that on the uncoated resin. These results illustrate that equilibrium adsorption of the model toxins was not affected by the presence of the polyHEMA encapsulation.

To test the feasibility of such results, an investigation of the adsorption of these model toxins was conducted, using novel macroporous hydrogel polymer particulates, based on the hydrogel polyHEMA. Since the removal of such high levels of amino acids, amines and urea, as those removed by the ion exchange resins, would not be required in clinical haemoperfusion, the ideal adsorbent is one possessing a lower affinity for these toxins. This adsorbent would also need to be biocompatible to

abolish the need for a suitable selectively permeable biocompatible coating. However, similar functional groups and properties of ion exchange resins, observed to be effective in the removal of these model toxins, would be important assets in such purposely designed adsorbents. With these points in mind, a range of hydrogels were synthesised (in a separate project) and evaluated for the adsorption of amino acids, amines and urea in this project. This study is presented in the following section.

6.4 Adsorption of Amino Acids and Amines on Particulate Hydrogels : Static Adsorption Studies

The effects of liver disease on amino acid metabolism [76] and false neurotransmitter formation [8] have received much attention, and their adsorption on materials such as charcoal and neutral polymeric uncharged resins [117] have been attempted. However, there is no record in the literature relating to the use of hydrogels in particulate form, as adsorbent materials for such nitrogen based hepatic toxins. This project employs a selection of hydrogels based on polyHEMA as adsorbent materials, and investigates their ability to adsorb amino acids (e.g. methionine, phenylalanine, tyrosine, histidine, glycine), false neurotransmitter amines (e.g. octopamine, phenylethanolamine, ethanolamine) and urea. The structure and properties of the hydrogels employed in this study are detailed in Table

Table 6.3 Macroporous Hydrogel Beads Used in Study

Code Number	Composition	Effective Size (mm)	Equilibrium Water Content EWC (%)	Synthetic Technique
S1	HEMA/AA/EDM (40:60:8.5)	<0.15	64	Suspension polymerisation (brine)
S2	HEMA/AA/EDM (50:50:10)	1.0-4.0	57	Freeze-thaw
S3	HEMA/EDM (100:10)	0.5-1.0	56	Suspension polymerisation (brine)
S4	AA/EDM (100:8.5)*	0.1-3.0	75*	Freeze-thaw
S5	HEMA/MAA/EDM (50:50:4.6)	0.025-0.125	58	Suspension polymerisation (brine)
S6	HEMA/NMACR/EDM (90:10:8.5)	0.5-1.0	61	Suspension polymerisation (brine)
S7	HEMA/DAA/EDM (100:25:9)	0.25-0.5	50	Suspension polymerisation (brine)
S8	ACR/NN'MBA (100:10)	1.0-1.5	80	Inverse suspension polymerisation (hexane)

*some possible doubt exists as to the precise identity of this specimen

6.3, and are the same as those used for the adsorption of ammonia (Chapter 5, Table 5.3).

By the conventional static adsorption studies, 1g of adsorbent material was shaken with 5 ml of model toxin solution, containing 15 mg Nl^{-1} for one hour, and the liquid samples decanted-off for analysis. Similarly, this procedure was repeated in desorption studies using distilled water, and the liquid samples analysed for the release of adsorbed model toxin.

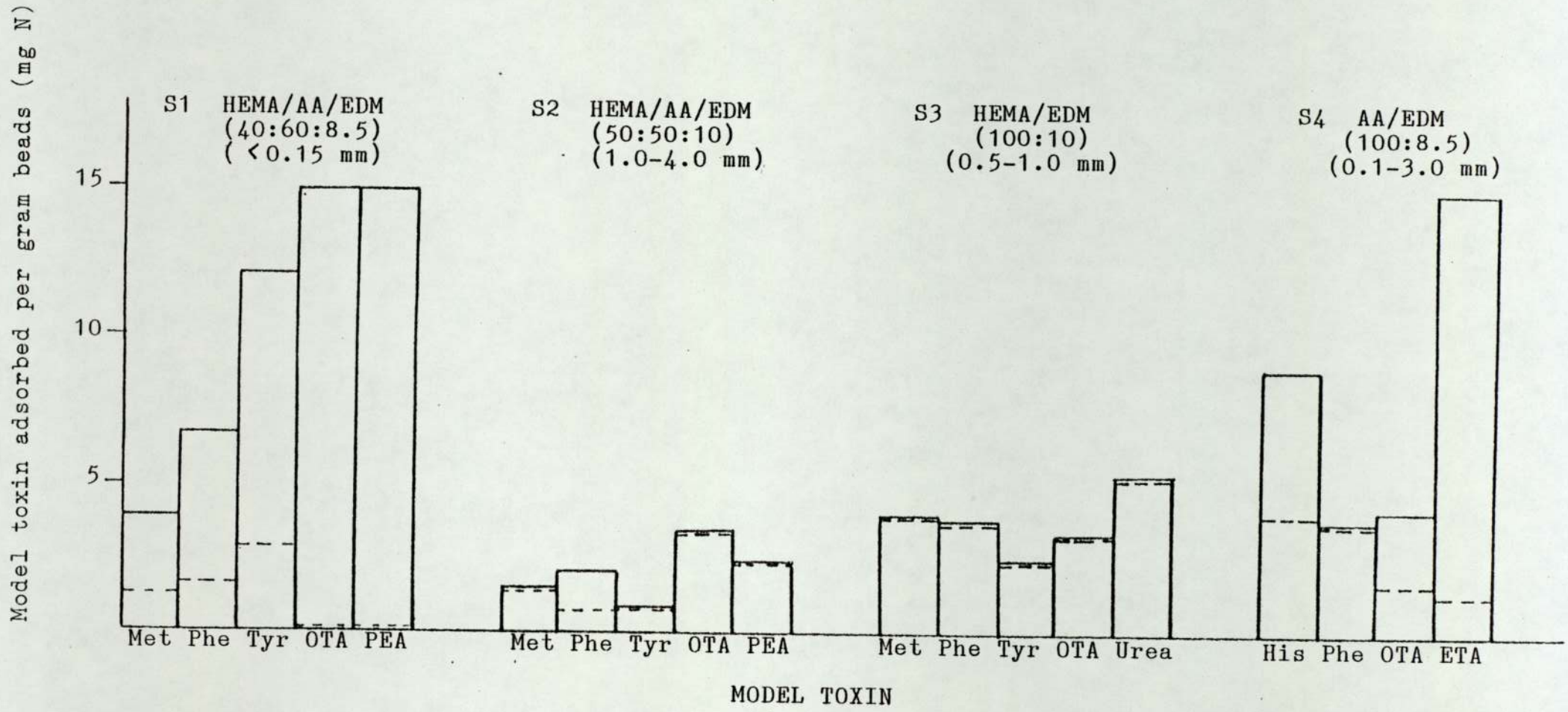
The observations obtained for the hydrogel systems S1 - S4, presented in Fig 6.5, demonstrate the ability of HEMA/AA/EDM(S1) particulates to adsorb the selected model toxins studied. The adsorbed toxins were also strongly bound to the adsorbent, as shown in the desorption studies, where only a small percentage of adsorbed model toxin was released. The fact that a large particle size conveys a small surface area, and thus a decrease in adsorption, is demonstrated by the adsorption on system S2, which is similar in composition to system S1, but larger in particle size.

Particulates of systems S3 (HEMA/EDM) and S4 (AA/EDM) also adsorbed the model toxins studied. In general, it was observed that the adsorption capacity of such particulates composed of individual monomers was inferior to that obtained with particulates containing both monomers, e.g. system S1 (HEMA/AA/EDM).

Fig 6.5 ADSORPTION OF AMINO ACIDS AND AMINES ON PARTICULATE HYDROGELS

CONDITIONS: $[\text{MODEL TOXIN}]_o \equiv 15$ mg Nl^{-1}; 1g adsorbent; 1hr static adsorption study;

—— adsorption study; --- desorption study



An additional study with particulates of system S4 was performed using a variation on the conventional static adsorption technique. This involved shaking the adsorbent with 5 ml model toxin solution containing 15 mg Nl^{-1}, for a period of time, and then analysing the liquid sample. A fresh model toxin solution was then added and the procedure continued until three such samples were collected. The adsorption of histidine and ethanolamine was investigated by this technique. The results presented in Fig 6.6 show that such model toxins were adsorbed by hydrogel particulates of acrylic acid (S4) and adsorption is comparable to that observed with the weakly acidic cation exchange resin, Amberlite IRC-50 (H^+)(Fig 6.1).

Other hydrogel particulates employed, composed of acidic or basic monomers, also possessed the ability to adsorb the model toxins studied (Fig 6.7). However, although the absolute accuracy of adsorption levels is open to some doubt, the trends are clearly shown to indicate a considerable difference in adsorption capabilities between monomer structures studied.

6.5 Adsorption of Amino Acids and Amines on Uncoated and Coated Adsorbents : Dynamic Adsorption Studies

Although the static adsorption experiments previously described provide a reasonable basis for the

Fig 6.6 ADSORPTION OF HISTIDINE AND ETHANOLAMINE ON NOVEL MACROPOROUS HYDROGEL AA(100) EDM(8.5) SYSTEM S4 : STATIC ADSORPTION STUDY

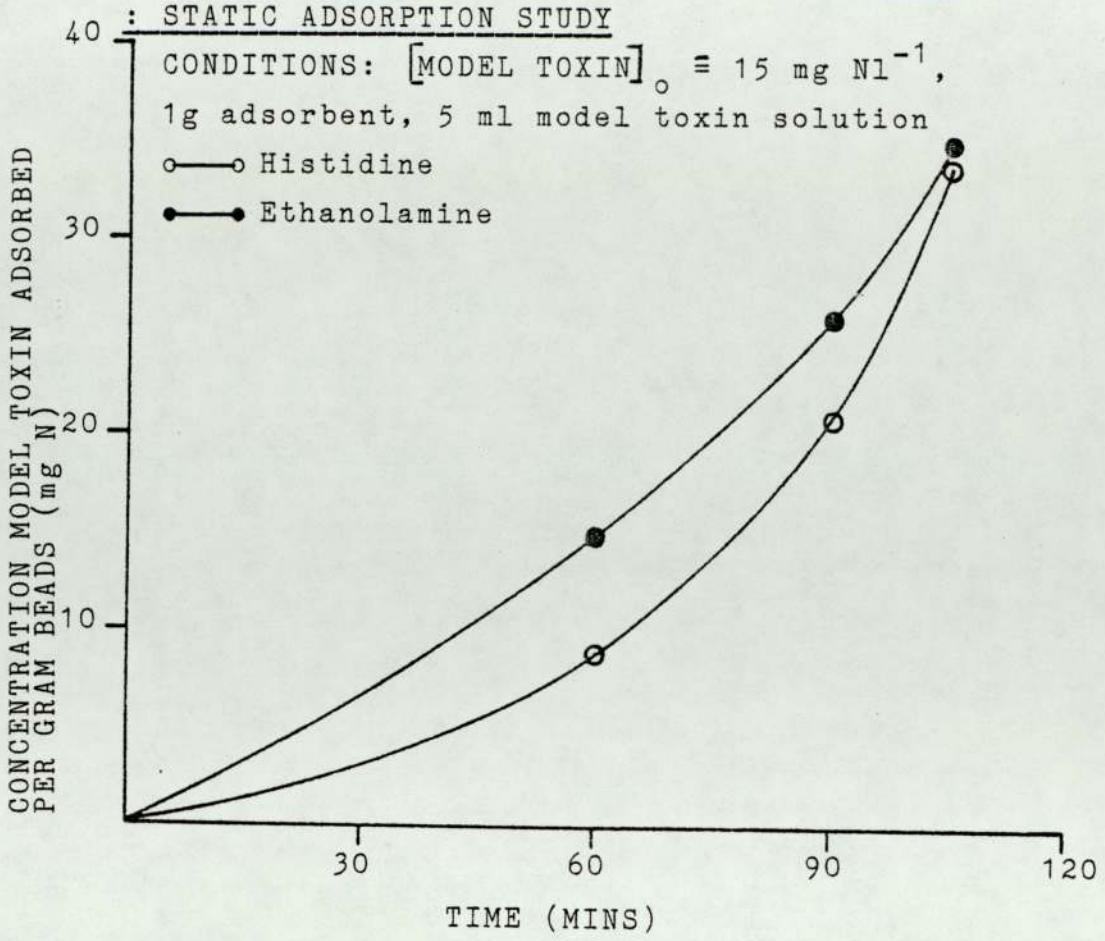
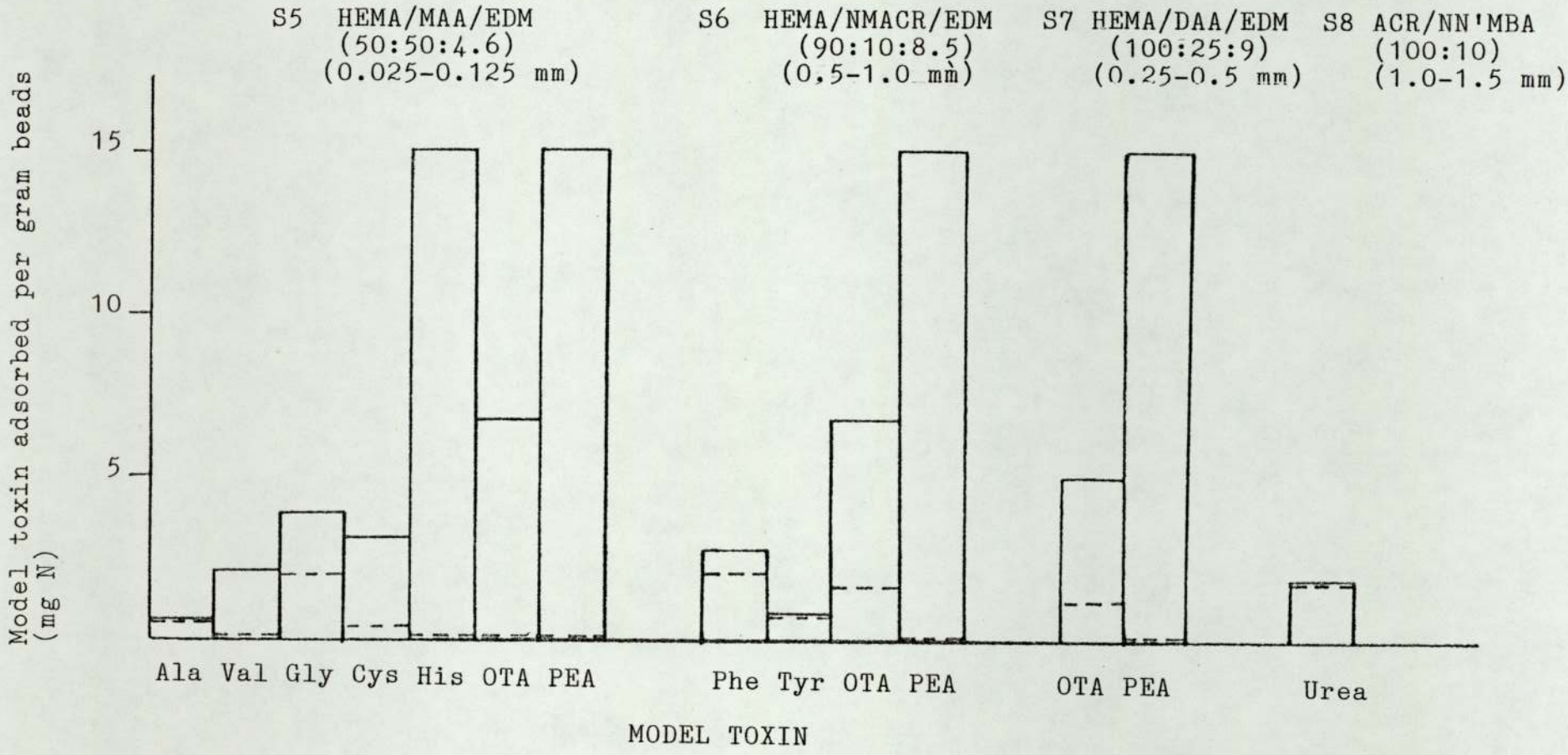


Fig 6.7 ADSORPTION OF AMINO ACIDS AND AMINES ON PARTICULATE HYDROGELS

CONDITIONS: $[\text{Model Toxin}]_o \equiv 15$ mg Nl^{-1}; 1g adsorbent;
1hr static adsorption study;
—— adsorption study; ---desorption study



comparison of relative adsorption efficiences, in order to investigate the adsorption of amino acids and amines under conditions which more closely mimic clinical haemoperfusion systems, dynamic adsorption studies were carried out. Such studies involved placing 5g adsorbent into the flow cell of the dynamic adsorption system (Plate 3.1) and perfusing the model toxin solution (containing 15 mg Nl^{-1}) over the adsorbent at the desired flow rate, for the desired length of time. Sample fractions were then analysed for unadsorbed model toxin.

6.5.(1) Adsorption on Acidic (Cationic) Exchange Resins

The acidic cation exchange resins were used in this study because they demonstrated a strong affinity for the model toxins studied under static adsorption conditions. The model toxins (e.g. amino acids and false neurotransmitter amines) employed in the dynamic adsorption studies include a selection from those significantly elevated in liver failure, e.g. phenylalanine, methionine, tyrosine, histidine, octopamine and phenylethanolamine, and also glycine and cysteine. The results from the dynamic adsorption study of amino acids on uncoated ion exchange resins are presented in Figs 6.8 - 6.15.

On the whole the ion exchange resins demonstrated a large adsorptive capacity for the amino acids studied, with adsorption by the strongly acidic resins being

Fig 6.8

ADSORPTION OF METHIONINE ON AMBERLITE IR-120 (Na^+) RESIN

CONDITIONS: $[\text{Methionine}]_o \equiv 15$ mg Nl^{-1}; 5g adsorbent; flow rate = 1.5 mls/min for 24 hours

—— amino acid adsorbed; --- cumulative amino acid adsorbed

Fig 6.9

ADSORPTION OF PHENYLALANINE ON AMBERLITE IR-120 (Na^+) RESIN

CONDITIONS: $[\text{Phenylalanine}]_o \equiv 15$ mg Nl^{-1}; 5g adsorbent; flow rate = 1.5 mls/min for 3 hours; 8mls/sample

—— amino acid adsorbed; --- cumulative amino acid adsorbed

Fig 6.8

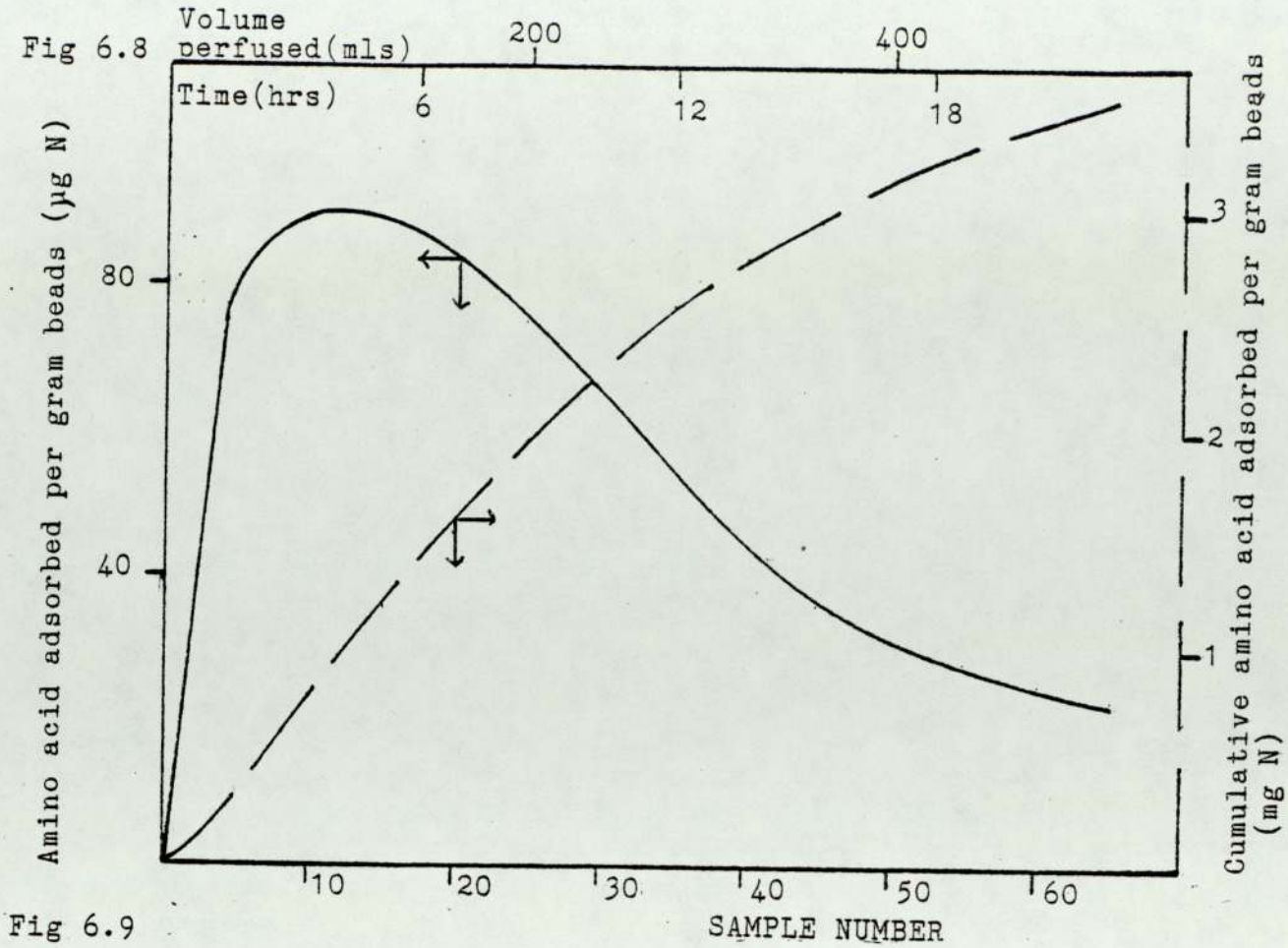

Volume perfused(mls)
200
400
Time(hrs)
6
12
18
80
40
3
2
1
10
20
30
40
50
60
SAMPLE NUMBER
Amino acid adsorbed per gram beads (µg N)
Cumulative amino acid adsorbed per gram beads (mg N)

Fig 6.9

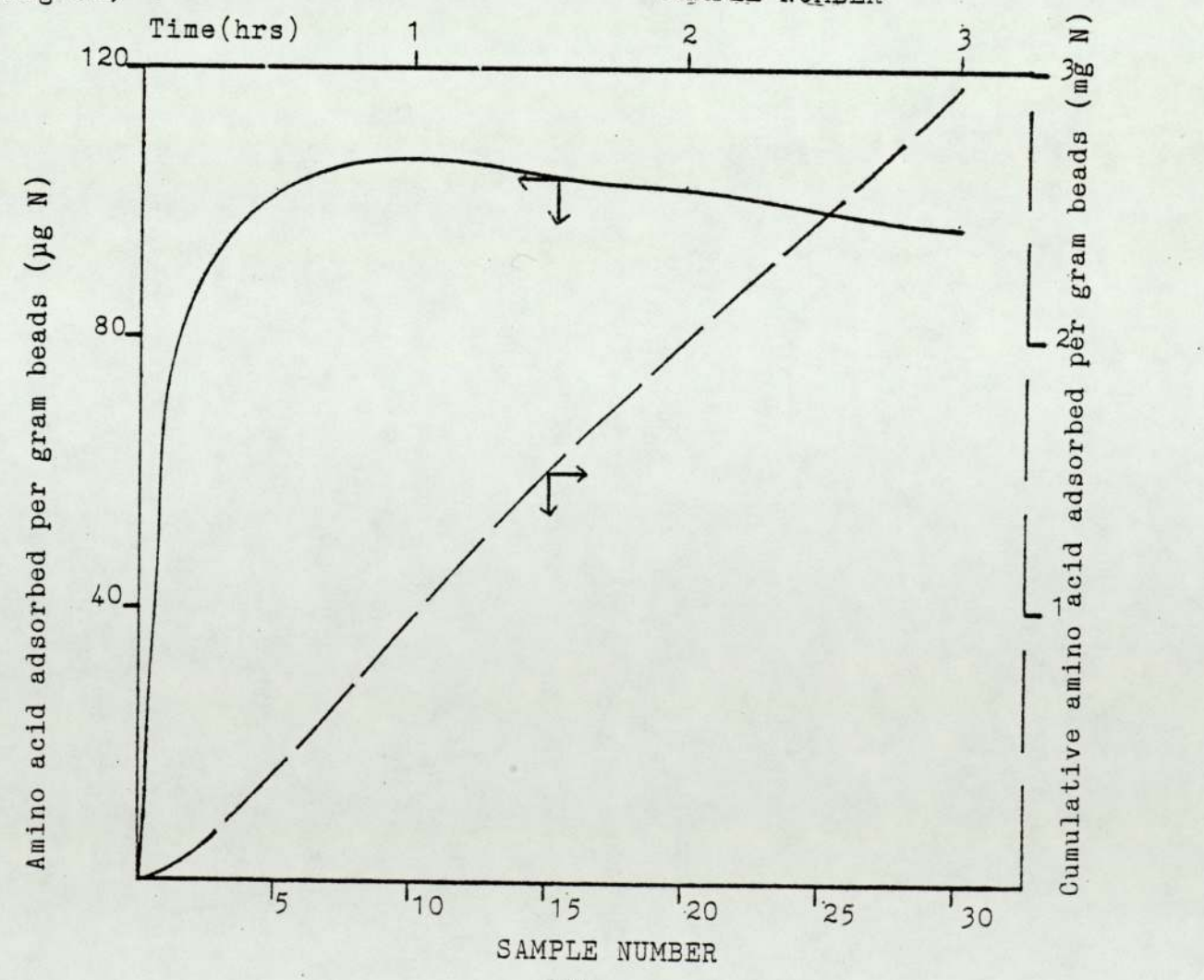

Time(hrs)
1
2
3
120
80
40
3
2
1
5
10
15
20
25
30
SAMPLE NUMBER
Amino acid adsorbed per gram beads (µg N)
Cumulative amino acid adsorbed per gram beads (mg N)

Fig 6·10

ADSORPTION OF PHENYLALANINE ON DUOLITE C255 (H^+) RESIN

CONDITIONS: $[\text{Phenylalanine}]_o \equiv 15$ mg Nl^{-1}; 5g adsorbent;

flow rate = 2 mls/min for 24 hours;

—— amino acid adsorbed; --- cumulative amino acid adsorbed

Fig 6·11

ADSORPTION OF GLYCINE ON DUOLITE C255 (H^+) RESIN

CONDITIONS: $[\text{Glycine}]_o \equiv 15$ mg Nl^{-1}; 5g adsorbent;

flow rate = 2 mls/min for 3 days; 8mls/sample

—— amino acid adsorbed; --- cumulative amino acid adsorbed

Fig 6.10

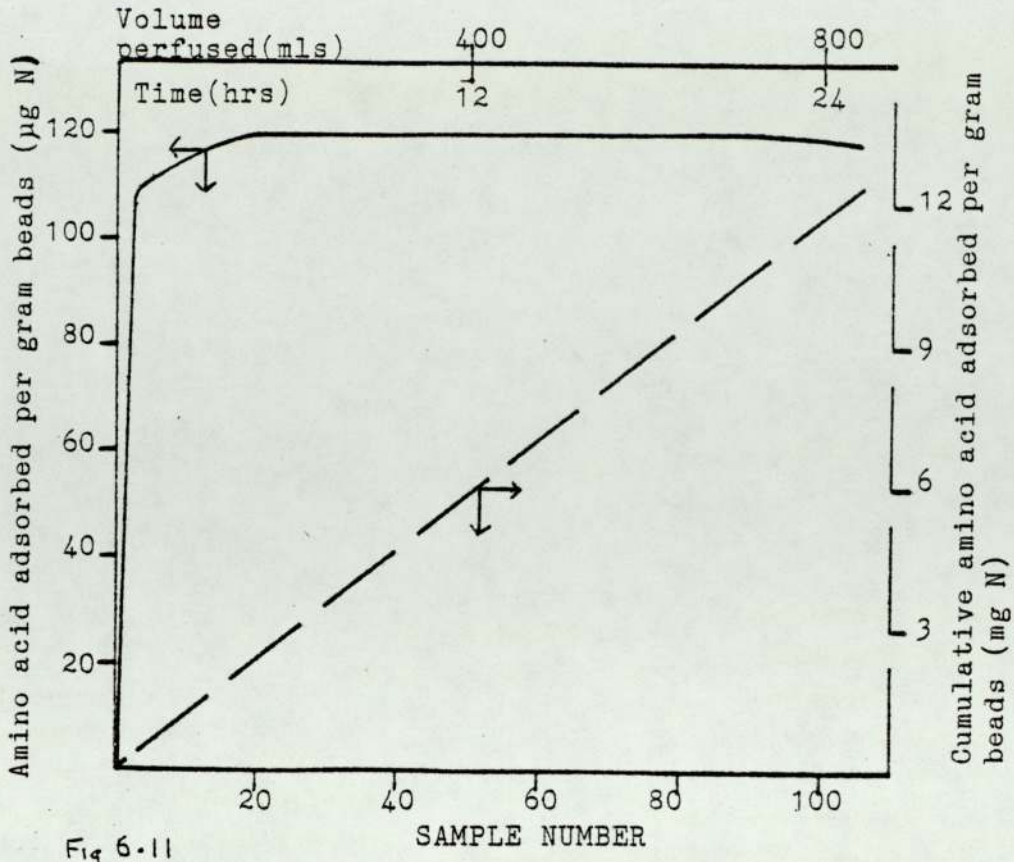


Fig 6.11

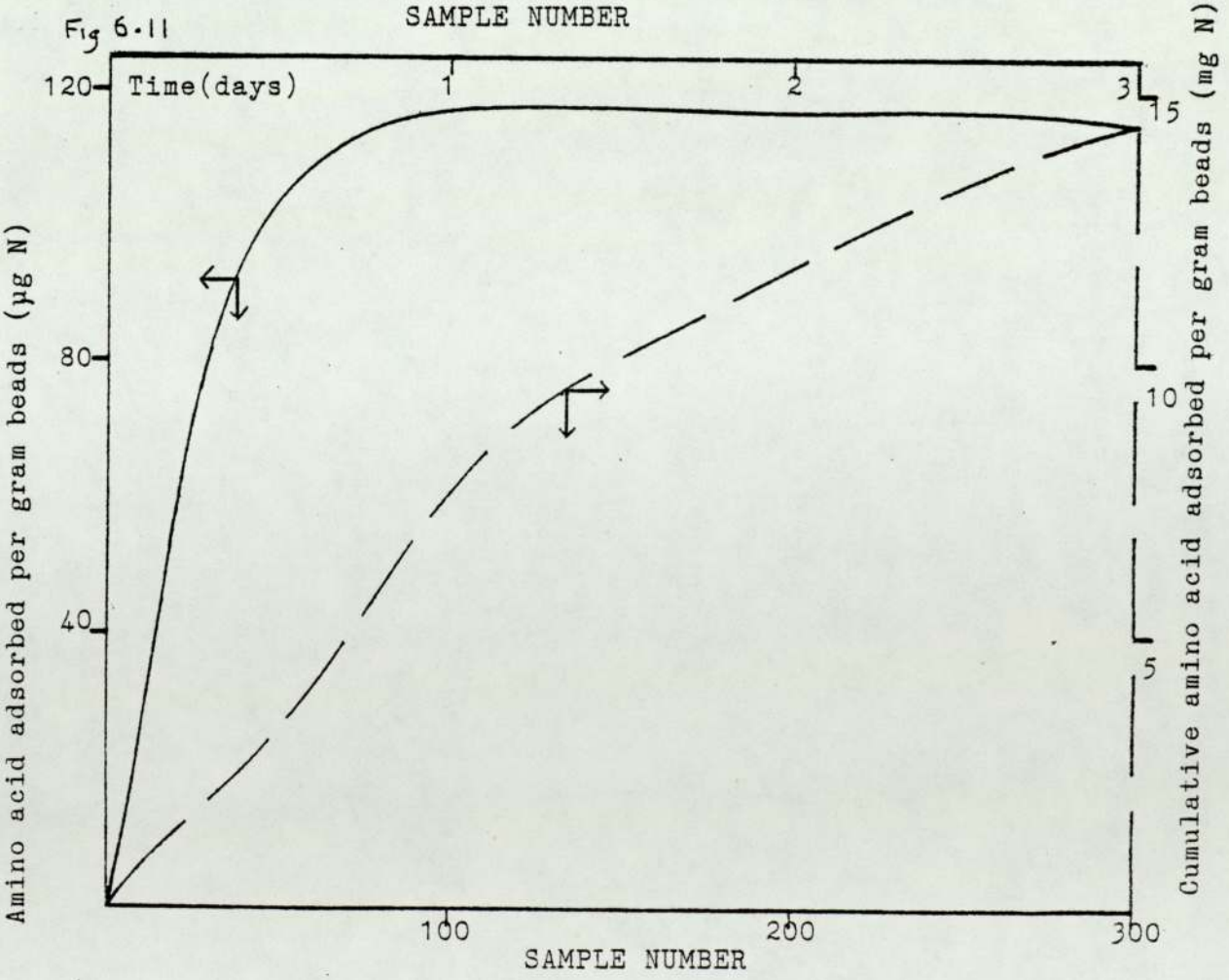


FIG 6·12

ADSORPTION OF CYSTEINE ON DOWEX 50W-X8 (H^+) RESIN

CONDITIONS: $[\text{Cysteine}]_o \equiv 15$ mg Nl^{-1}; 5g adsorbent; flow rate = 2 mls/min for 3 hours;

—— amino acid adsorbed; --- cumulative amino acid adsorbed

FIG 6·13

ADSORPTION OF TYROSINE ON AMBERLITE IR-120 (H^+) RESIN

CONDITIONS: $[\text{Tyrosine}]_o \equiv 15$ mg Nl^{-1} (in 0.9% saline); 5g adsorbent; flow rate = 2 mls/min for 24 hours;

—— amino acid adsorbed; --- cumulative amino acid adsorbed

3mls/sample

Fig 6.12

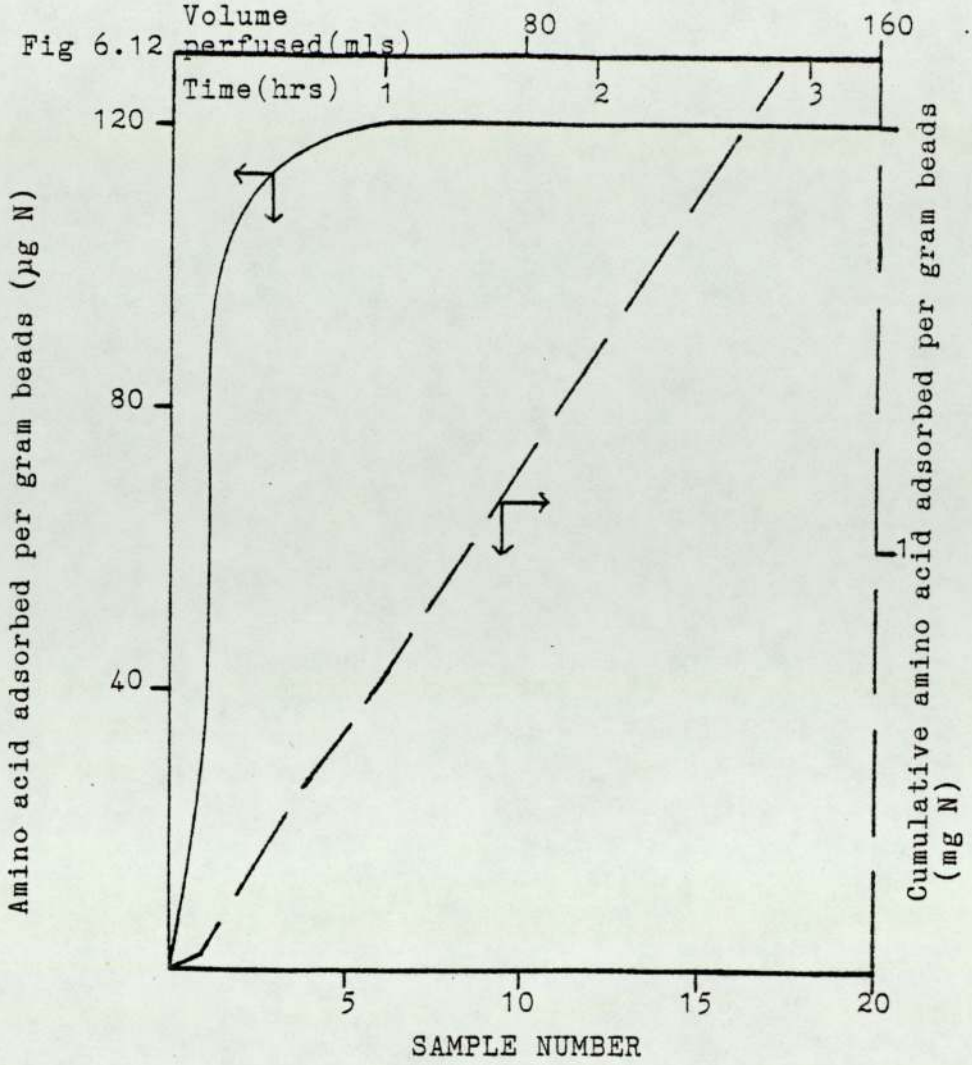


Fig 6.13

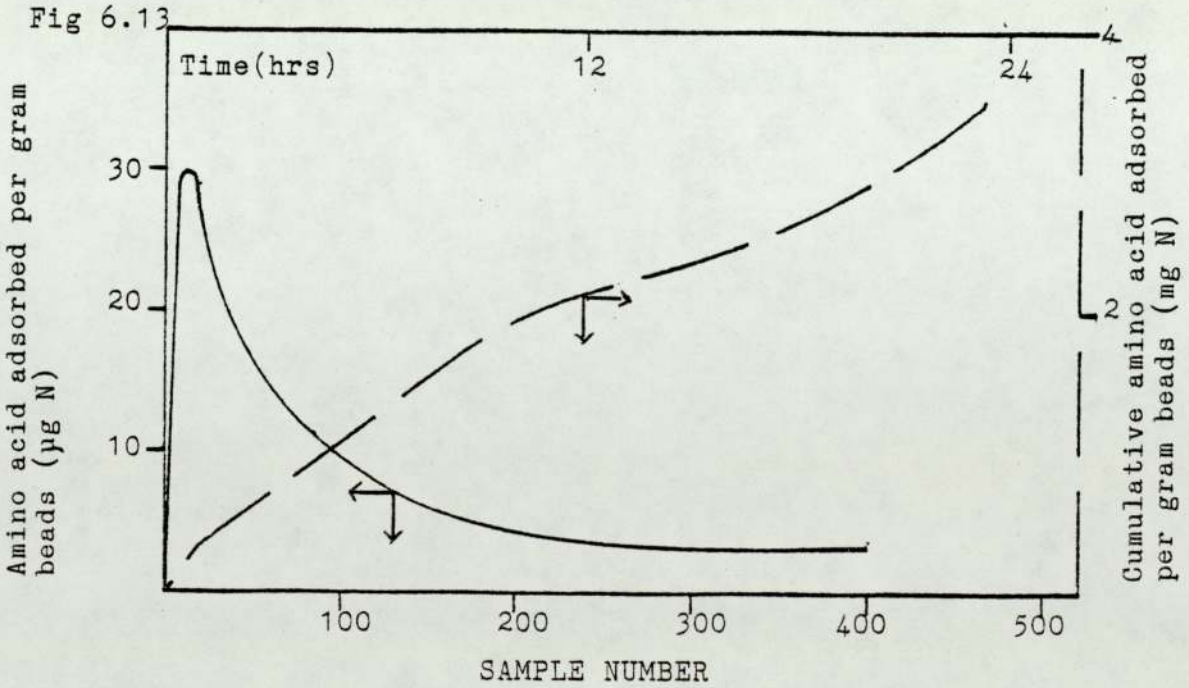


FIG 6·14

ADSORPTION OF HISTIDINE ON AMBERLITE IRC-50 (H^+) RESIN

CONDITIONS: $[\text{Histidine}]_o \equiv 15$ mg Nl^{1-}; 5g adsorbent;
flow rate - 4.5 mls/min for $2\frac{1}{2}$ hours;

—— amino acid adsorbed; --- cumulative amino acid adsorbed

FIG 6·15

ADSORPTION OF HISTIDINE ON DOWEX 50W-X8 (H^+) RESIN

CONDITIONS: $[\text{Histidine}]_o \equiv 15$ mg Nl^{-1}; 5g adsorbent;
flow rate = 2 mls/min for 24 hours; 8mls/sample

—— amino acid adsorbed; --- cumulative amino acid adsorbed

Fig 6.14

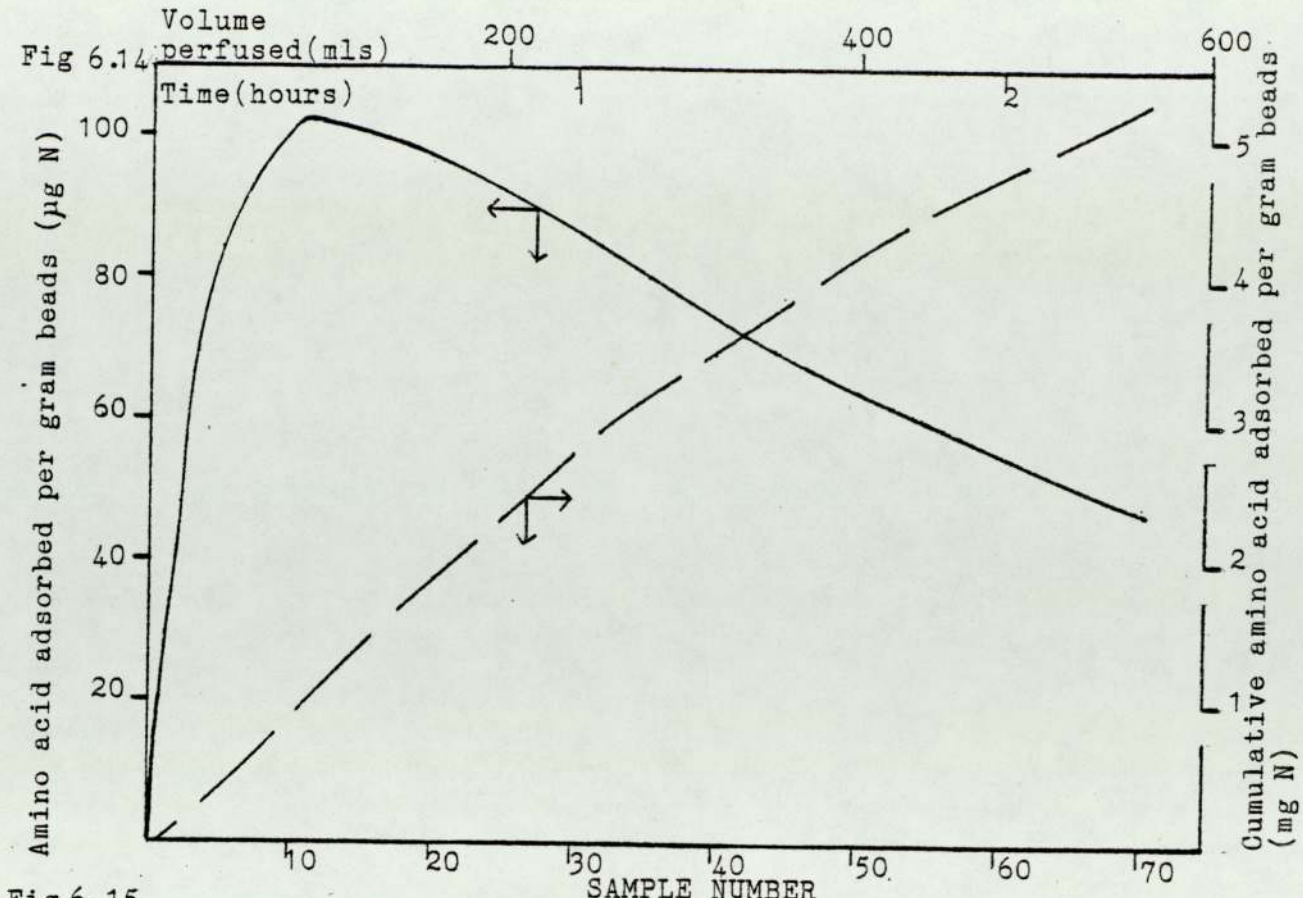
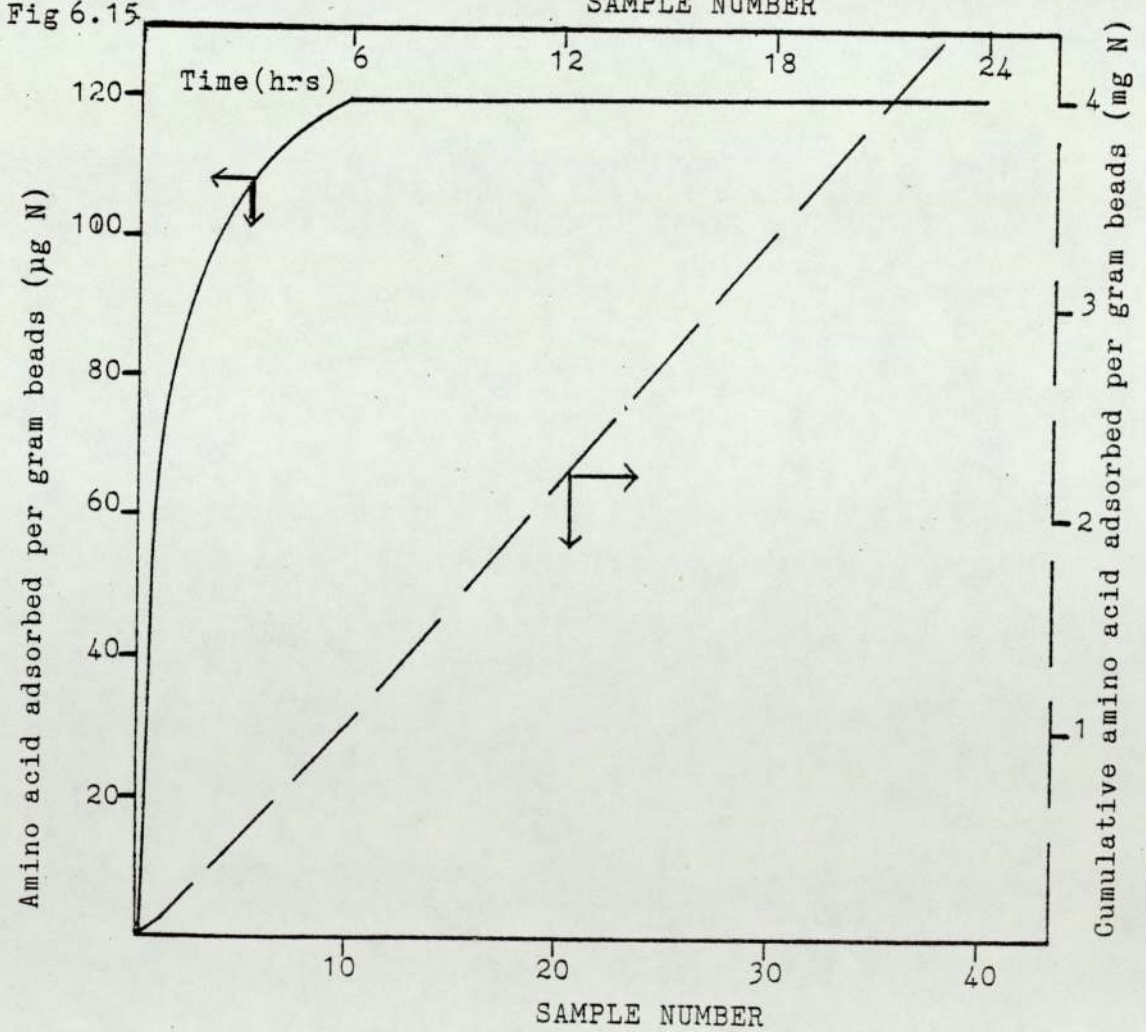


Fig 6.15



superior to that obtained for the weakly acidic resin. In addition, the acidic (H^+) form of the resins presented a greater adsorptive capacity for the amino acids, than that obtained with the sodium form (Na^+). Such results are consistent with those previously obtained in static adsorption studies.

In general the physiological and pathological amino acid levels cover a wide range, in different individuals. In hepatic failure, the removal of amino acids to levels within the normal physiological spectrum is desirable. However, the performance of the ion exchange resins in amino acid removal show that they possess a greater affinity than would be required, since it is not necessary,and furthermore, not beneficial to deplete amino acid levels during haemoperfusion treatment. A more detailed discussion of this point will be considered in the final discussion, where amino acid levels in normal subjects and hepatic failure patients will be presented in a table.

Despite this large adsorption capacity of ion exchange resins, however, further studies employing poly-HEMA coated Duolite C255 (H^+) resin were conducted. This evaluation was performed since a biocompatible coating is normally an essential requirement for most adsorbent materials used in haemoperfusion systems. Also some knowledge of the effect of the encapsulating material

on adsorption is required.

The dynamic adsorption study was carried out, using a slightly different approach. Ammonia solution (as ammonium chloride) containing 15 mg Nl^{-1} was perfused over polyHEMA coated Duolite C255 (H^+) resin, followed by a water wash and then a solution of phenylalanine, also containing 15 mg Nl^{-1}. Sample fractions were then analysed on the AutoAnalyser. The objective of this exercise was to observe the extent that competition and saturation of adsorptive sites for different toxins occur. The results of this study are presented in Fig 6.16. Duplicate studies in which the adsorbent was first perfused with water produced similar results and are presented in Fig 6.17. These studies, when taken in comparison with those involving uncoated resins (e.g. Fig 6.8 - 6.15) give some information of the role of polyHEMA coatings in impeding transport and adsorption.

The adsorption of false neurotransmitter amines over uncoated and coated resins was also studied under dynamic adsorption conditions. The results of this study are presented in Figs 6.18 and 6.19. By comparing these results with those obtained in similar studies with ammonia (Figs 5.12 - 5.17) the difference in adsorption of false neurotransmitter amines and ammonia on ion exchange resins can be seen. The adsorption capacity of the uncoated resin for the false neurotransmitter amine, octopamine, is comparable to that observed with

FIG 6·16

ADSORPTION OF AMMONIA AND PHENYLALANINE ON 5% POLYHEMA COATED DUOLITE C255 (H^+) RESIN

CONDITIONS: $[NH_4Cl]_o \equiv 15$ mg Nl^{-1} @ 2 mls/min; d.H_2O @ 5 mls/min; $[\text{Phenylalanine}]_o \equiv 15$ mg Nl^{-1} @ 2 mls/min; 5g adsorbent;

—— model toxin adsorbed; --- cumulative model toxin adsorbed

FIG 6·17

ADSORPTION OF AMMONIA AND PHENYLALANINE ON 5% POLYHEMA COATED DUOLITE C255 (H^+) RESIN

CONDITIONS: d.H_2O @ 2 mls/min; $[NH_4Cl]_o \equiv 15$ mg Nl^{-1} @ 2 mls/min; $[\text{Phenylalanine}]_o \equiv 15$ mg Nl^{-1} @ 2 mls/min; 5g adsorbent;

—— model toxin adsorbed; --- cumulative model toxin adsorbed

Fig 6.16

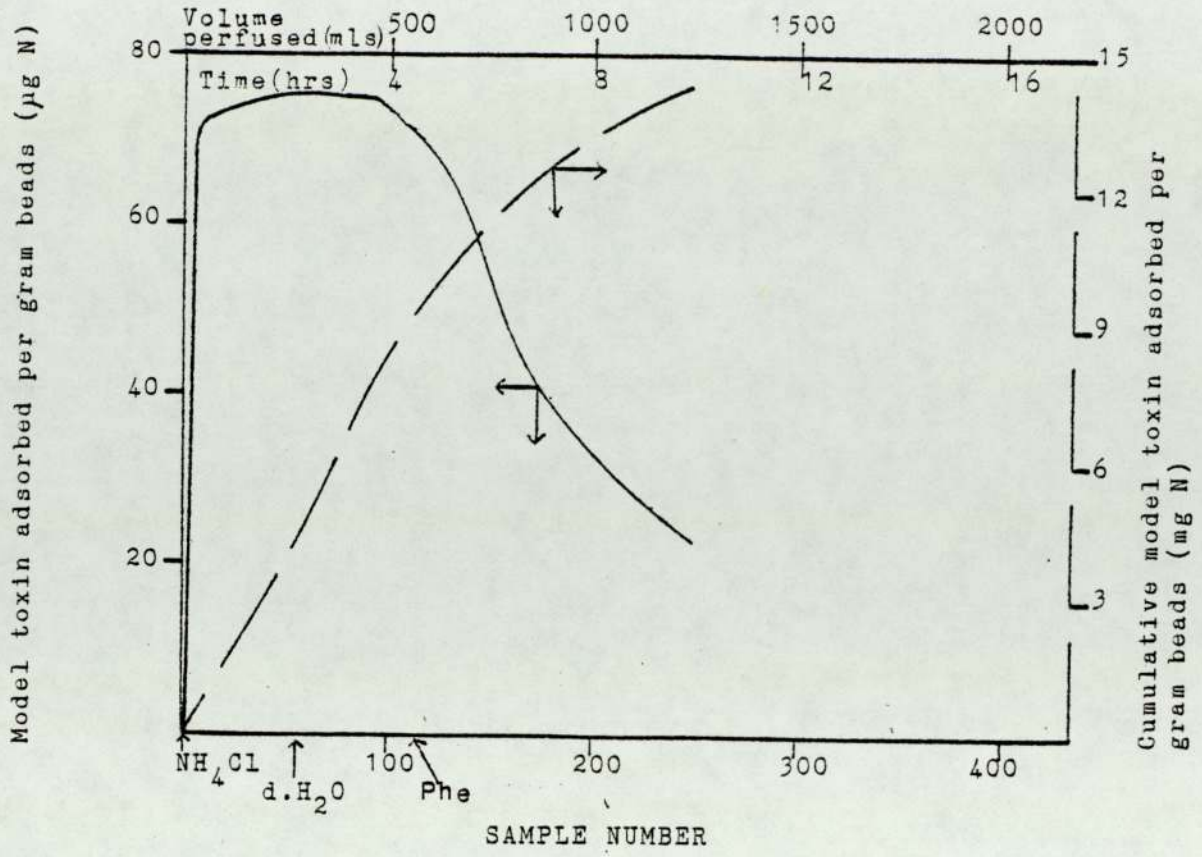


Fig 6.17

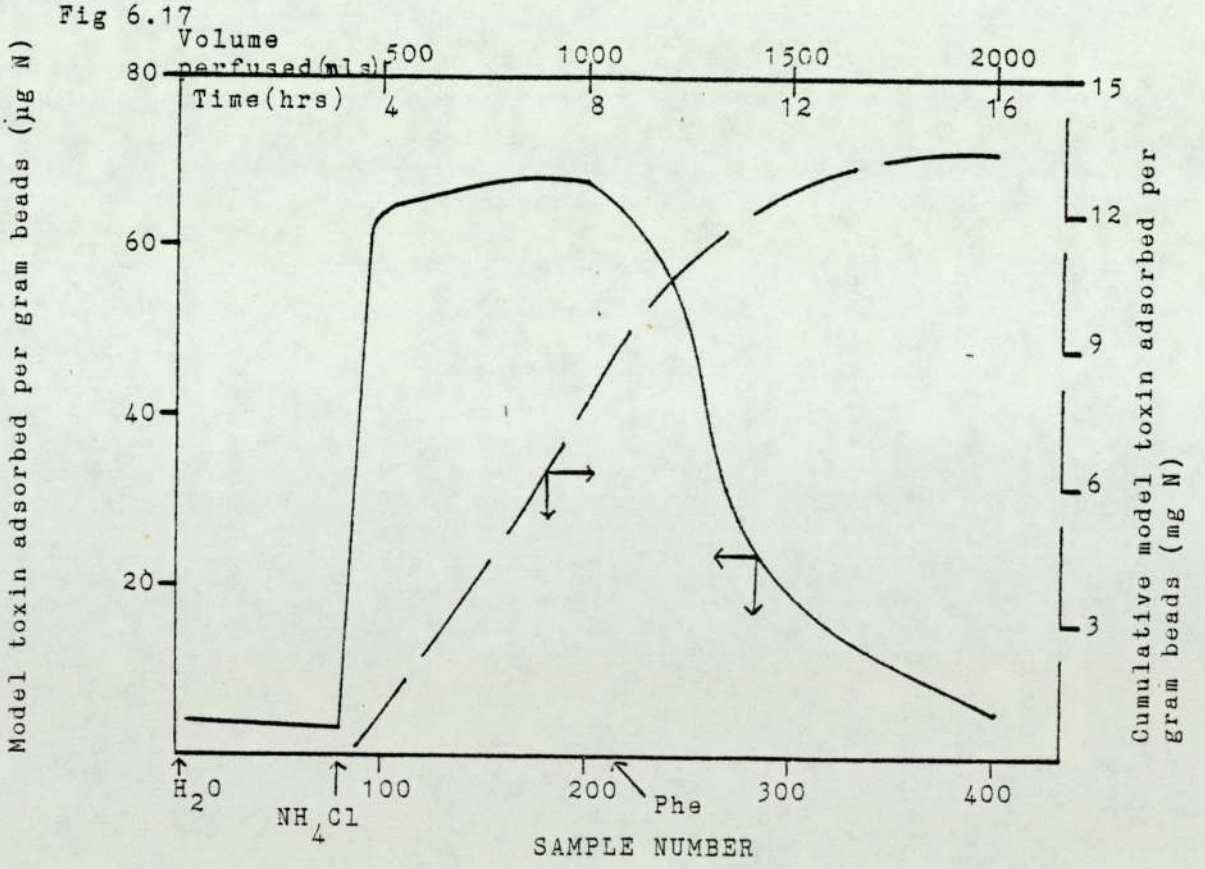


FIG 6·18

ADSORPTION OF OCTOPAMINE ON AMBERLITE IR-120 (H^+) RESIN

CONDITIONS: $[\text{Octopamine}]_o \equiv 15$ mg Nl^{-1}; (in 0.9% saline); 5g adsorbent; flow rate = 2 mls/min for 24 hours

—— amine adsorbed; --- cumulative amine adsorbed

FIG 6·19

ADSORPTION OF PHENYLETHANOLAMINE ON 5% POLYHEMA COATED DUOLITE C255 (H^+) RESIN

CONDITIONS: $[\text{Phenylethanolamine}]_o \equiv 15$ mg Nl^{-1} (in 0.9% saline); 5g adsorbent; flow rate = 2 mls/min for 12 hours; 3 ml/sample

—— amine adsorbed; --- cumulative amine adsorbed

Fig 6.18

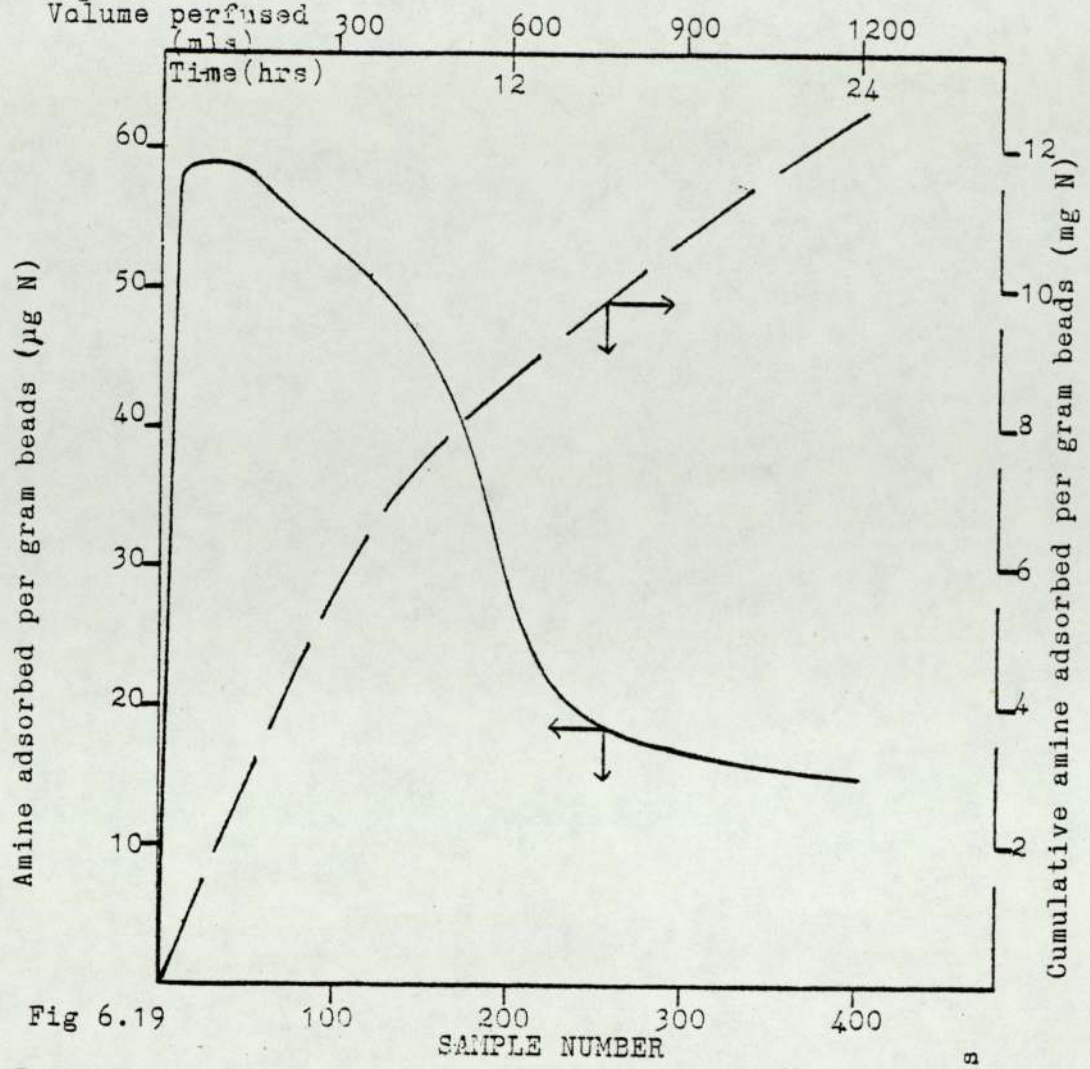
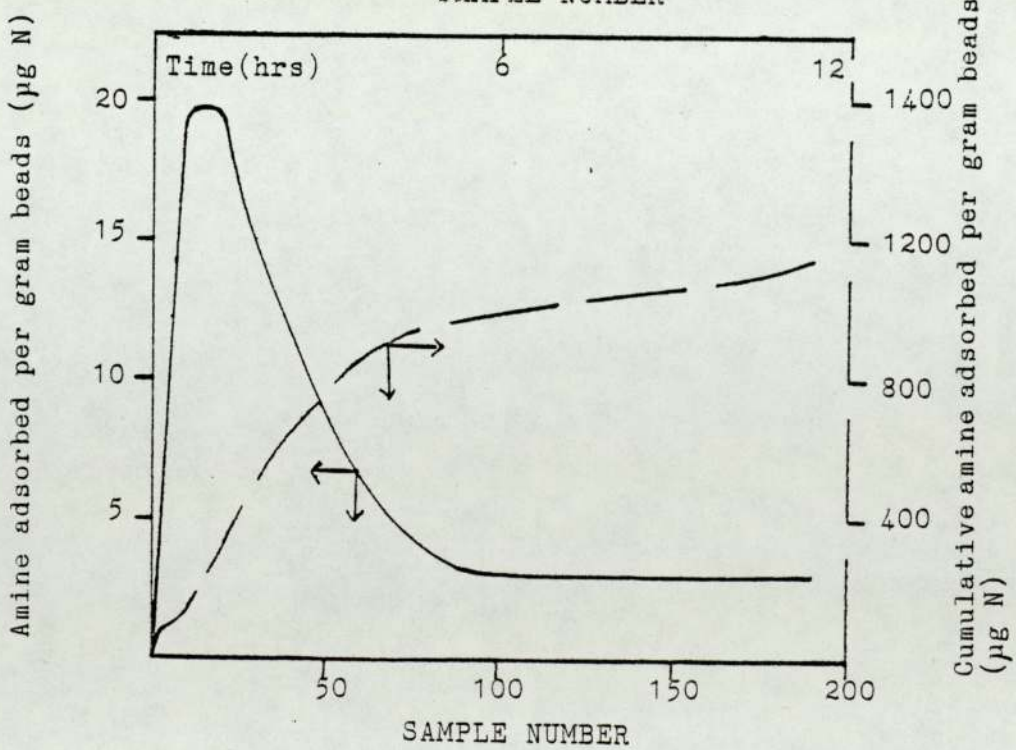


Fig 6.19



ammonia and amino acids. However, the adsorption of the false neurotransmitter amine, phenylethanolamine, on polyHEMA coated ion exchange resin is greatly reduced compared to amine adsorption on the uncoated resin and that observed for ammonia adsorption on coated ion exchange resins. This leads to the suggestion that the false neurotransmitters studied are not transported as readily through the polyHEMA coat to the adsorption sites of the resin. This point was indicated in Fig 6.16 and 6.17, where the adsorption of phenylalanine, an amino acid, was shown to be inferior to that of ammonia, and the reduced transport of molecules larger than and less soluble than ammonia through polyHEMA was indicated. The reduced adsorption of less soluble organic species such as false neurotransmitter amines also points to the suggestion that the rate of transport through dense polyHEMA diminishes with an increase in molecular size of the model toxin and the introduction of organic groups. The use of macroporous membranes or particulates would overcome this problem.

6.5.(2) Adsorption on Particulate Hydrogels

Novel macroporous hydrogels in particulate form are employed in this study (since they are themselves biocompatible) in an attempt to overcome the serious pro-

blems of biocompatibility generally encountered with the use of adsorbents in haemoperfusion systems. The hydrogel particulates have previously demonstrated a moderate and selective adsorptive capacity for the model toxins, under static adsorption conditions. This level of efficiency is desirable and more attractive than the over-efficient capacity presented by the ion exchange resins.

However, since static adsorption studies do not indicate the capacity of the beads, but really only compares the performance of the different adsorbents, dynamic adsorption studies are performed. Thus, the adsorption capacity for the model toxins is observed under conditons similar to that encountered in haemoperfusion systems.

The acidic and basic hydrogel particulates evaluated are shown in Table 6.4. Those particulates used show some differential adsorption efficiency for the model toxins under static adsorption studies.

Histidine and octopamine, both elevated to pathological levels in liver failure were perfused over the hydrogel particulates, and their adsorption observed. The results of this study are presented in Figs 6.20 - 6.23, and demonstrate that the adsorption of histidine and octopamine is related to the chemical configuration of both the adsorbent and model toxin, a point which will be subsequently discussed in more detail. In general, the

Table 6.4 Macroporous Hydrogel Beads Used in Dynamic Adsorption Studies

Code Number	Composition	Effective Size (mm)	Equilibrium Water Content EWC (%)	Synthetic Technique
D1 (=S4, Table 6.3)	AA/EDM (100:8.5)*	0.1-3.0	75*	Freeze-thaw
D2 (=S2, Table 6.3)	HEMA/AA/EDM (50:50:10)	1.0-4.0	57	Freeze-thaw
D3 (=S1 Table 6.3)	HEMA/AA/EDM (40:60:8.5)	< 0.15	64	Suspension poly-merisation (brine)
D4 (=S6, Table 6.3)	HEMA/NMACR/EDM (90:10:8.5)	0.5-1.0	61	Suspension poly-merisation (brine)

*some possible doubt exists as to the precise identity of this specimen

dynamic adsorption efficiency of the hydrogels, Figs 6.20 - 6.23 was consistent with that observed in the static adsorption studies (Figs 6.5 - 6.7).

It was apparent that the presence of acidic monomers such as acrylic acid in the composition of the hydrogel particulates, facilitated the adsorption of basic chemical species, such as histidine and octopamine Figs 6.20 - 6.23. The greater percentage by weight of acrylic acid in the particulates of system D1 (Fig 6.20) compared to that of system D2 (Fig 6.21) assisted the adsorption of histidine. With the substitution of the acidic acrylic acid (AA) group for the basic N-methylolacrylamide (NMACR) group, the adsorption of basic species such as octopamine, could be reduced (Figs 6.22 - 6.23). A small particle size and the water content of the hydrogel particulates are also important factors in the adsorption process.

The hydrogel particulates present a group of adsorbents which could be used to adsorb various levels of amino acids and amines. By the incorporation of the appropriate monomers in the hydrogel composition, such materials could be tailored to suit specific individual needs in artificial liver support systems.

FIG 6·20

ADSORPTION OF HISTIDINE ON AA/EDM (100:8.5) PARTICULATE HYDROGEL (D1)

CONDITIONS: $[\text{Histidine}]_o \equiv 15$ mg Nl^{-1}; 5g adsorbent; flow rate = 9 mls/min;

—— amino acid adsorbed; --- cumulative amino acid adsorbed

FIG 6·21

ADSORPTION OF HISTIDINE ON HEMA/AA/EDM (50:50:10) PARTICULATE HYDROGEL (D2)

CONDITIONS: $[\text{Histidine}]_o \equiv 15$ mg Nl^{-1}; 5g adsorbent; flow rate = 8 mls/min;

—— amino acid adsorbed; --- cumulative amino acid adsorbed

Fig 6.20

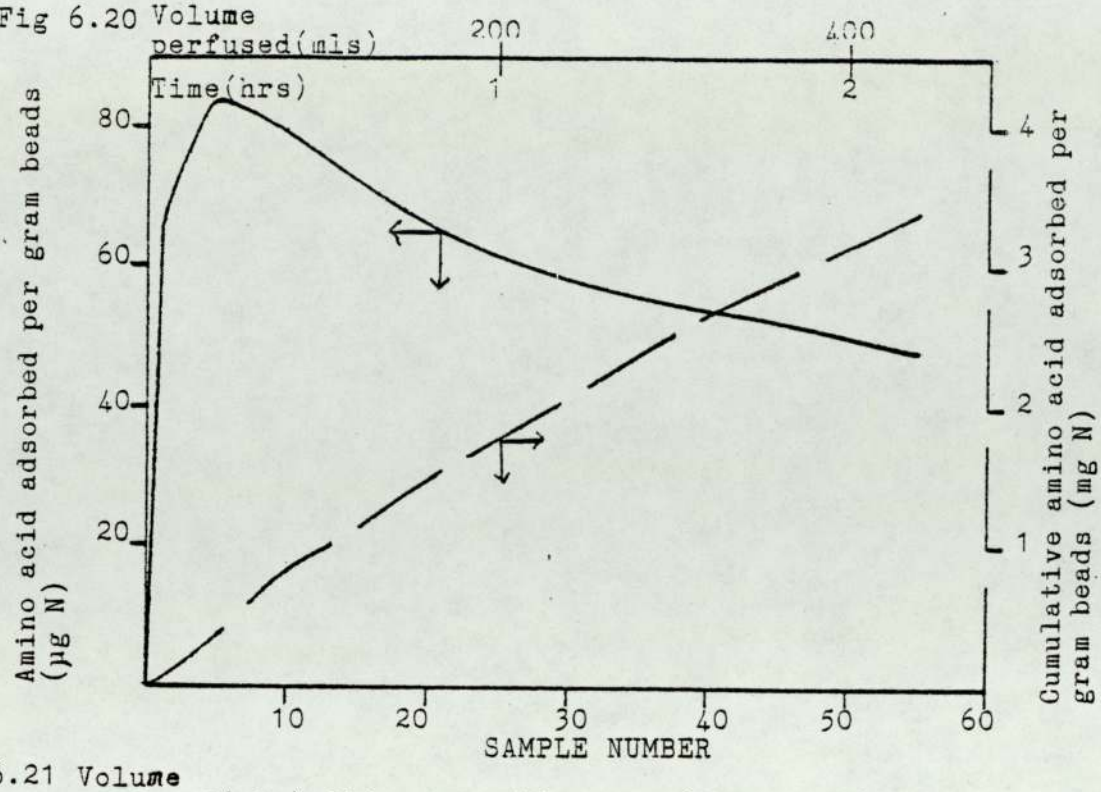


Fig 6.21

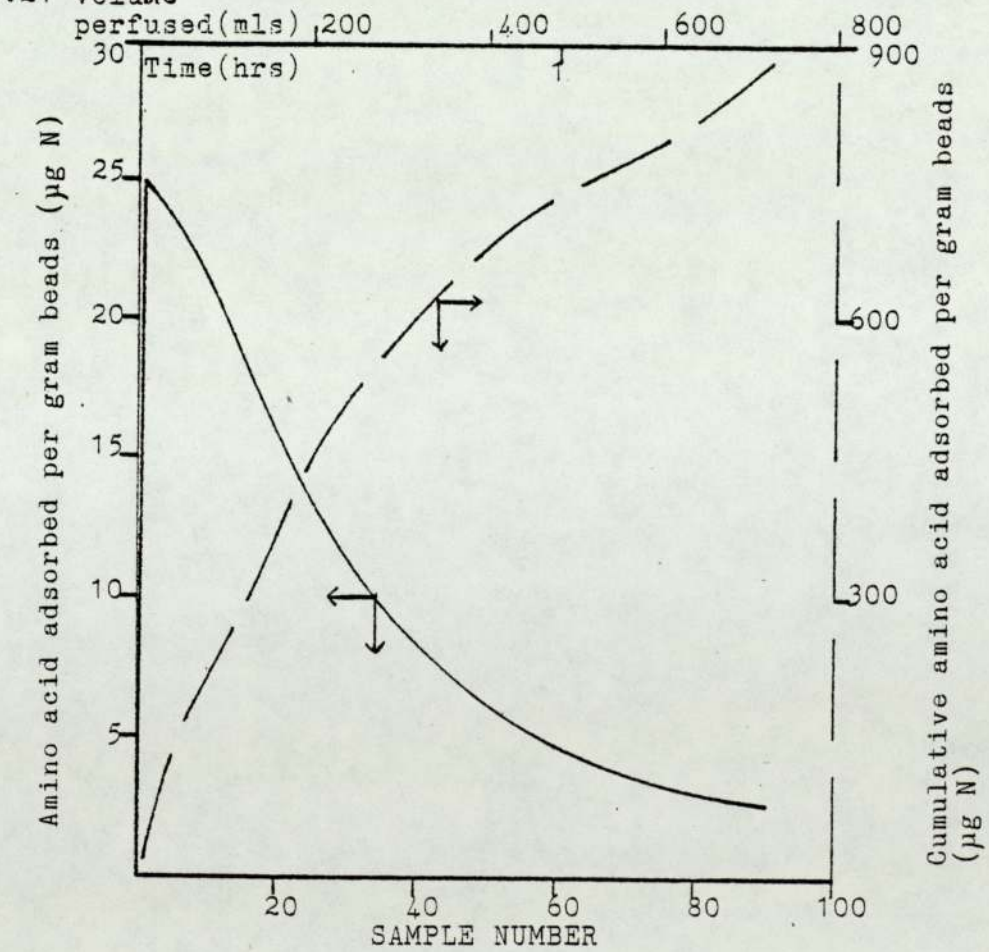


Fig 6.22

5g D4 HEMA/AA/EDM PERFUSED WITH A 15 mg Nl^{-1} OCTOPAMINE SOLUTION AT 4 mls/min FOR 5 HOURS

Fig 6.23

5g D4 HEMA/NMACR/EDM PERFUSED WITH A 15 mg Nl^{-1} OCTOPAMINE SOLUTION AT 4 mls/min FOR 1½ HOURS

Fig 6.22

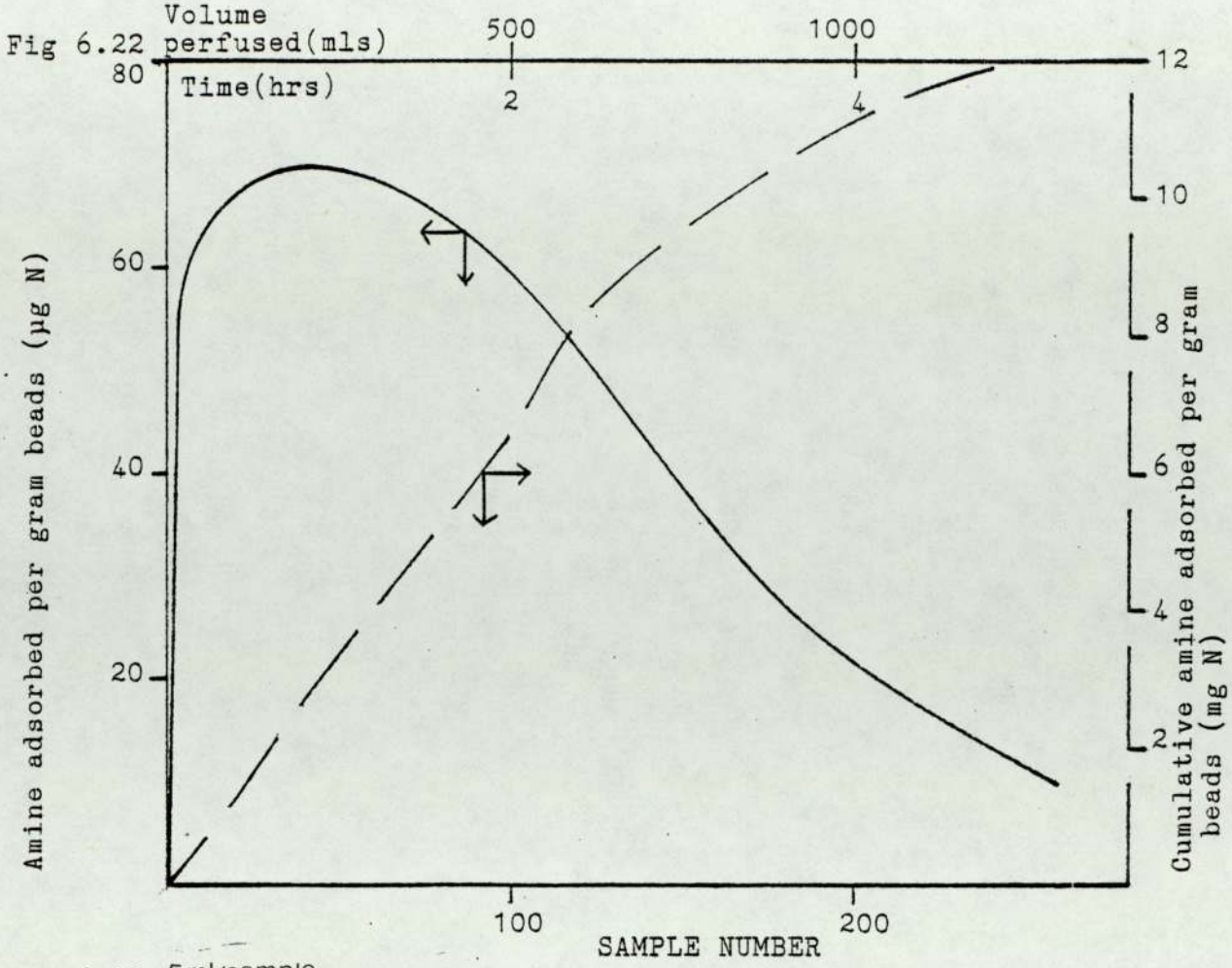
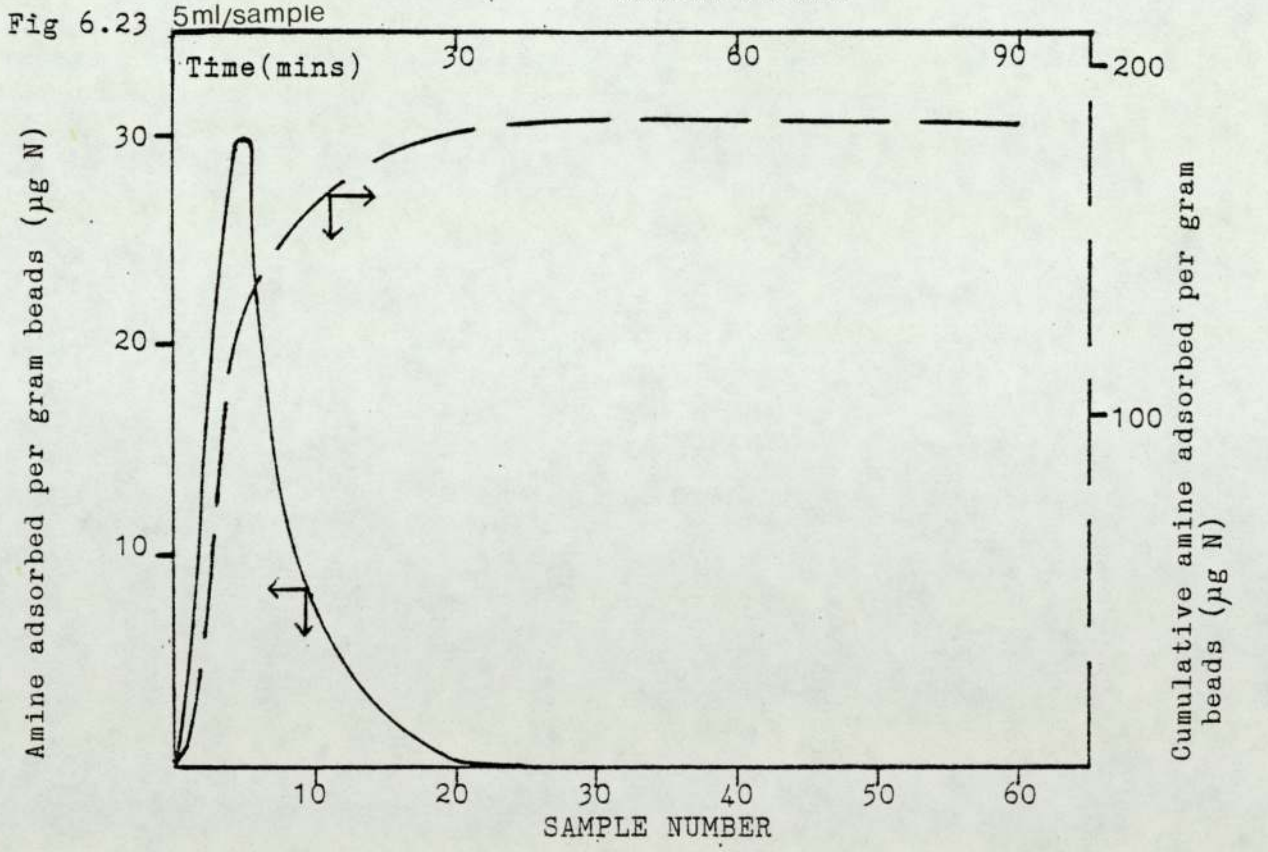


Fig 6.23



6.6 Discussion

At present, artificial liver support systems are being devised to provide temporary therapeutic assistance in acute fulminant hepatic failure. The rationale for such devices is that they will potentially remove accumulated toxins which may be related to the associated coma and/or may inhibit hepatic regeneration[204-214]. These potential toxins have not been well characterized and encompass lower molecular weight substances - the so-called 'middle molecules',and substances bound to plasma proteins. Since these potential toxins cover a broad chemical-physical spectrum, it is unlikely that any one device will effectively remove them all from a complex biological system, such as plasma[55]. For this reason this project has been interested largely in the removal of nitrogen based toxins and particularly the amino acids and false neurotransmitter amines in this chapter.

Hepatic encephalopathic patients have raised concentrations of all plasma amino acids, except for normal levels of branched chain amino acids : valine, leucine and isoleucine. Methionine, phenylalanine and tyrosine show the greatest proportionate rise and these are the precursors of the neurotransmitters implicated in the pathogenesis of hepatic coma[55,215,216].

A range of commercially available adsorbents was employed in this study in an effort to remove amino acids and false neurotransmitter amines. The adsorption trends observed for activated charcoal and the neutral uncharged polymeric resins were very similar. Some amino acids were adsorbed more strongly than others, and there was a spectrum of activity and no distinct difference in their adsorption. On the whole, the false neurotransmitter amines were more efficiently adsorbed, the capacity for them being about twice as that observed for the amino acids (Table 6.2). The neutral resins function much like carbon in their ability to adsorb, by bonding the hydrophobic portion of an organic molecule to the resin surface, and so adsorption of amines was more effective than that of the amino acids. Having said this, aromatic amino acids such as phenylalanine and tyrosine did show a slightly greater adsorption than did other amino acids on charcoal and on the neutral resins. Adsorption by charcoal and neutral resins is also due to their highly porous nature, hydrogen bonding and weak van der Waals' forces. However, since these materials were non-specific and non-selective in their adsorption behaviour, and the fact that they have the tendency to leach impurities into the perfusate, make them unsuitable for clinical haemoperfusion for liver failure patients, unless, of course, they are first coated with a biocompatible membrane.

The use of uncoated charcoal and polymeric resins is practised, but only for the relief of drug intoxication, where the major priority is the removal of the elevated drug level and the general condition of the patient is not as severe as that in coma from liver failure. Drug overdose patients are relatively 'healthy' and their liver can cope to some degree with platelet loss and the result of haemolysis arising from the use of such adsorbent materials. However, this is not so, for liver failure patients who depend on the biotolerance of the substrates employed. The need for biocompatible and purposely designed haemoperfusion substrates in such cases, cannot be over emphasised[186].

This project aimed to evaluate biocompatible systems for such use, which was more laudable than using available systems. Since the potential of activated charcoal and polymeric neutral resins was such that they were relatively unsuitable as adsorbents for amino acids and amines in liver failure, the design of biocompatible membranes for such adsorbents was not justified by their performance. Thus, no further investigations were made to develop coatings for activated charcoal and the neutral resins. However, studies were carried out using the clinically successful HSA-coated Amberlite XAD-7 resin[150,151]. The question of biocompatibility and adsorption potential was well illustrated in this case, since the coating

(Human Serum Albumin) was not only biocompatible, but also adsorbed amino acids and amines (Fig 6.24). Because the uncoated resin species does not adsorb such nitrogen-based hepatic toxins, it is suggested that albumin has an affinity for the amino acids and false neurotransmitter amines. This results in the attraction to the protein coat and binding by strong protein binding forces, hydrogen bonding and general polar interactions.

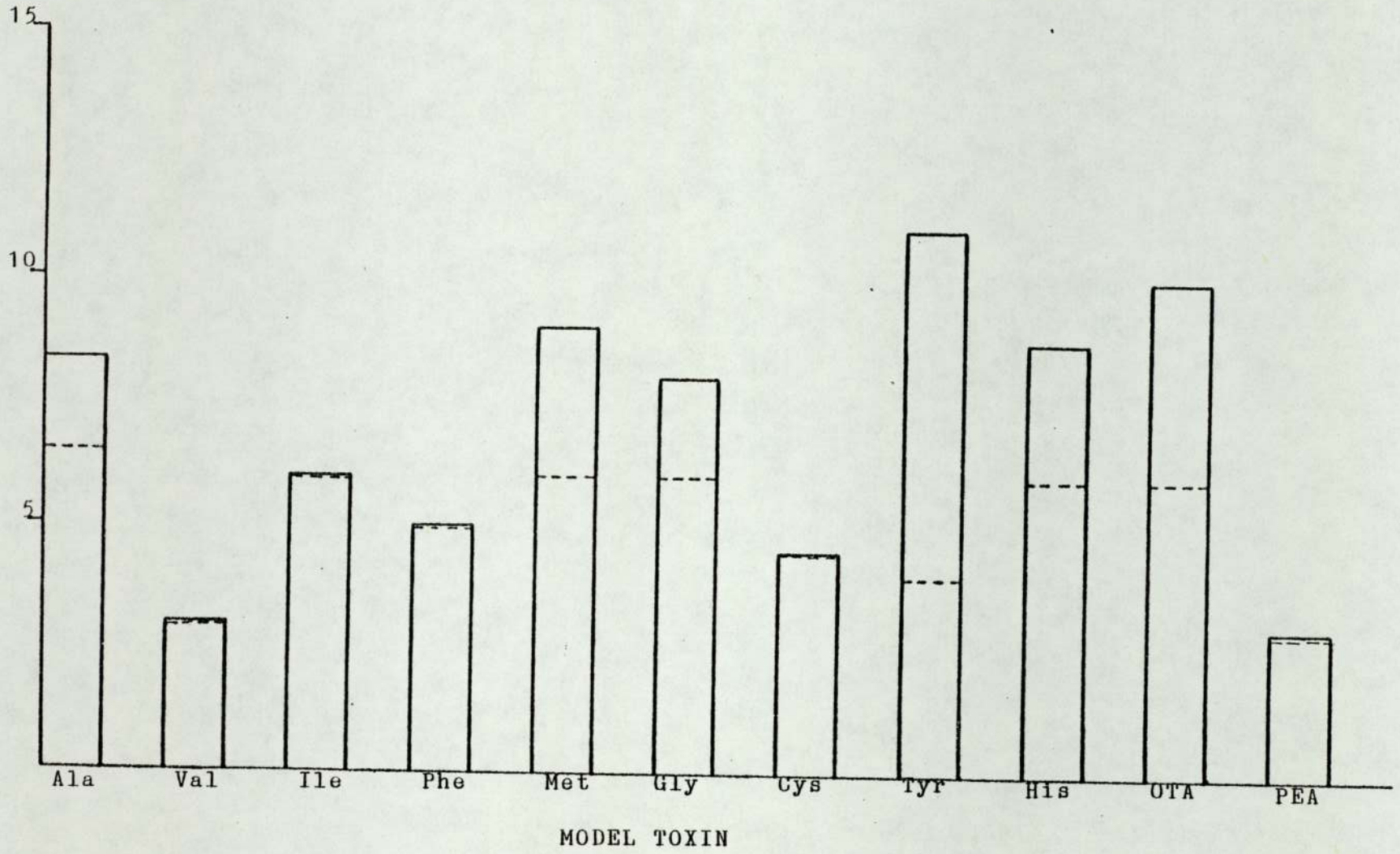
In the HSA-coated Amberlite XAD-7 system, the resin acts as a support for the adsorption coat, in much the same way as charcoal acted as a support for HEMA in the adsorption of ammonia (Chapter 5). This is a reasonable approach, but does not maximise the use of adsorbents. Furthermore, the capacity of the available surface coating in such a system may not be practical for the specific removal of amino acids and amines in liver failure. However, since HSA-coated XAD-7 resin is currently being used for the reduction of bile acid and bilirubin levels, perhaps it may make some contribution to correcting elevated amino acid and amine levels in artificial liver support systems.

Studies were also carried out using ion exchange resins. Although ion exchange has proved useful in the processing as well as for the separation of certain amino acids[148], its' use in the removal of amino acids in fulminant hepatic failure is not a widely established

Fig 6.24 ADSORPTION OF AMINO ACIDS AND AMINES ON HSA-COATED AMBERLITE XAD-7 : STATIC ADSORPTION STUDY

CONDITIONS: $[\text{Model Toxin}]_o \equiv 15$ mg Nl^{-1}; 1g adsorbent, 5 ml model toxin solution, 1 hour study.

—— adsorption study ; --- desorption study



practice. Adsorption studies with amino acids and amines, using ion exchange resins produced encouraging results. The strongly acidic cation exchange resins, in the acidic (H^+) form, demonstrated the greatest affinity for such model hepatic toxins, when compared to the resins of the sodium (Na^+) salt form (Figs 6.2 - 6.4). The binding of amino acids to ion exchange resins occurs via electrostatic ionic bonding between ionized groups of specific charge.

In summary, the ion exchange resins are very effective as adsorbents for amino acids and amines. Despite this, however, their lack of selectivity and more particularly, their great affinity for, and efficiency in amino acid and amine adsorption, makes them unsuitable for use in artificial liver support systems. Of course, the removal of such model toxins by polar mechanisms is desirable, but weakly ionic groups are more favourable than the strong ion exchange mechanism occurring with ion exchange resins. In addition, certain disadvantages do arise with the use of ion exchange resins in biomedical applications. These are largely concerned with their bioincompatibility, since they also remove blood platelets, red and white blood cells and essential inorganic cations (such as Na, K, Ca, Mg) from the blood. Furthermore, thrombocytopenia already present in liver failure, makes biocompatibility requirements much more stringent than for haemoperfusion applications, as in acute poisoning. However,

the cation exchange resins were used as a support material for the application and assessment of biocompatible membranes, and not to develop them for use as coated adsorbents. In this case, the ion exchange resin acted as a useful 'sink' and the adsorption of amino acids and amines on polyHEMA coated Duolite C255 (H^+) was investigated.

In general, the polyHEMA encapsulation material used to confer some biocompatibility to the ion exchange resin did not affect the equilibrium adsorption of amino acids and false neurotransmitter amines (Figs 6.3, 6.4) under static adsorption conditions. However, although the use of polyHEMA coated ion exchange resins seem promising for the use in artificial liver support systems, the adsorption of such large amounts of amino acids would make them less than useful for this application.

Normal amino acid levels show a wide variation between individuals; this variation being caused by diet, genetic differences, age, sex, etc.[76,81]. Likewise, pathological amino acid levels also vary in patients, as illustrated in Table 6.5[76]. To reduce elevated amino acid levels to levels lower than physiologically accepted, as would be achieved by the use of ion exchange resins, would be detrimental to the patient. Table 6.5 does not show the expected pattern of elevated amino acid levels in the liver failure patients, compared to the normal subjects. Other workers have quoted much higher pathological con-

Table 6.5 Plasma Amino-Acid Concentrations

Amino-acid	Control subjects (n=57) Median + range (mg/100ml)	Chronic active hepatitis (n=26)	
		Median + range (mg/100 ml)	mg equivalents of Nitrogen (mgN/100ml)
Alanine	3.1 (1.5-7.8)	2.9 (2.0-5.6)	0.46
Valine	2.8 (1.7-5.0)	1.9 (0.8-3.0)	0.22
Isoleucine	1.2 (0.6-2.2)	0.8 (0.4-2.0)	0.08
Phenylalanine	1.2 (0.7-2.8)	1.2 (0.8-2.0)	0.1
Methionine	0.5 (0.2-1.2)	0.9 (0.4-3.5)	0.08
Glycine	1.9 (0.7-3.5)	1.8 (1.2-3.0)	0.34
Cysteine	0.9 (0.2-2.7)	1.0 (0.5-2.3)	0.12
Tyrosine	1.4 (0.6-2.8)	1.7 (0.9-4.5	0.13
Histidine	1.6 (0.6-3.6)	2.2 (0.9-4.0)	0.58

centrations, but this table is presented since it illustrates that amino acid levels, which are physiological for one individual, may well be pathological in another.

Dynamic adsorption studies employing polyHEMA coated Duolite C255 (H^+) resin showed that although small molecular species such as ammonia and amino acids are adsorbed, some degree of competition occurs between such chemical species. Adsorption studies with ammonia demonstrated that ammonia is very strongly bound to the acid functionality of ion exchange resins and such materials have a large, almost infinite capacity for ammonia. Ammonia penetrates the polymer matrix, as the availability of the interaction sites apparently remains constant and independent of the amount of compound previously adsorbed. However, although adsorption sites are not saturated with ammonia, the subsequent adsorption of amino acids such as phenylalanine on HEMA coated ion exchange resins was impeded in the presence of adsorbed ammonia (Figs 6.16, 6.17). Although this experiment was not a definitive one and not intended to be, it illustrates competitive binding and the difference in transport between two chemically different species.

Ammonia, being a very soluble small molecule, is more easily transported through the polyHEMA encapsulation, and thus adsorption on the ion exchange resin occurs more readily than for phenylalanine. The transport of phenylalanine (a larger molecule) through the encapsulating membrane (and thus adsorption on the ion exchange resin) may

have been reduced because its' transport depends on its' size and solubility. In addition, the reduction in phenylalanine adsorption may have been a result of the saturation of polyHEMA with phenylalanine. This experiment illustrated that the transport of large molecular species such as amino acids (relative to ammonia) through hydrogels, is affected by the size and solubility of such chemical species, and the fact that transport through a dense coating layer of hydrogels affects amino acid adsorption on otherwise effective adsorbents.

To expand on this study, the adsorption of amino acids was investigated under static adsorption conditions, using macroporous hydrogel particulates based on polyHEMA. The results presented in Figs 6.5 - 6.7 show that amino acids were adsorbed, but no general trend was observed for their adsorption on the various hydrogel compositions employed. The binding of amino acids to such macroporous hydrogel beads may be attributed to the degree of hydrogen bonding of such species to polyHEMA and to other monomers of the bead composition if present; the ability of the amino acids to ionize in aqueous solution (polarity), and their ability to be transported in the water phase of the beads. Adsorption is also a result of the macroporous structure of the beads, since large discrete pores facilitate diffusion [217].

Because of their amphoteric/amphiprotic character, amino acids can exist in three ionic forms, e.g. cations,

anions and zwitterions. Thus, adsorption on acidic, basic or neutral hydrogel forms can take place. Adsorption of amino acids on hydrogels containing acidic monomers (e.g. acrylic acid (AA), methacrylic acid (MAA) or basic monomers (e.g. N-Methylol acrylamide (NMACR), diacetone acrylamide (DAA)) occur via ionic forces, whilst adsorption on the neutral hydrogels (e.g. polyHEMA) occurs via hydrophilic and polar forces. The observation that there was no great structural selectivity in amino acid adsorption on the hydrogels, may have great implications in artificial liver support systems, especially in cases where the whole amino acid profile is deranged and the reduction in their concentration is a desirable goal. In other cases, where certain amino acids are elevated, these hydrogels can be tailored to suit the specific needs of such individuals, and a haemoperfusion column containing hydrogels with complementary adsorption spectra, would be a useful tool.

The incorporation of acidic groups in the hydrogel structure would (as in the case of ion exchange resins) promote the adsorption of the more basic amino acids and false neurotransmitter amines, e.g. histidine and octopamine (Figs 6.20 - 6.22). Similarly, the incorporation of basic groups would promote the adsorption of the more acidic amino acids, whilst the adsorption of basic amino acids would be reduced. Thus the nature and structure of the monomers in such hydrogel particulates would then contribute to their adsorption abilities, together with other

factors such as the particle size, porosity and water content of the hydrogel particulates, which can all be controlled accordingly.

Since the levels of amino acids in liver failure patients vary, a range of adsorbent materials such as the hydrogels that can deal with the adsorption of different elevated amino acid levels would be of great value in artificial liver support systems. The biocompatibility of these hydrogels is also an advantage, since this abolishes the need for biocompatible encapsulation membranes. The adsorption capacity of the macroporous hydrogel particulates (although not as large as that observed with the ion exchange resins) may be adequate for the reduction of elevated amino acid and false neurotransmitter amine levels in artificial liver support systems. The dynamic equilibrium between the body compartments, means that any alteration in plasma concentration, however low this concentration is, will produce a shift in the other compartments (e.g. brain). Thus, a relatively small amount of toxin removed may be sufficient to produce a pharmacological effect[62].

The hydrogels employed have also been shown to adsorb ammonia (Chapter 5), and could be used to correct hyperammonaemic levels simultaneously. Such membranes and macroporous beads have also previously been shown to adsorb much larger chemical species such as Bromosulphophthalein

(BSP) and bilirubin[145] and their adsorption potential may also be extended to accomodate the adsorption of large acidic chemical species[198]. However, before such adsorbents can be used in artificial liver support systems, numerous in-vitro and in-vivo trials have to be carried out, which remains the next step for the continuation of this work.

CHAPTER 7

GENERAL DISCUSSION, MISCELLANEOUS SUPPLEMENTARY EXPERIMENTS, CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

CHAPTER 7

General Discussion, Miscellaneous Supplementary Experiments, Conclusions and Suggestions for Further Work

The original aim of this research project was to evaluate polymer based adsorbents by in-vitro techniques for their potential use as adsorbents in artificial liver support systems, for the removal of elevated nitrogen-based toxins.

It must be appreciated that the investigations did not concern the actual use of biological samples (such as whole blood or plasma) since the interest in this project was directed towards developing adsorbents suitable for the selective removal of ammonia, amino acids and amines elevated in liver failure. Investigations were conducted by firstly examining quantitatively their adsorption on commercially available adsorbents as it was necessary to establish that the prospective materials had useful adsorption properties, before the difficult problems of biocompatibility assessments could be undertaken. Adsorption studies performed indicated factors important for the adsorption of nitrogen-based hepatic toxins.

In general, it became apparent that acidic functional groups were important assets for the adsorption of such hepatic toxins. The ion exchange resins were a particular group of adsorbents showing a great potential

in the adsorption of such compounds. (See Chapter 5, Figs 5.5 - 5.6 and Chapter 6, Figs 6.2 - 6.4). However, their adsorption capabilities were too great for the nitrogen based hepatic toxins, for them to be ideal for use in artificial liver support systems. Furthermore, vital blood elements (such as platelets and red and white blood cells) are also adsorbed by ion exchange resins. In addition there is a reduction in essential ionic levels confirmed in supplementary experiments (which will be discussed subsequently).

In view of the bioincompatibility of such adsorbents and their aggressive adsorption properties observed, this research project was not interested in developing such materials for use in artificial liver support systems. However, they were used as a support material for the application and development of biocompatible membranes in much the same way as charcoal and Amberlite XAD-7 and other adsorbents have been used by previous workers(1).

The transport properties of such biocompatible encapsulating membranes for the nitrogen-based hepatic toxins were investigated. Adsorption studies using polyHEMA coated ion exchange resins demonstrated that the adsorption of ammonia was as efficient as that on the uncoated adsorbent (Chapter 5, Figs 5.12 - 5.17). PolyHEMA did not in any way impede the adsorption of ammonia under dynamic adsorption conditions, and the trans-

port of ammonia through polyHEMA was broadly similar to that of oxygen. Other studies also indicated the adsorption of ammonia on polyHEMA (Chapter 5, Figs 5.1, 5.2). However, although such encapsulating membranes, which take the form of a dense layer covering the adsorbent were adequate for ammonia adsorption, the transport (and not capacity) of larger molecular weight species (such as amino acids and amines) through such membranes was impeded to some extent (Chapter 6, Figs 6.16, 6.17, 6.19).

It was logical to expect that hydrogel coatings would not necessarily impede equilibrium adsorption of such larger hepatic toxins on strong adsorbents as ion exchange resins, but that their rate of transport would be reduced as their size increased. This rate effect would be partially controlled by increasing the water content of the hydrogel coating, since it is known that the transport of water soluble species is directly related to the water content of the hydrogel[192-193]. In addition, the larger the hydrogel pore size, the faster the transport[197].

The most appropriate way of obtaining a large pore size and thus faster transport, without losing strength in the hydrogel, is to use macroporous hydrogel material[197].

One of the greatest points in favour of the novel macroporous hydrogel structure is the size of the pores, which approach 1.0 μm. With the use of macroporous hydrogel particulates, adsorption is not limited to the pore

diameter of hydrated polyHEMA which is in the order of a few angstroms ($< 10\text{Å}$) and so amino acids could be adsorbed. Because of the adsorption and transport potential of such HEMA coatings, they were then developed further and used as adsorbents in the form of macroporous hydrogel beads for the adsorption of ammonia and other larger nitrogen based hepatic toxins, e.g. amino acids and amines.

The structure of macroporous hydrogel particulates could be modified in much the same way as ion exchange resins, to incorporate acidic groups (which were most desirable for the removal of basic species), basic groups (for the removal of acidic species) or neutral groups. Such adsorbents could also be designed to accomodate the adsorption of chemically different species. Factors such as a small particle size and large pore size and the structure and nature of monomers used, all contribute to their adsorption ability.

Such hydrogel beads were previously shown by P.J. Skelly[145] to be capable of adsorbing organic molecules such as bilirubin. The incorporation of ion exchange groups in the structure of such beads, facilitated the adsorption of ionically charged species in this project. This observation was particularly important since the ionic groups shown to be important for the adsorption

of nitrogen-based toxins on ion exchange resins, were also important for their adsorption on the purposely synthesised novel macroporous hydrogel particulates. Of course strongly acidic functional groups such as sulphonic acids ($-SO_3H$) were not incorporated in the hydrogel structure, since adsorption by such groups was shown to be too dramatic. (This argument was developed in Chapters 5 and 6, where the relationship between structure and adsorption was observed). Furthermore, such acidic groups in hydrogels adsorb a large amount of water. Instead, weaker carboxylic (-COOH) acid groups such as acrylic acid and methacrylic acid were incorporated by their copolymerisation with HEMA. (Such weak acidic groups adsorb less water).

Together with the macroporous structure and a small particle size, the nature of the monomers in the hydrogel particulates proved to be effective in the adsorption of ammonia, amino acids and amines (Chapter 5, Figs 5.18 - 5.23 and Chapter 6, Figs 6.5 - 6.7 and 6.20 - 6.23). Ammonia being a very basic species, very soluble in water and also small in size and molecular weight is easily adsorbed. However, to extend this work to the adsorption of hydrophobic species, which are less soluble in water and larger in size than ammonia, the macroporosity of such novel hydrogel adsorbents would be important.

The scanning electron micrograph of the surface of a novel macroporous hydrogel particulate is presented in Plate 7.1

Plate 7.1 NOVEL MACROPOROUS HYDROGEL PARTICULATES
HEMA(100) EDM(10) (X2750)

Further work in this area would involve investigating the adsorption of other hepatic toxins using such macroporous hydrogel particulates.

The fact that hydrogel beads could be developed as selective adsorbents, is important as a range of substances with different adsorptive capabilities is desired in the artificial liver support field. Such materials would enable a more selective adsorption to take place in individual cases of liver failure, thus allowing the adsorption of toxins to be more closely tailored to suit the individual patient. Of course, biocompatibility requirements would have to be met and numerous experimental trials performed on blood samples and then animals before the biomedical application to man.

Although the work in this research project has been concerned with nitrogen-based hepatic toxins, the potential use of these adsorbents can be extended to the adsorption of other toxins. Many of the principles can be extended to larger acidic molecules, such as BSP and bile acids. BSP or more commonly Bromosulphophthalein is often used as a model toxin for liver failure[6] and bile acids (BA) are usually elevated in liver failure. Based on the reliability of the relationship of structure and adsorption capabilities of adsorbents, such acidic species may be adsorbed on basic hydrogel adsorbents.

Preliminary supplementary adsorption studies on different classes of commercially available adsorbents showed that BSP, unlike the nitrogen-based toxins, was not adsorbed on the strongly acidic cation exchange resins, but was adsorbed on activated charcoal, the neutral polymeric resins, and basic and weakly acidic ion exchange resins (See Fig 7.1).

In parallel to this study, synthetic work has been carried out in a supplementary project by Mr. U.S. Atwal (198), where the importance of molecular size and HEMA pore size in the rate of adsorption of large acidic species (such as BSP and BA) has been demonstrated. This particular work illustrates the importance of macroporous particulates for such adsorption, since dense HEMA (as coatings) does not transport large molecules as efficiently. In addition, the observations reflect the requirements for the adsorption of acidic species.

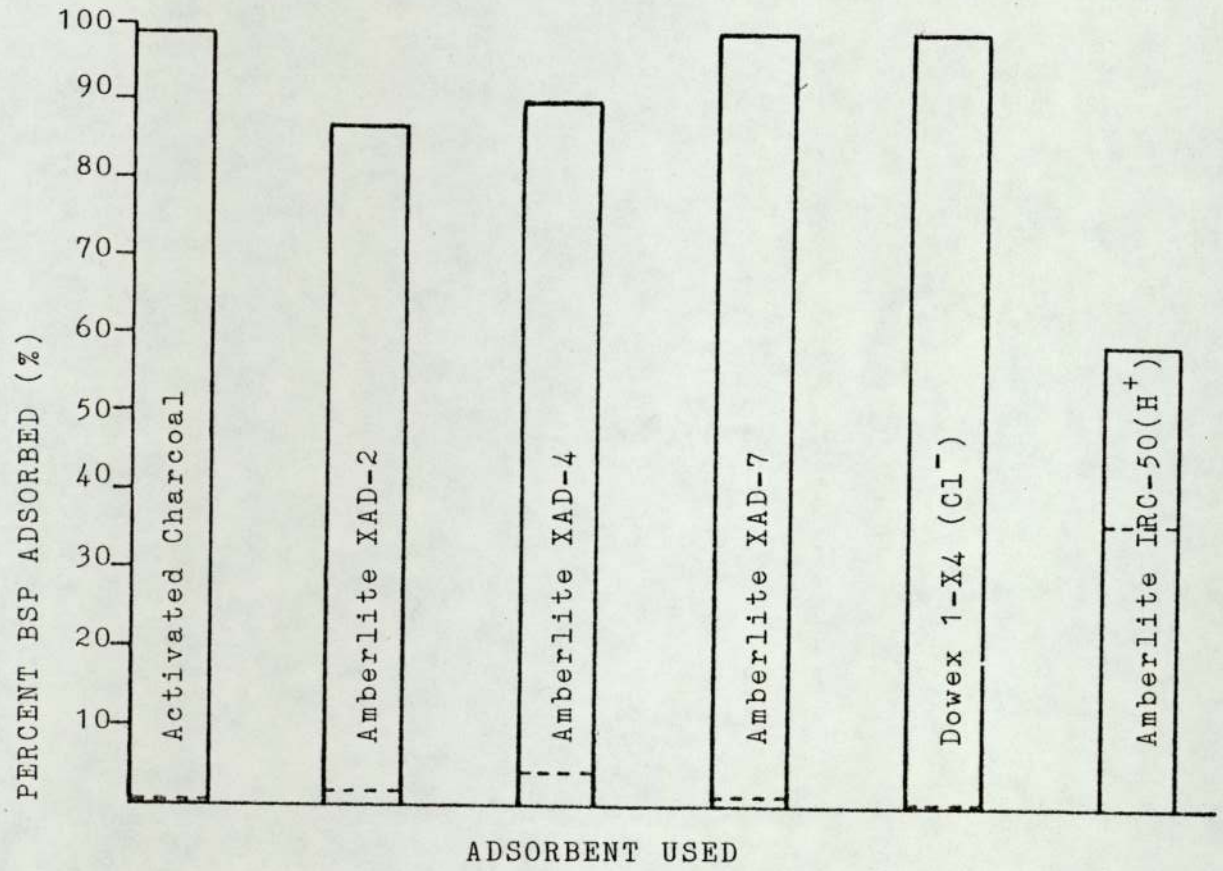
The preliminary work on various classes of adsorbents (Fig 7.1) pointed to the fact that macroporous hydrogel beads, with basic groups were required for enhanced adsorption of acidic species. Broadly speaking, this was the general opinion from this project , and the work performed by Mr. U.S. Atwal is consistent and in accordance with this.

Biological samples have not been employed in the in-

Fig 7.1 ADSORPTION OF BSP ON VARIOUS ADSORBENTS : STATIC ADSORPTION STUDY

CONDITIONS: $[BSP]_o \equiv 1 \times 10^{-3}$M; 1g adsorbent, 5ml BSP solution (in 0.9% saline), 1 hour study

—— adsorption study; --- desorption study Read on SP 600 Spectrophotometer



vestigations in the project, since it was essential first to obtain results in an aqueous environment. However, if and when adverse results do occur with the eventual use of biological samples, they can then be counteracted, using the knowledge gained from these studies.

In addition to the previously described experiments, investigations were carried out on ion exchange resins, to determine their extent of inorganic cation (e.g. Na, K, Ca, Mg) adsorption on perfusion, and to investigate the removal of ammonia, amino acids and amines when contained in a mixture. 5g of Duolite C255 (H^+), a strongly acidic cation exchange resin, (previously washed and soaked in 0.9% physiological saline for 7 days) were perfused at 2 mls/min for six and a half hours with a physiological media of Krebs Mammalian Ringer Solution[218] made up of solutions all isotonic with serum (See Table 7.1) and containing a mixture of ammonia, amino acids and false neurotransmitter amines, all at an initial concentration of 15 mg Nl^{-1} (see Table 7.2). The concentrations of electrolytes and organic acids were similar to mammalian serum, and contained intrinsic substances. Analysis of the nitrogen-based hepatic toxins was performed on an Amino Acid Analyser and the concentration of cations (Na, K, Ca, Mg) was determined by atomic adsorption spectrophotometric analysis using the Perkin Elmer Spectrophotometer Model 400. Such essential cations were shown to be

Table 7.1

Physiological Media : Krebs Mammalian Ringer Solution [218]

(Krebs Improved Ringer 1)

SOLUTION (All approximately isotonic with serum)		PROPORTION BY VOLUME
0.154M	NaCl (0.90%)	80
0.154M	KCl (1.15%)	4
0.11M	$CaCl_2$ (1.22%)	3*
0.154M	KH_2PO_4 (2.11%)	1
0.154M	$MgSO_4.7H_2O$ (3.8%)	1
0.15M	$NaHCO_3$ (1.3%)	21‡
0.16M	Na pyruvate (or L-lactate) (1.76%)	4
0.1M	Na fumarate (1.6%)	7
0.16M	Na-L-glutamate (2.9%)	4
0.3M	Glucose (5.4%)	5

* Twice the conc. of ionized Ca in serum

‡ Gassed with 100% CO_2 for 1 hr. before mixing with other solutions

Table 7.2

Contents of Model Toxin Mixture; [15 mg Nl^{-1}].

Ammonia	Methionine	Octopamine
Alanine	Glycine	Phenylethanolamine
Valine	Cysteine	Urea
Isoleucine	Tyrosine	
Phenylalanine	Histidine	

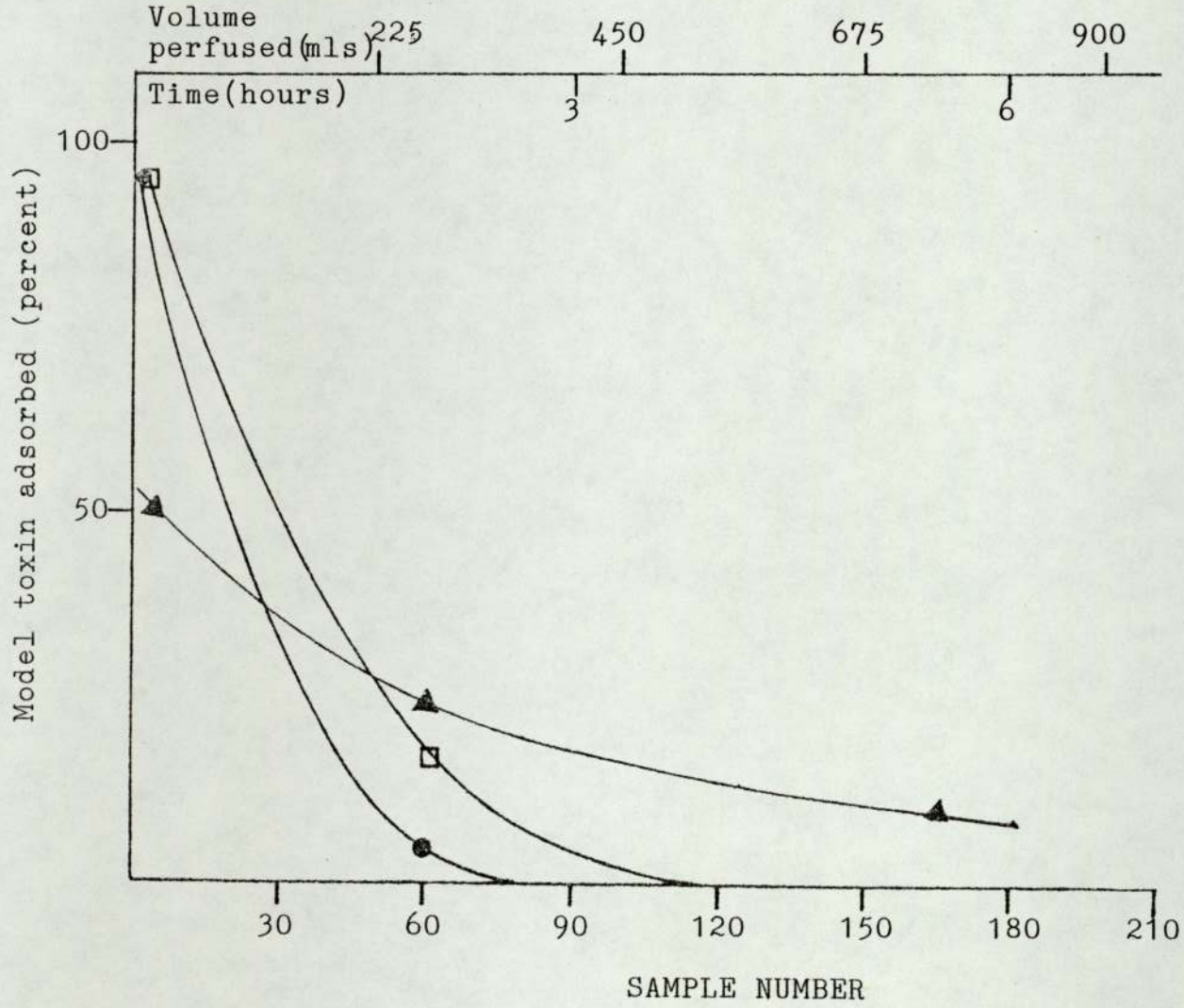
adsorbed by Duolite C255 (H^+) resin and highlights some of the problems that would be encountered in the use of uncoated ion exchange resins in haemoperfusion systems.

The analysis of the model toxins in sample fractions showed that ammonia, urea and the amino acids were all adsorbed on Duolite C255 (H^+) resin, with adsorption decreasing with time, as illustrated in Fig 7.2 The concentration of each amino acid was assayed, and because they were all very similar to each other, their adsorption profile is expressed as one curve. Competitive adsorption between the model toxins is not indicated from the results, although it is interesting to note that equilibrium adsorption for the amino acids is achieved within three hours of perfusion, followed by that for urea within four hours of perfusion. In comparison, ammonia was still being adsorbed after six hours of perfusion, (although slowly diminishing),since adsorption sites were more easily accessible to ammonia, a smaller molecule.

Fig 7.2 ADSORPTION OF MODEL TOXINS ON DUOLITE C255 (H^+) RESIN

CONDITIONS: $[\text{Model Toxin}]_o \equiv 15$ mg Nl^{-1}; 5g adsorbent; flow rate = 2 mls/min for 6½ hours

●—● amino acid; ▲—▲ ammonia; □—□ urea



Semi-Conductor Based Ammonia Sensors

The adsorption studies using polyHEMA coated ion exchange resins revealed that ammonia, amino acids and false neurotransmitter amines could be transported through such microencapsulating membranes. This observation bore particular importance to the closely related system of semi-conductor based ammonia sensors. Such devices, capable of detecting ammonia have been used for the detection of ammonia in the head space of water-bound waste, but may also show great potential in the use in liver failure, to monitor ammonia levels in the blood of patients during haemoperfusion. Of course, such a device would require a biocompatible encapsulation for protection and permselective control before use. Since HEMA, the hydrogel coating material employed for the encapsulation of ion exchange resins was permeable to ammonia, it was again used as a membrane coat to confer some degree of biocompatibility to the semi-conductor ammonia sensors.

An appreciable amount of work was carried out in this area. However, for reasons of commercial security, details of the study are not given here. But, to keep to the general theme of work performed, only a brief account of this part of the work will be given.

The semi-conductor based microchip devices, constructed by Thorn EMI (CRL), Hayes, Middlesex (UK) consist of a miniature ammonia sensor, mounted centrally on

a thin electrical grid plate, through which an electric current is passed when in use.[219] The coating procedure of such devices was relatively simpler than that employed for coating ion exchange resins, and involved immersing the device in a hydrogel (e.g. polyHEMA) solution of different polymer concentrations (8-10%). The dip-coated devices were then dried.

The selection of such membranes followed on logically from the results of Chapter 5. Thus, ammonia, a small water soluble, 'oxygen-like' molecule was readily transported through polyHEMA, and hydrogels with a moderate water content provided excellent membranes for this type of work.

Several areas of investigations are suggested for further research in the field of ammonia sensors. Of course, observations in an ammonia environment remain the major priority. (Studies of this type were not performed, although initially intended, since the nature of the project changed on the part of the collaborating body, who were responsible for such evaluations). This would be followed by in-vitro, then in-vivo evaluations of such devices. If success permits, the devices could be further developed for use in haemoperfusion systems for monitoring ammonia levels in perfused blood. Eventually, the devices could be implanted in arteries of liver failure patients for the direct and continuous monitoring

of ammonia levels. Such monitoring of ammonia levels in artificial liver support systems in the future would revolutionise the practise of medicine in as profound a manner as the introduction of the automated multi-analyser.

Certain parameters such as the extent of clot formation and protein adsorption are usually the biggest problems to be solved during in-vivo use of such sensors [220,221]. Investigations have already demonstrated the adequate biocompatibility of the hydrogel coating material[145]. Further research would involve the design of more ammonia selective permeable biocompatible membranes and numerous tests before their practical application in the clinical and biomedical field. The use of the novel macroporous hydrogel particulates as preferred adsorbents for the reduction of elevated ammonia levels in liver failure, together with the use of such semiconductor based ammonia sensors, would provide a potentially valuable technique for controlling hyperammonaemia in liver failure patients. Such adsorbents, at the same time, could also be modified to reduce elevated amino acid and false neurotransmitter amine levels for individual patients.

In summary, the use of synthetic macroporous hydrogel beads offer ways of providing purposely designed

adsorbents for the use in biomedical application for patients in acute liver failure. The logical pathway of future work in this area would therefore involve:

a) adsorption studies employing plasma or whole blood
b) studies on animals

Investigations using biological samples and animals are important and will be very complex. Such investigations were not omitted in this project because of foreseen difficulties, but because it was important first to develop selective adsorbents and to understand adsorption processes.

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