DRUG/WATER INTERACTIONS
IN HYDROGEL MATRICES

by

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SUMMARY

The primary aim of this research has been the investigation of the role of water structuring effects in the widely different extents of irritancy displayed by certain antibiotics.

The compounds involved were members of the Lincomycin group of antibiotics. The aqueous solution behaviour of these compounds was studied using techniques such as vapour pressure osmometry and differential scanning calorimetry (D.S.C.).

The effects of the antibiotics on water structure in hydrogel membrane preparations in which the equilibrium water content (E.W.C.) and constituent amounts of freezing and non-freezing water were varied were also investigated using D.S.C. The permeability of water swollen hydrogel preparations to aqueous antibiotic solutions as well as other solutes were studied. A series of hydrogel preparations into which the antibiotics had been incorporated during polymerisation were developed and used in studies of the effects of the antibiotics and their water structure modifications on the permeation of a range of solutes.

Key phrases:
Solute/water interactions
Water structuring effects
Hydrogel permeability
Differential scanning calorimetry
Lincomycin antibiotics

This work was carried out between 1979 and 1982 in The University of Aston in Birmingham. It has been done independantly and has not been submitted for any other degree.

N D Atherton

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"I am the Alpha and the Omega, the Beginning and the End. I will give unto him that is athirst of the fountain of the water of life freely."

Revelation of St John
21.6

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LIST OF ABBREVIATIONS

AA	Acrylic acid
ACM	Acrylamide
ATP	Adenosine triphosphate
C/M	Clindamycin hydrochloride
C/M/P	Clindamycin phosphate
DCQC	2,6-Dichloro-p-benzoquinone-4-chlorimine
DEAEMA	Diethyl aminoethyl methacrylate
DMF	Dimethyl formamide
DMAEMA	Dimethyl aminoethyl methacrylate
DNA	Deoxyribonucleic acid
DSC	Differential scanning calorimetry
DTNB	5,5'-Dithiobis(2-nitrobenzoic acid)
EDMA	Ethylene dimethacrylate
E/M	Erythromycin
E/M/G	Erythromycin glutamate
E/M/L	Erythromycin lactobionate
EWC	Equilibrium water content
HEMA	Hydroxy ethyl methacrylate
INC(inc)	Incorporated
L/M	Lincomycin hydrochloride
MAA	Methacrylic acid
MMA	Methyl methacrylate
NVP	N-vinyl pyrrolidone
pACM	poly(acrylamide)
pGMA	poly(glycerol methacrylate)
pHEMA	poly(hydroxy ethyl methacrylate)
pHPA	poly(hydroxy propyl acrylate)

List of Abbreviations Continued

pHPMA	poly(hydroxy propyl methacrylate)
pVP	poly(vinyl pyrrolidone)
STY	Styrene
TEGDA	tetra ethylene glycol dimethacrylate
VPO	Vapour pressure osmometry
wt	weight
xt	Thickness (1xt = 1 gasket)
MBACM	METHYLENE BIS ACRYLAMIDE

CHAPTER I

INTRODUCTION AND BACKGROUND TO WATER STRUCTURE AND THE ANTIBIOTICS STUDIED

I.1 General Introduction

Ordinary water of the type with which we are all familiar and rarely pause to give a second thought to is far from being the well understood substance we might have assumed it to be, as many authors have in the past pointed out.[1,2,3,4,5] It is most certainly indispensible for the maintenance of life but can hardly be considered to be abundant in the ecosphere in a suitable form for such a purpose.

Although many measurements of the physical properties of water have been made over many years, which revealed many of the anomolous properties of water, the structure of water has not been precisely determined. The properties of water and aqueous solutions must ultimately be described in terms of the intermolecular forces present. The literature, however, contains several contradictory theories of the structure of pure liquid water and, in addition, the structure of ice is also far from being straightforward. If the structure of pure liquid water is unclear then even less is known about the structure of water in moderately concentrated aqueous solutions and the structure of water near interfaces.

These difficulties have in recent years led to something of a divide between theorists and experimentalists with few workers managing to bridge the gap. This situation although by no means satisfactory is nevertheless understandable since the complex interactions occurring in physico-chemical and biological systems as studied by experimentalists are not suitable for the work of theoretical physicists and mathematicians.

In order to make at least some progress both camps have continued their separate ways despite attempts to reconcile them.

The term "water structuring" refers to modifications of the three-dimensional network of water molecules by different degrees of hydrogen bonding to ions, macromolecules or surfaces. The properties of water in such systems can be so drastically affected that distinct water fractions can be identified. The types of water structure are complex and have been described later but can be considered to fall between two extreme situations. These are often known as the "bulk" or "free" and "bound" water states. Bulk water is that which behaves "normally", for example, it freezes at 273K. The bound water state is that in which the properties of water, as measured by various techniques, are markedly different from those of "normal" water. It will, for example, not freeze even if cooled to the temperature of liquid nitrogen (about 77K). The definitions vary both in the way they describe the types of water involved and in the way they are determined.

These different states of water have been identified in inert model systems and biological systems alike and have been implicated in the control of cellular processes.[6,7,8] It was in this context that the studies reported here were originally proposed. The work concerned the study of Lincomycin antibiotics which were almost identical in chemical structure but which were reported to produce widely different extents of irritancy.[9] Unfortunately the most potentially effective of the compounds studied was also the most irritant.

Since only small changes in the chemical nature of these compounds resulted in large differences in irritancy the irritant effects were considered to be of physico-chemical rather than purely chemical origin.

Bearing in mind the types of water structure modifications already described that are found in aqueous solutions and biological systems, an investigation of the water structuring effects of the antibiotics concerned here was proposed with a view to explaining the differing extents of irritancy of the compounds in terms of their water structuring capabilities.

The investigation involved not only the study of the water structuring effects of the antibiotics in aqueous solution but also their influence on established water structures in model systems. The model systems employed were hydrogel preparations which are water swollen polymer networks. The type of water environment in hydrogels can be controlled by the selection of appropriate monomers or comonomers in the polymerisation mixture. The use of such model systems simplifies the evaluation of the water structuring effects of the antibiotics which could be considered to be of a more complex nature in biological systems. Another advantage of the use of hydrogels as model systems was that in recent years the properties of such water swollen polymer networks have been extensively characterised by members of this research group.[10,11,12,13,14,15,16] The use of such model systems allowed direct comparisons of the antibiotic/water interactions of almost identical compounds to be made in a range of water environments. These studies therefore have a direct relevance to the types of interactions that could occur in biological systems in which the antibiotics are present. The importance of water in such systems was stressed by Szent-Gyorgyi who described it as the "matrix of life".[17]

It has taken a long time for theories involving water structuring to gain credibility, even after the importance of water in the functioning of biological systems had been recognised. Perhaps the reason for this lies in the words of Sir Oliver Lodge who reportedly said that the very last thing a deep-sea fish would discover is water![17]

I.2 The Structure and Properties of Water

Water has been shown for many years to demonstrate a large number of unexpected properties. Some of the more widely known properties will be mentioned here but for an extensive treatment of the subject the reader is recommended to the work of Franks.[18] There are other monographs to be found such as those of Kavanau,[19] Jellinek[20] and Drost-Hansen[2] each of which places a slightly different emphasis on the type of system in which water properties are discussed, the most recent of such collections being those of Rowland[21] and Drost-Hansen and Clegg.[22]

I.2.1 Physical Properties of Water and "Bulk" Water Structure

The following are some of the more notable physical properties of water:[3]

(i) Negative volume of melting

(ii) Density maximum in the normal liquid range (at 4°C)

(iii) Isothermal compressibility minimum in the normal liquid range (at 46°C)

(iv) Numerous crystalline polymorphs (at least nine, including those formed at elevated pressure)

(v) High dielectric constant

(vi) Anomolously high melting, boiling and critical temperatures for a low molecular weight non-ionic, non-metallic substance

(vii) Increasing fluidity with increasing pressure

and (viii) High mobility transport for H^+ and OH^- ions.

As has been already mentioned the occurrence of so many peculiarities must eventually be explained in terms of the intermolecular forces present and the key to understanding the properties of water lies in the concept of the hydrogen bond. Hydrogen bonds between electronegative atoms such as nitrogen, oxygen, fluorine or chlorine and hydrogen atoms are weak electrostatic chemical bonds that are strongly directional. The chemical bond containing the hydrogen atom that is donated to the hydrogen bond points directly at the nucleus of the hydrogen accepting electronegative atom. The strengths of such bonds are intermediate between Van der Waal's interactions (about 0.3 kcal mol^{-1}) and covalent bonds (about 100 kcal mol^{-1}). Units of kcal mol^{-1} are frequently used in the literature, hence, their use here although S.I. units of kJ mol^{-1} are more appropriate (1 cal = 4.184 J).

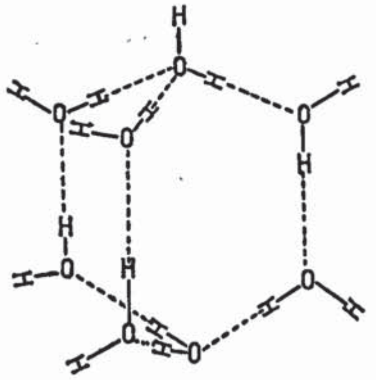
A single water molecule may be attracted to adjacent molecules by zero, one, two, three or four hydrogen bonds, the non-linear water molecule able to act both as a donor and acceptor of hydrogens. These abilities result in the structure of ordinary hexagonal ice as seen in Figure I (1). In this crystal structure each water molecule has four nearest neighbours to which it is hydrogen bonded in a tetrahedral manner. The bond angle of the free water molecule (104.5^{0})[3] is actually less than the ideal tetrahedral angle (109.5^{0}) required for strictly linear hydrogen bonding in an ice crystal.

The more highly developed hydrogen bond network of water than found for HF and NH_3 thus leads to its high melting point. Bifurcated and even trifurcated hydrogen bonds have been postulated to exist in some systems where the formation of simultaneous hydrogen bonds to two or more acceptor atoms occurs. Quantum mechanical calculations have been widely employed in the study of water structure as well as monte carlo simulations of solute/water systems.[23,24,25] Studies of water structure using x-ray diffraction,[26] neutron diffraction[27] and various spectroscopic methods[28,29] are to be found as well as extensive use of dielectric[30,31,32,33] and nuclear magnetic resonance techniques.[34,35,36] The use of differential scanning calorimetry (D.S.C.) has been discussed later, but this technique enables the determination of the freezing water content of a given sample.

The equilibrium freezing of an aqueous system requires that an ice crystal nucleus be made available at the equilibrium freezing point of the system and that appreciable temperature differences do not exist. If these requirements are not met then supercooling may arise. The non-equilibrium freezing behaviour of aqueous systems will be discussed in Chapter IV but the supercooling of water can lead to homogeneous nucleation at about -41°C.[37,38] Stillinger[3] comments on the work of Angell which shows that virtually all the measured physical properties of water point to an anomoly at about -45°C, just below the limit of attainable supercooling. This is known as the Lambda anomoly.

FIGURE I (1)

THE BICYCLIC OCTOMER OF HEXAGONAL ICE (I_h)[23]



The various models developed to account for the properties of water have been summarised by Safford and Leung (1971) and their work was reproduced by Drost-Hansen.[17] Such models can be roughly divided into three groups, continuum models, specific structural models and cluster models. Bernal and Fowler's irregular four co-ordinated tetrahedral structure of hydrogen bonding falls into the continuum model category.[1] Samoilov's model (see reference 17) was a specific structural model, water having a distorted or expanded ice I_h (hexagonal or "normal" ice) structure in which defects can occur through which other water molecules may pass and take up interstitial positions. An example of a cluster model is that of Frank and Wen[39] in which the formation of hydrogen bonds is co-operative, short lived (10^{-10}, 10^{-11} seconds) ice-like "flickering clusters" of varying extent were proposed. The co-operativity process depends on changes in the charge distribution of the water molecules involved in hydrogen bonding so that the hydrogen acceptor molecule becomes potentially an even better hydrogen donor than before, ie successive hydrogen bonding reinforcement encourages the formation of clusters of water molecules.

For the purpose of progressing to a discussion of structured water systems it can be said that it is generally accepted that long-lived water structures do not exist in bulk water but a certain amount of intermolecular order similar to that found in ice is present. Co-operativity of hydrogen bond formation is also generally accepted, and increased stability of one cluster, perhaps by a water structure promoting region, could also increase the stability of a neighbouring cluster. Such an effect could be propagated some distance, a concept utilised by Pauling.[40]

I.2.ii Structured Water Systems

There are many factors which can influence water structure. These can strengthen, weaken or completely break water structure and so depending on the effect of a "solute" on water structure it can be classified as either a structure maker or a structure breaker.[41] Water around non-polar molecules or non-polar segments of macromolecules which act as structure makers, forms cage-like "clathrates". Molecules with centres of high charge density or inorganic ions cause the formation of so-called "soft-ice" structures when in water. These ions can also act at a greater distance as structure breakers whilst acting locally as structure makers.

An increase in water structure will increase the chemical potential of solutes within that water fraction. If the chemical potential of a species is greater in one area of a system than another it will migrate down the chemical potential gradient. The result of such a process is that water structure "makers" and "breakers" can determine the distribution of solutes and the solution properties of the system.

Clathrate hydrates have been known for over 150 years. They are solid solutions of a minor component in a water lattice which becomes stabilised on forming cages around small solute molecules with no dipole moment. Another widely known interaction is that of hydrophobic bond formation. This was at first attributed to purely Van der Waal's forces between non-polar groups. However, the introduction of non-polar moieties into aqueous systems has been shown to result in unfavourable entropy changes which are postulated to result from changes in the hydrogen bonding and water molecule arrangement about solute molecules.[42] The phenomena is known as "Hydrophobic Hydration" and the formation of a hydrophobic bond between non-polar residues can thus be regarded as a partial reversal of the above process and so would be thermodynamically favoured.[43,44] Such processes are important in the tertiary structure of proteins such as haemoglobin, myoglobin and lysosyme.

Water structure near an interface is also different from bulk water structure and two extreme views of the number and extent of molecules affected by proximity to a surface tend to be taken:[45,46]

(i) The surface perturbs water molecules for only two or three molecular diameters where normal bulk properties are resumed, or

(ii) The surface can exert an influence on water structure up to 0.1 μm into the liquid phase.

Long range water structure concepts are generally unpopular, partly because of the "polywater" or "anomolous" water episode where the strange properties of water condensed into small quartz capillaries were ascribed to the long range effects of the quartz surface upon water structure. Such observations were later found to be due to impurities.[5]

In addition to such types of water structure there is another level of structuring which results in "bound" water. Bound water does not exhibit the properties associated with bulk water and since there can be no sharp dividing line between bound and bulk or "free" water states, values of the amounts of these water types present in samples are very much dependant on the experimental techniques and definitions used. The various definitions of bound water have been summarised by Berendsen:[47]

(i) Water bound in equilibrium at a certain relative humidity and temperature or water retained after a certain drying procedure.

(ii) Water that does not freeze at the normal freezing point of water.

(iii) Water that gives no orientational contribution to the dielectric constant at high frequencies and hence is not free to rotate.

(iv) Water that shows a slower rate of rotation as measured by magnetic resonance techniques.

and (v) Water that can be shown by infra-red or Raman spectroscopy to be engaged in hydrogen bonding to a macromolecule.

However, different results may be obtained by using different techniques since different aspects of the properties of water may be employed.

These have again been considered by Berendsen:[47]

(a) THERMODYNAMIC ASPECTS - enthalpies and entropies of interactions between macromolecules and water and influences on the activity of solutes (cases (i) and (ii) above).

(b) DYNAMIC ASPECTS - ranging from details of molecular motion to hydrodynamic properties (cases (iii) and (iv) above).

and (c) STRUCTURAL ASPECTS - relating to the average positions and orientations of water molecules with respect to each other and macromolecules (case (v) above).

A wealth of evidence on the importance of water structuring in relation to various phenomena has accumulated in recent years and a number of terms describing the various states of water have come into use. As can be seen from Table I.A, states of water in between the "free" and "bound" water states have been postulated and will be considered here under the term "interfacial" water although such water can also be described as "vicinal" water.

TABLE I.A[10]

Bound Non-Freezing Primary		Free Freezing Secondary	
Z W_3 Bound	Y W_2 Interfacial	X W_1 Bulk	
Primary Bound	Secondary Bound	Free	Bulk

Nuclear magnetic resonance (n.m.r) and differential scanning calorimetry (D.S.C.) are perhaps the two most common techniques used to study bound water. The n.m.r. technique detects the mobility of protons in various energy states, hydrogen atoms in bound water being at a different energy level from hydrogen atoms in free water due to the reduced mobility of bound water relative to free water. Broadline and pulse n.m.r. techniques may be encountered and, whilst the principles involved are similar, the types of samples used, the instrumentation and the results are different. The broadline technique can be used for the study of samples in the solid state whereas the pulse method is better for samples in the liquid state. n.m.r. measurements may be made at many temperatures but the equipment is expensive and considerable sample preparation is required. It is important to realise that bound water as measured by n.m.r., whilst being relatively immobile as compared to free water, is still more mobile than pure ice and that there can be rapid exchange between the bound and free water states.

The D.S.C. technique is simpler and more convenient than n.m.r. The technique is described in detail in Chapter II but suffice it to say that D.S.C. measures the amount of heat absorbed or evolved during a thermal transition in a sample and that with suitable calibration the amount of freezing water can be determined. The amount of bound or "non-freezing" water can be found by difference if the total water content of the sample is known. Since the D.S.C. technique was extensively used during this research the free and bound water states will often be referred to as "freezing" and "non-freezing" water respectively and a continuum of water states between these extremes is envisaged (see later).[10]

I.2.iii Structured Water in Biological Systems

One of the most important properties of water is the density maximum at 4°C which allows the freezing of rivers and lakes to take place from the surface downwards. The effect of this is to allow life to continue undisturbed by climatic changes below an insulating ice layer. Water is also important in biochemical reactions associated with metabolism and in the correct folding of enzymes necessary for their activity. Water also stabilises the double helix of D.N.A. In fact over 60% of human body weight is made up of water. Brain and muscle tissues have the highest water contents whilst bone and fat tissues have the lowest water contents. Approximately 20% of tissue water can be demonstrated to be non-freezing.

The dependance of metabolic activity on hydration level has been shown by a number of workers who demonstrated that varying amounts of water can be removed from bacterial and mammalian cells without stopping cell division and growth. Clegg[8] demonstrated three metabolic domains of hydration dependant metabolic events in artemia cysts. These were:

0 - 30 g H_2O/100 g dried cyst, ametabolic
30 - 60 g H_2O/100 g dried cyst, restricted metabolism
60 -140 g H_2O/100 g dried cyst, coventional metabolism

Wiggins has produced much work concerning the selectivity coefficients of silica gel, toad bladder and rat renal cortex (see Chapter VI) for potassium and sodium ions. Selective K^+ uptake over Na^+ was demonstrated and a model for a universal mechanism of active transport involving changes in the solvent properties of intracellular water was developed (see Chapter VII). The selectivity studies also demonstrated maxima at temperatures of 15°C, 30°C and 45°C. Such maxima were also observed by Peschel and Adlfinger[48,49] for the disjoining pressure and viscosity of water between two highly polished quartz plates. Drost-Hansen has also reported abrupt changes in the growth behaviour of many biological species at these same characteristic temperatures[50,51,52] and Trincher developed a "mathemetic-thermodynamic" basis to these anomolies based on quasi-crystalline, fluid and ice-relic components.[53]

All known proteins hydrate, globular proteins hydrating a single layer of water due to internal saturation of the bulk of backbone NH and CO groups whereas fibrous proteins absorb more water than can be accounted for by their polar side chains. These features have been used by Ling[54,55,56] in his "Association - Induction" hypothesis. This is based on long range order of water molecules and permits the models to demonstrate an all-or-none type of permeability change as proteins co-operatively alter their conformations and liberate water in a reversible manner. A feedback process was also anticipated whereby water could impress its structure on polypeptide conformation. When in an appropriate conformation multiple layers of cell water would be polarised by the proteins which could specifically absorb or exclude solutes depending on their hydration requirements (see Chapter VI). The co-operatively linked protoplasmic protein-water-solute assembly would function as a unit.

An alternative model has been put forward by Clegg[8] based on a co-ordinating system of networks of "vicinal" water structures generated by intracellular surfaces. Since the surfaces are under cellular control, so are the water networks. The model uses the widely demonstrated water structures at interfaces in which macromolecules are purported to have more affinity for "vicinal" water than "bulk" water. The molecules would be retained within the ordered water layer for an appreciable time on a molecular scale and this would allow close association of some or all of the components of a given enzyme pathway.

Concepts such as those of Ling and Clegg rely heavily on the presence of intracellular interfaces which have recently been identified by electron microscopy. Such studies were initiated by the consideration of cytoplasmic integration by Peters.[57] Keller and Riley[58] later observed large numbers of microfilaments throughout the nuclei of some eukaryotic cells and the massive surface area of such networks and their modification during cellular activity suggested an important cellular function.

Etzler and Drost-Hansen[52] expanded earlier theories and suggested a mechanism whereby metabolic pathways could be regulated in a manner which explained the anomolous behaviour of many systems at 15°C, 30°C, 45°C and 60°C. When the cell involved was not near one of these transition temperatures for "vicinal" water cellular metabolism was considered to be orderly. Enzymes would be in complexes near various intracellular interfaces and bulk water "channels" exist for the transport of small molecules within the cell. When the temperature of the cell approaches vicinal water transition temperatures the enzyme complexes dissociate and the bulk water "channels" become less well defined. Hence metabolism would slow down and result in the observed growth minima at these temperatures. Upon passing through a transition zone new enzyme complexes would form and metabolism would become orderly again but the "vicinal" water structure would be different to the previous one.

There are other theories of the importance of water and water structures in biological systems to be found in the literature. One such recent report was that of Macovschi[59] who proposed the existence of "Biostructured" water. In the "Biostructural" concept water in living tissue exists in free, biostructured(not to be confused with interfacial water) and bound states. However, dead tissue only has free and bound water, the biostructured water being water that is integrated into living tissues and can apparently be shown to be distinct from free water and yet is released when the tissue dies.

New theories of the role of structured water and its properties are usually greeted with scepticism bearing in mind the lessons learnt from the "Polywater" episode. However, it was in this context that the importance of water structure in the integrity of cellular processes and the effects of Lincomycin antibiotics on water structure was investigated. Differing antibiotic water structuring effects might provide the basis of an explanation of the different extents of irritancy of these very similar compounds since any influence on or disruption of water structure in biological systems could lead to the breakdown of membrane processes, uncontrolled metabolism and eventually irritation.

I.3 An Introduction to the Antibiotics Concerned

Drug-induced irritation is a widespread problem, especially with compounds that are administered parenterally (external to the intestine). However, the antibiotics studied during this research were an example of cases in which compounds although of similar structure or general classification lead to quite different extents of irritancy.

It was the need to investigate the physico-chemical basis of such effects that led to the involvement with Upjohn Limited in the study of lincomycin hydrochloride (L/M), clindamycin hydrochloride (C/M) and clindamycin phosphate (C/M/P) as well as limited studies involving erythromycin lactobionate (E/M/L) and erythromycin glutamate (E/M/G). The structures of these compounds and physical-chemical properties are presented in Chapter II and Chapter III respectively.

The erythromycins were used as reference materials with which to compare the properties of the other antibiotics. Throughout this thesis lincomycin hydrochloride has often been referred to as Lincomycin or Lincomycin.HCl and clindamycin hydrochloride as Clindamycin or Clindamycin.HCl.

Lincomycin and Clindamycin are chemically unlike any other antibacterial preparations and the group is known as the Lincomycins. Lincomycin was derived from an actinomycete - streptomyces lincolnensis var lincolnensis n.sp - by Lewis, Clapp and Grady in 1962[60] and Clindamycin was produced by 7-chloro substitution of the 7-hydroxyl group of Lincomycin.[61] The antibacterial activity of a number of analogues of Lincomycin had been studied but none were as active as Clindamycin.[62] Clindamycin has the same bacterial spectrum as Lincomycin but is more active in vivo and in vitro,[63] unfortunately it is also more irritant than Lincomycin.[9] The compounds are indicated in serious infections caused by susceptible gram +ve organisms and can be used when penecillin is contra-indicated. Aerobic gram-ve bacterial are nearly all resistant but activity against anaerobic gram-ve bacteria is important (eg the Bacteroides species).

The most common disadvantages of the antibiotics are diarrhoea (which can be severe) and colitis (which can be fatal) and so they are advisedly used only in severe infections. Clindamycin is mainly indicated in the treatment of severe anaerobic sepsis or pelvic inflammatory disease where its range of antibacterial activity is advantageous. It may also be very effective in severe infections caused by multi-resistant staphylococci, especially where the site of infection is the bone, into which high penetration is observed.[64,65,66,67]

The antibiotics may be injected intramuscularly to enable prompt absorption and later may be found widely distributed in the body. Clindamycin's mode of action is the inhibition of the synthesis of bacterial nucleic acids acting specifically on the 50S subunit of the bacterial ribosome, probably affecting the process of peptide chain initiation and thus causing a cessation of protein synthesis.[63,68]

Apart from the initial soreness about the site of an intramuscular injection there is a later general degradation of muscle tissue which can be followed by elevated levels of serum creatine kinase.[9] Increased levels of K^+ loss from red blood cells incubated in Clindamycin containing buffer than was found using Lincomycin containing buffer has also been demonstrated, the K^+ loss apparently occurring prior to cell lysis.[7]

Serum protein binding studies have also been reported, antibiotic bound to serum protein has no antibacterial activity. Gordon, Regamey and Kirby[69] showed that 72% of Lincomycin and 94% of Clindamycin was bound to serum protein in their experiments.

Although the actual extents of irritation due to Lincomycin and Clindamycin are hard to quantify, for the purpose of comparison Lincomycin will be regarded as "non-irritant" and Clindamycin as "irritant" throughout this work.

I.4 Hydrogel Applications to Water Structuring Studies

Having to some extent implicated water structure in the smooth running of cellular processes and having seen that certain types of water molecules possess their own water structuring abilities, the possible water structuring effects of Lincomycin and Clindamycin required investigation. Such studies were first carried out in aqueous solution but later studies employed model membrane systems. Such model systems were water swollen polymer networks or hydrogels.

In recent years the majority of the work within this research group has concerned the general biomedical applications of polymers and their biocompatibility. This has centred on the exploitation of the ability to produce hydrogels of desired water content and freezing/non-freezing water composition by selection of appropriate monomers (hydrophilic and hydrophobic) and cross-linking agents . Hydrogels are widely used in fields such as extended wear contact lens design, artificial liver support systems and reverse osmosis applications. The work of the group to date has meant that hydrogel materials are well characterised and understood.

The term "hydrogel" can be used to describe any water containing hydrophilic network and so includes gelatin, polysaccharides, polyelectrolyte complexes, cross-linked polyacrylamide polymers, cross-linked hydroxyl containing methacrylic or acrylic ester polymers etc.

Water in hydrogels is generally treated in terms of a three state model. Bound water is strongly associated with the polymer, probably as water hydrating the hydrophilic groups of the polymer and at the other extreme free water has properties very similar to bulk water in aqueous solution. It is generally accepted that some form of interfacial water exists between these two states and has been proposed to be associated with hydrophobic interactions between polymer segments. However, work carried out within this research group suggested a continuum of water states between the free and bound extremes. The characterisation and properties of hydrogels used during this research have been described in more detail in Chapter III.

I.5 Scope and Objectives

The project involved the study of the physico-chemical properties of Lincomycin antibiotics and an investigation of their water structuring abilities as a possible explanation of the widely different irritant effects of apparently very similar compounds.

The properties of aqueous solutions of Lincomycins were studied using techniques such as vapour pressure osmometry and differential scanning calorimetry. A range of hydrogel preparations covering a range of free/bound water compositions were synthesised and the effects of the antibiotics on water structure in these preparations were studied.

The permeability characteristics of water swollen hydrogels and hydrogels into which antibiotics had been incorporated during polymerisation were also investigated using aqueous solutions of simple solutes, aqueous antibiotic solutions and aqueous solutions of biologically important compounds that might be implicated in the irritation process.

CHAPTER II

SYNTHESIS OF HYDROGEL POLYMERS AND EXPERIMENTAL TECHNIQUES

II.1 Introduction

The synthesis of hydrogel polymers, copolymers and terpolymers and the methods by which these hydrogels were prepared in membrane forms are described in this chapter. An account of the methods employed for the subsequent characterisation of these preparations with regard to their water content, the nature of the water associated with the hydrogel matrix and factors directly related to or influenced by the extent and composition of water within the hydrogel matrix, is also included in this chapter.

II.2 Reagents

II.2.1 Monomers

The monomers used during the course of this research are shown in Table II.A, and their chemical structures are shown in Figure II (1). The liquid monomers were purified by conventional reduced pressure distillation. The purity of the monomers was established by gas liquid chromatography using a Pye series 104 model 23/34 gas chromatograph fitted with a silicon gum SE 30 column and a flame ionisation detector. The purified monomers were then stored in a refrigerator until required.

The only solid monomer used was acrylamide and this was recrystallised from ethylacetate.

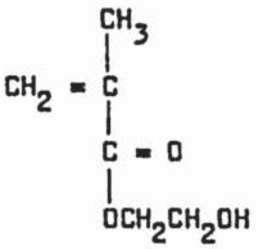
The purification of 2-hydroxyethyl methacrylate is especially difficult since it undergoes disproportionation to ethylene glycol dimethacrylate and methacrylic acid even at low temperatures.

TABLE II.A

Monomer	Molecular weight	Abbreviation	Supplier	Physical state at room temperature
2-Hydroxyethyl Methacrylate	130	HEMA	B.D.H	Liquid
Methacrylic acid	86	MAA	Koch-light	Liquid
Styrene	104	STY	Koch-light	Liquid
Acrylamide	72	ACM	Koch-light	Solid

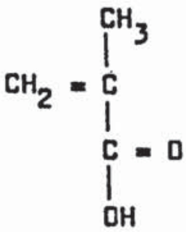
FIGURE II (1)

CHEMICAL STRUCTURES OF MONOMERS



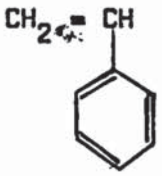
$CH_2=C(CH_3)-C(=O)-OCH_2CH_2OH$

(i) HEMA

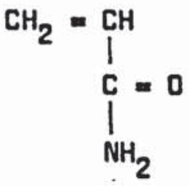


$CH_2=C(CH_3)-C(=O)-OH$

(ii) MAA



(iii) STY



$CH_2=CH-C(=O)-NH_2$

(iv) ACM

II.2.ii Cross-linking agents

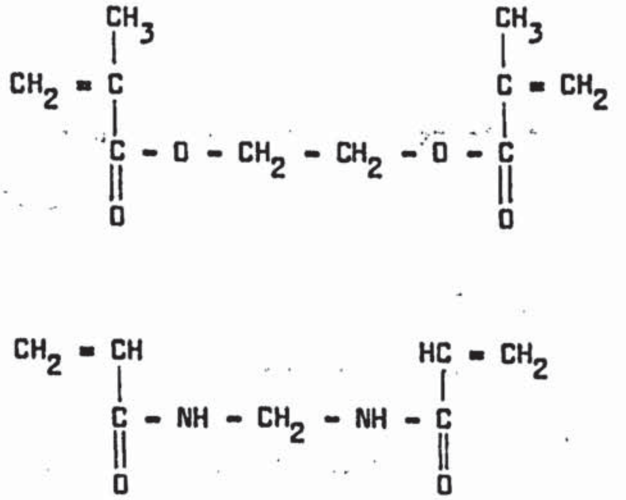
The reagents employed as cross-linking agents during this research are shown in Table II.B, and their chemical structures are shown in Figure II (2). All cross-linking agents were used as supplied without any further purification, and were stored in a refrigerator.

TABLE II.B

Cross-linking agent	Molecular weight	Abbreviation	Supplier	Physical state at room temperature
Ethylene glycol dimethacrylate	198	EDMA	B.D.H	Liquid
Methylene bis acrylamide	154	MBACM	Cambrian	Solid

FIGURE II (2)

CHEMICAL STRUCTURES OF CROSS-LINKING AGENTS



(i) EDMA

(ii) MBACM

II.2.iii Initiators

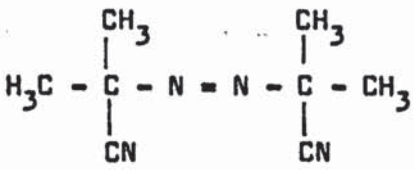
The free radical initiators used during this research are shown in Table II.C and their chemical structures are shown in Figure II (3). All initiators were used without further purification and were stored in a refrigerator.

TABLE II.C

Initiator	Molecular weight	Abbreviation	Supplier	Physical state at room temperature
α-Azo-bis isobutyronitrile	164	AZBN	B.D.H	Solid
Ammonium persulphate	228	Am.P.S	Hopkin & Williams	Solid

FIGURE II (3)

CHEMICAL STRUCTURES OF INITIATORS



$$H_3C - \underset{CN}{\overset{CH_3}{C}} - N = N - \underset{CN}{\overset{CH_3}{C}} - CH_3$$

(i) AZBN

$(NH_4)_2S_2O_8$ (ii) Am.P.S

II.2.iv Solvents

The solvents used during the course of this research are shown in Table II.D.

TABLE II.D

Solvent	Density g/cm^3	Molecular weight	Supplier
Xylene (1,4-Dimethylbenzene)	0.866	106	Hopkin & Williams
Tetrachloromethane	1.594	154	B.D.H
Dimethyl Sulphoxide A.R	1.101	78	B.D.H
1,4-Dioxan A.R	1.034	88	B.D.H

II.2.v Pharmaceutical Compounds

The antibiotics used during this research were all supplied by Upjohn Limited in crystalline form. The purity of the compounds was established by Upjohn Limited as meeting regulatory standards.

The chemical structures of the two groups of antibiotics are shown in Figure II (4). Lincomycin hydrochloride, clindamycin hydrochloride and clindamycin phosphate were generally 95 to 98% pure, the major impurity being water. Erythromycin was supplied in both the lactobionate and glutamate forms. As has already been mentioned in Chapter I, throughout this work lincomycin hydrochloride and clindamycin hydrochloride may be commonly referred to as Lincomycin and Clindamycin respectively unless otherwise stated.

II.2.vi Miscellaneous Chemicals

A number of miscellaneous chemicals were employed during the course of this research and these are shown in the Table II.E.

The chemical structures of Glycine, Phenylalanine and Creatine phosphate are shown in Figure II (5).

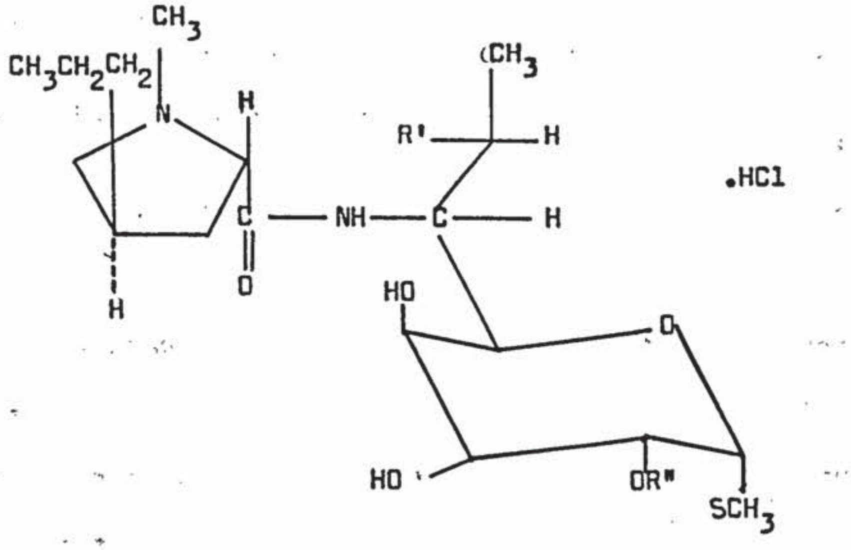
II.3 Method of Polymerisation

The hydrogel polymers, copolymers and terpolymers were all prepared in membrane form by bulk thermal free-radical polymerisation. Hydrogel polymers may be synthesised in membrane (or film), rod or powder form, however, because of the nature of the work undertaken, especially with regard to permeability studies, only hydrogel membranes were prepared.

The technique of hydrogel membrane preparation has previously been described by Barnes,[12] Ng,[11] Pedley,[13] Skelly,[14] Middleton[15] and Jolly[16]; the general method used is as follows.

FIGURE II (4)

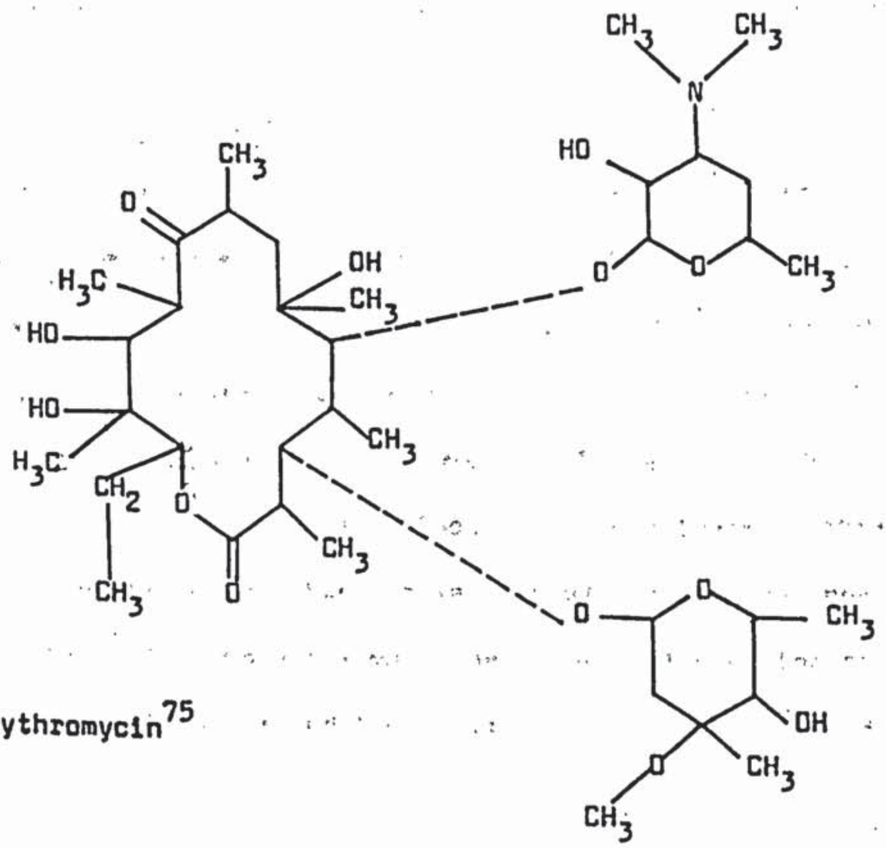
CHEMICAL STRUCTURES OF ANTIBIOTICS



(i) R' = OH (R), R" = H, = Lincomycin hydrochloride[70,65,71]

(ii) R' = Cl (S), R" = H, = Clindamycin hydrochloride[72,73]

(iii) R' = Cl (S), R" = H_2PO_3 = Clindamycin phosphate[74]



(iv) Erythromycin[75]

TABLE II.E

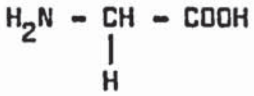
Substance	Abbreviation (Where Applicable)	Supplier
5,5 Dithiobis (2-nitrobenzoic acid)	D.T.N.B	B.D.H
2,6-Dichloro-p-benzoquinone-4-chlorimine	D.C.Q.C	B.D.H
Ammonium molybdate	-	May & Baker
Sodium arsenate	-	Hopkin & Williams
Sodium chloride A.R	-	Fisons
Potassium chloride	-	B.D.H
Calcium chloride A.R. Dehydrate	-	Fisons
Tris Buffer (Tris(hydroxymethyl)aminomethane)	-	Sigma
D-Glucose	-	B.D.H
Glycine	-	Hopkin & Williams
Phenylalanine D.L	-	Koch-light
Creatine phosphate (Phosphocreatine) disodium salt	-	Sigma

II.3.i Membrane Preparation

Hydrogel membranes were prepared by bulk polymerisation of the monomer or mixture of monomers in a suitable mould. The moulds consisted of two glass plates (12.5 cm x 10 cm), each covered with a melinex sheet (poly(ethylene terephthalate)) as an aid to mould release, placed together (melinex protected face inwards) with a poly (ethylene) gasket separating the two plates. A syringe needle was inserted through the edge of the mould so that its tip was protruding into the cavity formed by the gasket. The whole assembly was tightly clamped together by five spring clips fastened around the edges of the assembled mould. A sixth

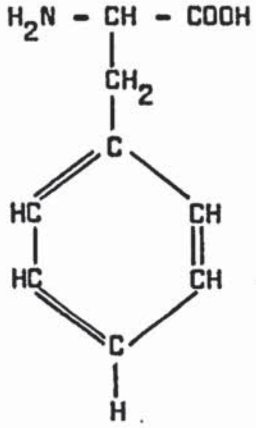
FIGURE II (5)

CHEMICAL STRUCTURES OF SOME MISCELLANEOUS COMPOUNDS

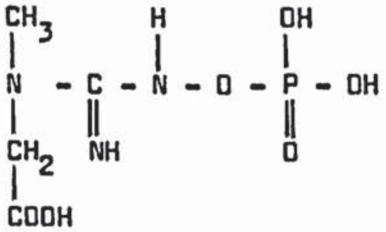


$H_2N - \underset{\displaystyle H}{\underset{|}{CH}} - COOH$

(i) Glycine



(ii) Phenylalanine



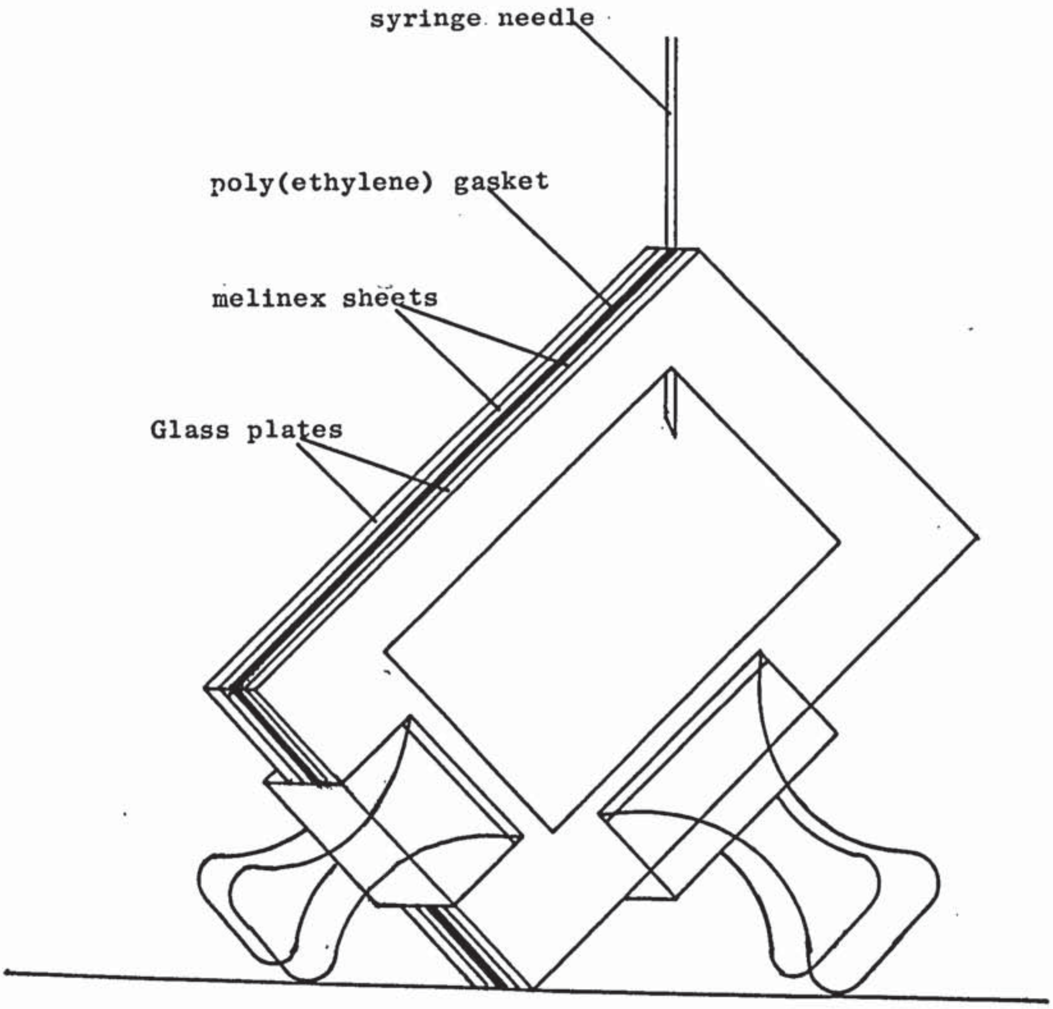
(iii) Creatine phosphate

spring clip was positioned where the syringe needle had entered the mould, after the monomer mixture had been injected into the mould and the syringe needle removed. The assembly is illustrated in Figure II (6).

In a typical synthesis a monomer mixture of 2-hydroxyethyl methacrylate and styrene in a molar ratio of 90:10 with ethylene glycol dimethacrylate (1.0% by wt total monomer) and α-azo-bis isobutyronitrile (0.1% by wt total monomer) was prepared as a homogeneous solution. After purging with nitrogen, the solution was introduced into the mould cavity formed by the poly (ethylene) gasket by means of the syringe, carefully avoiding the inclusion of air bubbles into the mould. This was best achieved by

FIGURE II.6

Membrane Making Apparatus



tilting the mould so that as it was filled air escaped through the gap created by the syringe needle. Once the mould cavity was full, the syringe needle was carefully removed and replaced by the final spring clip. The filled mould was then placed horizontally in an oven at 65°C for 70 hours, during which time polymerisation took place, followed by 2 hours post-curing at 90°C to ensure complete polymerisation. On removal of the spring clips, the mould was opened and the membrane was carefully removed from the melinex sheets. On a few occasions where mould release presented a problem, the membrane could be gently teased away from the melinex sheet as the adhering surfaces were lightly sprayed with distilled water.

Hydrogel membranes thus prepared were swollen and soaked in distilled water, with frequent changes of the distilled water to aid the removal of any residues present in the membrane, until an equilibrium hydration was reached (at least two weeks).

II.3.ii Modification of soaking and swelling treatments of hydrogel membranes

To suit the needs of the experimental work undertaken it was desirable to introduce various solutes (predominantly the antibiotics) into hydrogel matrices. This was carried out in a number of ways, by adaption of the soaking and swelling procedures of freshly prepared membranes and by modifying the polymerisation mixture composition. The main categories are shown below:

Type (i)	Hydrogel membranes swollen in water and then soaked in antibiotic solutions.
Type (ii)	Hydrogel membranes swollen and soaked in aqueous antibiotic solutions.
Type (iii)	Hydrogel membranes prepared with aqueous

antibiotic solution added to the monomer mixture before polymerisation. The resultant preparations were swollen and soaked in the same solution that had been incorporated into the preparation. In each case the solutions in which the preparations were swelling and soaking were frequently changed.

II.3.iii Type (i) Hydrogel/Antibiotic Preparations

In this type of preparation any antibiotic which entered the matrix had to displace water molecules already absorbed by the hydrogel upon swelling as well as encountering the diffusional barrier presented by the network itself. The extent to which the antibiotics could penetrate the matrix would also be dependant on the hydrogel's water content and the constituent amounts of freezing and non-freezing water, varying according to the nature of the hydrogel composition.

The level of antibiotics found in such preparations was not envisaged to be very high and when placed in a water environment these preparations would suffer fairly rapid depletion of antibiotic.

For these reasons this type of membrane preparation was not considered suitable for use in permeability studies, the amount of antibiotic present in the membrane during such studies would vary considerably over a period of time when used in a permeability cell.

II.3.iv Type (ii) Hydrogel/Antibiotic Preparations

In this type of preparation it was envisaged that the level of antibiotic present in the matrix would be greater than in Type (i) preparations. As the hydrogel swelled it would imbibe antibiotic present in the solution. However, once swollen and then soaked until equilibrium levels were reached, this type of preparation would still suffer from similar drawbacks to those encountered for Type (i) hydrogel preparations.

II.3.v Type (iii) Hydrogel/Antibiotic Preparations

This type of hydrogel preparation was considered to be the most desirable since it would contain high levels of antibiotic and, since antibiotic would be present during cross-linking, it was envisaged that a substantial proportion of the antibiotic molecules would be "incorporated" or "locked" into the hydrogel network.

Evidence that the antibiotics were indeed incorporated into the matrix is presented in Chapter III.

II.3.vi Development of Homogeneous Antibiotic Incorporated Hydrogel Membranes

References for the preparation of "preswollen" hydrogels of pHEMA, where polymerisation was carried out in an aqueous medium, are to be found in the literature[76,77]. Depending on the amount of water in the starting mixture the polymerisation yielded either an homogeneous hydrogel ($<$ approximately 40% water content) or a heterogeneous hydrogel ($>$approximately 50% water content)[78,79]. The formation of such heterogeneous structures was accounted for in terms of the phase separation of the polymer from water since water is a thermodynamically poor solvent for pHEMA. Polymerisation in thermodynamically better solvents (e.g. ethyleneglycol or ethanol) enabled the preparation of homogeneous hydrogels.

In the type of antibiotic incorporated system that was to be synthesised, as high a level of antibiotic in the matrix as could reasonably be achieved was desired. This would maximise the effects of the antibiotics on the water environment within the hydrogel and enable more well-defined comparisons to be drawn between hydrogel/antibiotic incorporated preparations of different antibiotic types.

Using an EDMA cross-linked pHEMA system it was decided to aim at incorporation of 35% (by wt total monomer) of a 1.0M antibiotic solution, this being slightly lower than the level at which the onset of heterogeneity had been reported. The synthesis of hydrogels of the following compositions were thus attempted.

(i) pHEMA incorporating 35% (by wt) 1.0M Lincomycin.HCl solution (aq).

(ii) pHEMA incorporating 35% (by wt) 1.0M Clindamycin.HCl solution (aq).

(iii) pHEMA incorporating 35% (by wt) distilled water.

The appearance of the resulting preparations are shown in Plates 1, 2 and 3 respectively. It was apparent that these compositions lay above the transition between homogeneity and heterogeneity, and the transition seemed to have been exceeded to the greatest extent for the incorporation of distilled water. The difference in appearance of antibiotic incorporated preparations and water incorporated preparations can be accounted for by the fact that a 1.0M antibiotic solution contained only approximately 45-50% (by wt) water. Results presented in later chapters might suggest that a combination of the high aqueous solubility of the antibiotics and their water structuring influences might also contribute to a stabilisation of starting solution structure and reduce the extent of heterogeneity.

Although it was apparent from the work of Refojo and Yasuda[78] that the water content of homogeneous pHEMA after soaking in water was independant of the amount of water in the starting mixture, the maximum amount of

PLATE 1

pHEMA incorporating 35% (by wt) 1.0M Lincomycin.HCl (aq)

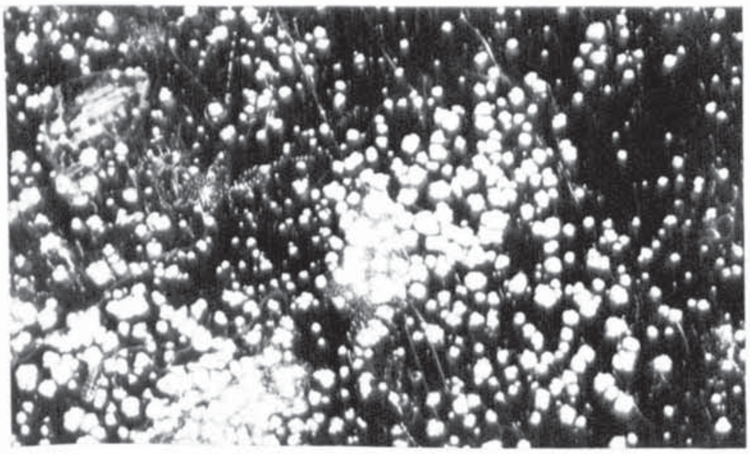


PLATE 2

pHEMA incorporating 35% (by wt) 1.0M Clindamycin.HCl (aq)

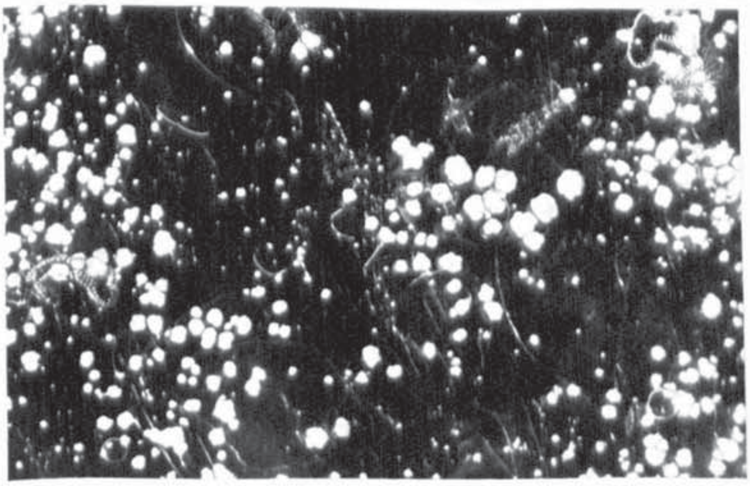
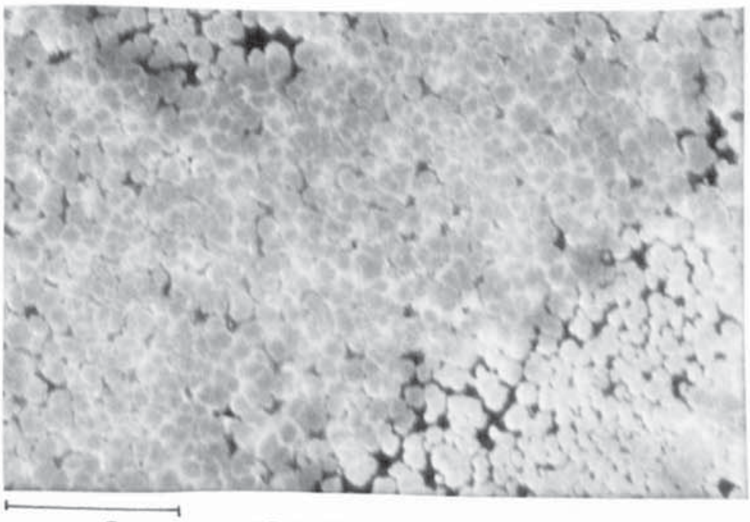


PLATE 3

pHEMA incorporating 35% (by wt) distilled water



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water alone that could be added to the starting mixture without resulting in heterogeneity was not clear.

The next step in the development of homogeneous antibiotic incorporated hydrogels was to decrease the amount of antibiotic solution added to the starting mixture.

The synthesis was repeated in the same manner as was described previously, this time for the preparation of hydrogels of the following compositions;

(i) pHEMA incorporating 25% (by wt) 1.0M Lincomycin.HCl solution (aq).

(ii) pHEMA incorporating 25% (by wt) 1.0M Clindamycin.HCl solution (aq).

(iii) pHEMA incorporating 25% (by wt) distilled water.

The appearance of the resulting preparations are shown in Plates 4, 5 and 6 respectively. Similar observations to those recorded earlier were made although a marked increase in the extent of the homogeneous regions of the hydrogels had been achieved. Once again incorporation of distilled water into the hydrogel resulted in more marked heterogeneity of the preparation than was found for antibiotic solution incorporation. However, an improvement in the 25% (by wt) water incorporated preparation was found over the 35% (by wt) composition.

It was now possible to speculate about the nature of the composition at which 1.0M aqueous antibiotic solutions could be successfully incorporated and which would result in a completely homogeneous hydrogel. The level of antibiotic solution to be incorporated into this preparation was chosen as 10% (by wt), and so the following membrane compositions were synthesized (under the same conditions as before);

(i) pHEMA incorporating 10% (by wt) 1.0M Lincomycin.HCl solution (aq).

PLATE 4

pHEMA incorporating 25% (by wt) 1.0M Lincomycin.HCl (aq)

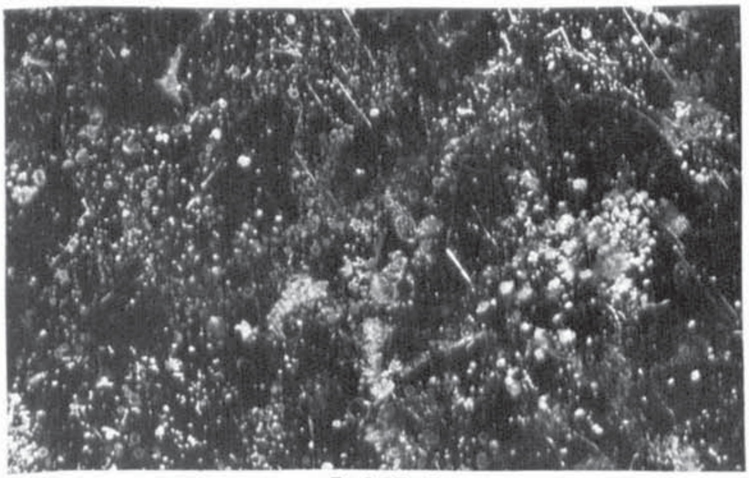


PLATE 5

pHEMA incorporating 25% (by wt) 1.0M Clindamycin.HCl (aq)

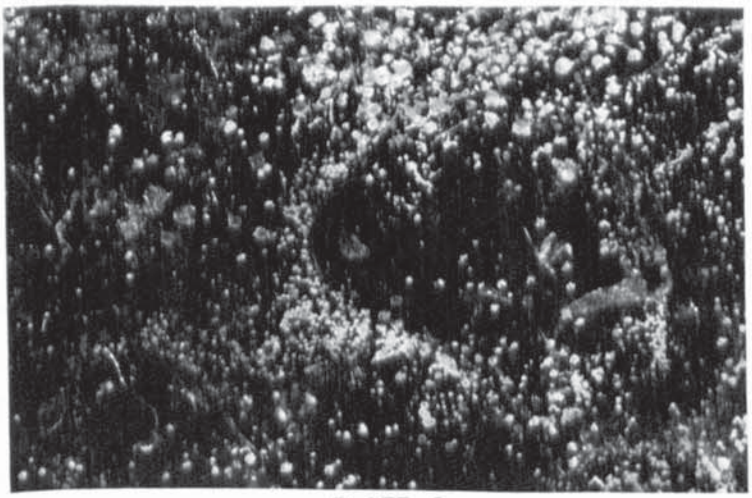
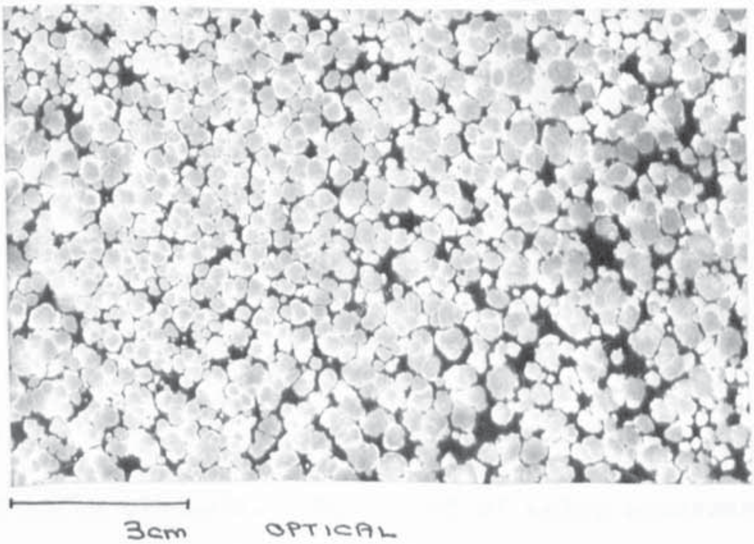


PLATE 6

pHEMA incorporating 25% (by wt) distilled water



(ii) pHEMA incorporating 10% (by wt) 1.0M Clindamycin.HCl solution (aq).

(iii) pHEMA incorporating 10% (by wt) distilled water.

Preparations (i) and (ii) above, in which 10% 1.0M lincomycin hydrochloride and clindamycin hydrochloride solutions had been incorporated were now of a completely homogeneous appearance. However, the preparation in which 10% distilled water had been incorporated ((iii) above), although markedly improved with regard to the extent of homogeneity, was still not a completely homogeneous preparation.

Having achieved a satisfactory antibiotic incorporated hydrogel composition, the range of such preparations was extended to include other copolymer and terpolymer compositions. These were also synthesised so as to incorporate 10% (by wt) of 1.0M aqueous solutions of either lincomycin hydrochloride or clindamycin hydrochloride into completely homogeneous hydrogel membranes.

The nature of these preparations, together with details of their characterisation and experimental systems in which they were employed are presented and discussed in later chapters.

II.4 Properties of Hydrogels

The presence of hydrophilic groups in hydrogel preparations gives rise to the uptake of water from an aqueous solution. The three-dimensional network of the cross-linked polymer suppresses any overhydration or dissolution of the polymer in water. There is a point at which the tendency to swell (involving the osmotic pressure of the polymer segments) is equally opposed by elastic retractive forces arising as the chains between cross-links elongate. The amount of water absorbed by a polymer in this equilibrium state is known as the equilibrium water content or E.W.C. of the hydrogel.

II.4.1 Experimental Determination of Equilibrium Water Content

The equilibrium water content of a hydrogel may be defined as the ratio of the weight of water contained in the hydrogel to the weight of the hydrogel at equilibrium hydration, and was expressed as a percentage. The weight of water contained in the hydrogel was determined by subtraction of the weight of a dehydrated sample of hydrogel (dehydrated to constant weight) from the previously recorded weight of the same sample when at equilibrium hydration. This can be expressed in the form seen in equations II.1 and II.2[80]

$$\%\ \text{E.W.C.} = \frac{\text{weight of water (Ww)}}{\text{weight of hydrogel (Wg)}} \times 100$$

Equation II.1

OR

$$\%\ \text{E.W.C.} = \frac{\text{weight of hydrated sample} - \text{weight of dehydrated sample}}{\text{weight of dehydrated sample}} \times 100$$

$$= \frac{Wg - Wp}{Wg} \times 100$$

Equation II.2

where Wp = weight of dehydrated polymer.

The hydrogel membranes were allowed more than sufficient time (at least two or three weeks) for equilibrium at room temperature (23°C) in distilled water to be reached. Several disc samples were cut from each of the hydrogel membranes using a cork-borer (approximately 1 cm in diameter). The surface water of each hydrated sample was carefully removed with a soft tissue paper and the samples transferred to previously weighed sample bottles. After weighing, the samples were dehydrated under vacuum at 60°C until a constant weight was attained. The equilibrium water content of each sample was then calculated according to Equation II.2.

The E.W.C's of several samples of each type of membrane preparation were determined and the average, generally better than ± 1% (by wt), was taken as the value of the E.W.C. of that preparation.

The factors affecting the E.W.C's of various hydrogels are discussed in Chapter III.

II.4.ii Determination of Hydrogel Densities

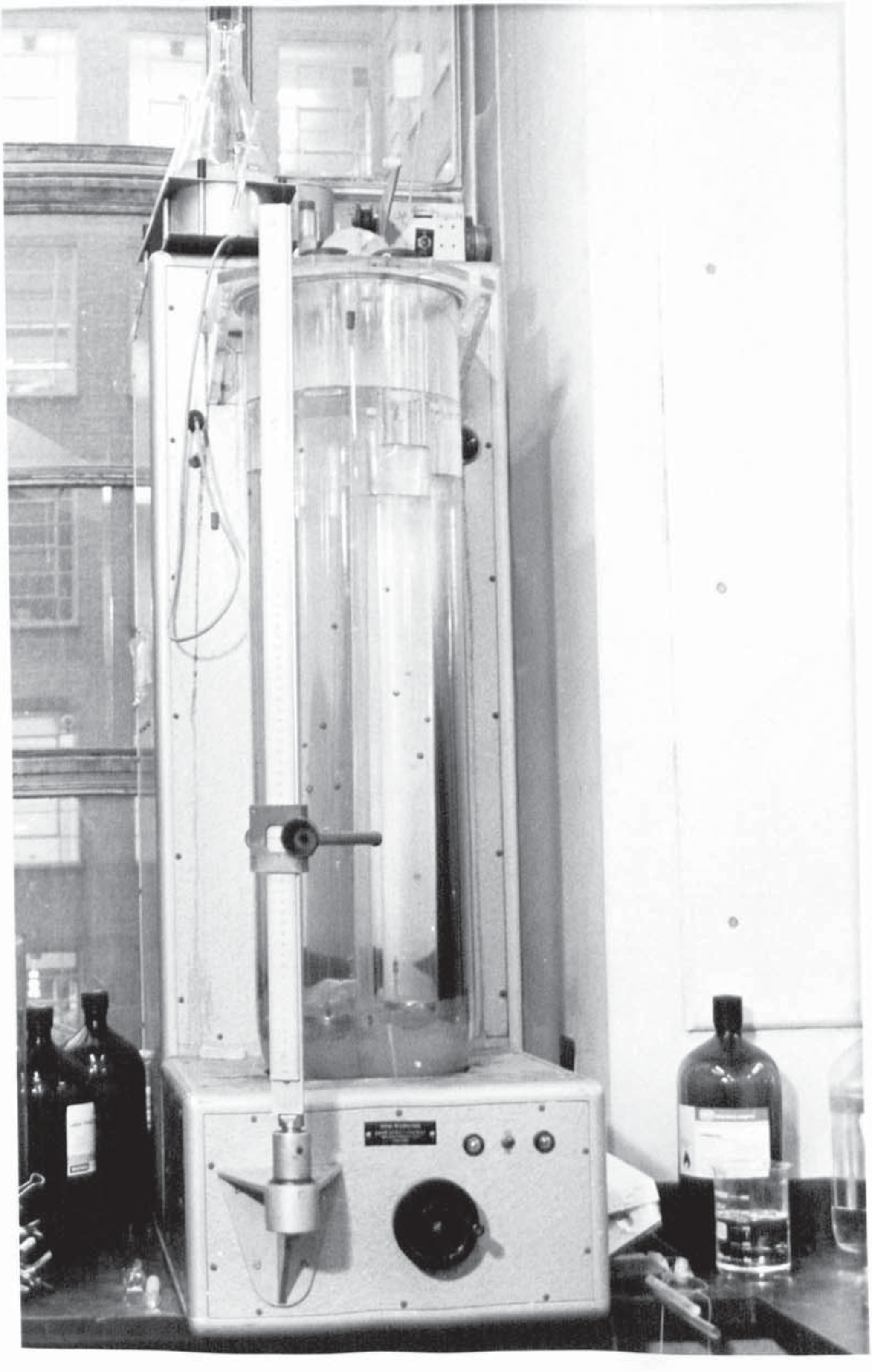
The determination of the densities of hydrogel membrane preparations, in both hydrated and dehydrated states, was carried out using a Davenport density gradient column apparatus (Plate 7). This apparatus enables the construction of density gradient columns of selected density range depending on the densities of the solvents chosen. Once prepared, columns can be maintained at constant temperature (23°C).

Columns were constructed, according to the procedure stated in the instruction manual,[81] with a mixture of two miscible liquids (in this case xylene and tetrachloromethane) so as to produce a useable density range of approximately 0.95 to 1.45 cm^{-3}. The Davenport density gradient column apparatus allows the mixing of the liquids used and their introduction into the column to be carried out automatically, thus producing a column where the density of the liquid mixture increased from the top to the bottom in a linear manner.

The columns were calibrated using coloured marker floats of known densities, selected so as to extend over the density range of the column and the positions of the floats were measured with a cathetometer once they had reached equilibrium with the column. The marker floats were introduced into the column automatically by a slow speed motor which lowered a "sweep basket", held on a piece of cord, into the column. The marker floats were placed in the sweep basket beforehand. This technique causes minimal disturbance of the density gradient and allows the column

PLATE 7

Davenport Density Gradient Column Apparatus



to be "swept" of samples and marker floats, after the sample densities have been determined, and then recalibrated and used again.

The determination of the density of a hydrogel sample in the hydrated state was carried out after removing surface water from a small hydrogel sample with soft tissue paper. The sample was moistened with tetrachloromethane and then carefully introduced into the top of the density gradient column. The position of the sample was monitored with time and the final height reading taken when equilibrium density was reached. Height readings for several samples of the same preparation were recorded and the average was used in conjunction with the calibration curve to find the density of the preparations.

The determination of the density of a hydrogel sample in the dehydrated state was carried on a sample cut from a hydrated sheet and dried under vacuum at 60°C in the same manner as for the E.W.C. determination of a sample. The dehydrated sample was moistened in tetrachloromethane and introduced into the top of the density gradient column in the same way as described above. Once again an average height reading was taken for several samples of the sample preparation and used to ascertain the density of the preparation in the dehydrated state.

The density of each preparation was obtained by reading off the density, corresponding to the average height value determined from a density calibration curve constructed from the cathetometer readings of the marker floats of known densities. Considerations such as non-linearity of density gradient columns, stability of the column with use and the effects of the solvents used on the hydrated and dehydrated hydrogel samples are discussed in Chapter III.

...

II.4.iii Determination of the E.W.C. of an Hydrated Polymer from the Densities of Hydrated and Dehydrated Samples

Ng[11] derived an equation which theoretically enabled the determination of the E.W.C. of an hydrated polymer from a knowledge of its densities in both hydrated and dehydrated states (Equation II.3). This was based on the assumptions that the volume of the hydrogel was the sum of the volumes of the polymer and water in the network, and the volume of the polymer was the ratio of the weight of the polymer to the density of the dehydrated polymer.

$$\frac{Ww}{Wg} = \frac{\left(1 - \frac{dg}{dp}\right)}{dg\left(\frac{1}{dw} - \frac{1}{dp}\right)} \times 100 \qquad \text{Equation II.3}$$

Wg and dg were the weight and density of the polymer in the hydrated state, dp was the density of the polymer in the dehydrated state, and Ww and dw were the weight and density of water in the hydrogel.

The values of densities obtained and the differences between experimentally determined E.W.C's and E.W.C's calculated from density measurements are discussed in Chapter III.

II.4.iv Differential Scanning Calorimetry in the Study of Hydrogel Properties

A complete account of the technique and uses of differential scanning calorimetry is contained in the following section of this chapter. However, it is appropriate to mention here, under "properties of hydrogels" that differential scanning calorimetry enables the determination of the constituent amounts of "freezing" and "non freezing" water found in hydrogels.

These different states of water have already been described in Chapter I. Differential scanning calorimetry can be used to determine the amount of freezing water present in a sample and the amount of non-freezing water present can be considered as the difference between the equilibrium water content of the sample and the amount of freezing water determined.

II.5 Experimental Techniques

This section contains information relating to a number of techniques used or developed during the course of this research. Results obtained from the use of these techniques will be presented and discussed in Chapter III where additional information necessary for the evaluation of results can also be found.

II.5.1 Differential Scanning Calorimetry (D.S.C.)

It has already been mentioned that a quantitative assessment of the amounts of freezing and non-freezing water can be obtained through the use of differential scanning calorimetry (D.S.C.). D.S.C. falls into the thermodynamic technique category discussed in Chapter I.

D.S.C. is one of a number of techniques which can all be described as forms of thermal analysis. Thermal analysis in its broadest sense is a measure of the changes in the physical or chemical properties of samples as a function of temperature. Thermal transitions taking place in a sample can be either exothermic or endothermic and D.S.C. enables the measurement of the amount of heat absorbed or evolved during a transition. There is a distinguishing feature of D.S.C. as compared with, for example, differential thermal analysis (D.T.A.). D.T.A. is primarily used to provide qualitative rather than quantitative information,[82] the difference in temperature between a sample and an inert reference material being recorded as a function of temperature.[83] However, in D.S.C. the instrument senses the heat absorbed or evolved by a sample during a transition and alters the distribution of heating energy between the sample and the reference material so as to maintain a thermal balance.[84] In D.S.C. the area under the output curve is directly proportional to the total amount of energy into or out of the sample and the ordinate is therefore proportional to the rate of heat transfer.[83,84]

The instrument used during the course of this research was a Perkin-Elmer D.S.C. -2 Differential Scanning Calorimeter in conjunction with a Servoscribe Is RE 542.20 potentiometric recorder with DISC integration facility and temperature event marker. Chart speeds of 30 mm min^{-1} or 600 mm hr^{-1} were generally used (Plate 8).

The Perkin-Elmer D.S.C - 2 was equipped with a liquid nitrogen subambient accessory permitting the use of the instrument over a temperature range of -175°C to 725°C. The instrument allows the operator to bring the sample to a desired temperature, select a programmed rate of either heating or cooling of the sample (0.313^{o} min^{-1} to 320^{o} min^{-1}) and thus programme the change in average temperature of the sample holders to a desired final temperature. Sensitivities ranging from 20 to 0.1 mcal sec^{-1} can be selected to enable full scale deflection of the recorder pen.

II.5.ii Preparation of Samples for D.S.C. and their Subsequent Analysis

Hydrogel membranes to be studied (approximately 0.5 mm in thickness) were cut into small discs of approximately 1.0 to 2.0 mm diameter using a cork borer, carefully wiped with soft tissue paper to remove surface water, and then hermetically sealed in aluminium sample pans.

D.S.C. traces were obtained for sample sizes of the range 1.0 to 7.0 mg weighed out using a Perkin-Elmer AD 2 - Z Autobalance. Samples were cooled to -50°C at a rate of 40^{o} min^{-1} and were then heated generally at either 1.25°C min^{-1} or 5°C min^{-1}, at sensitivities generally of either

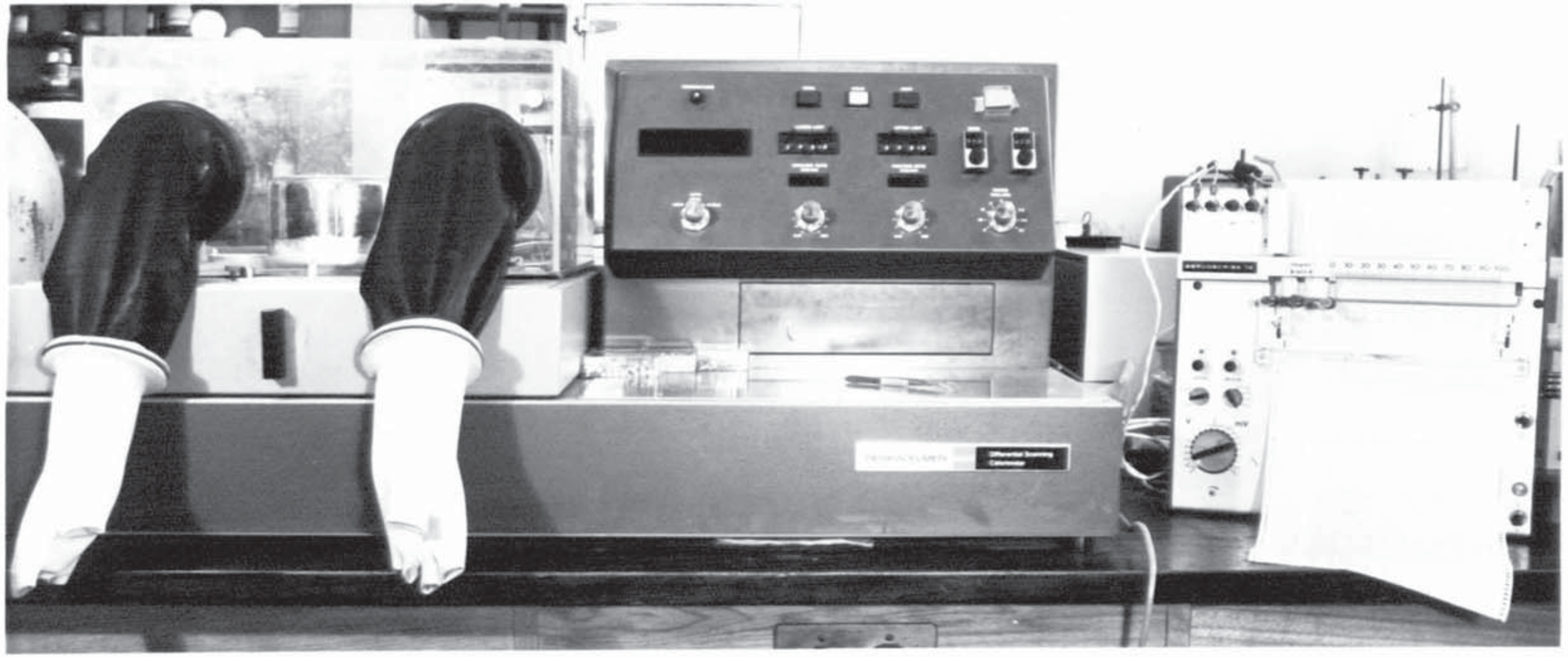
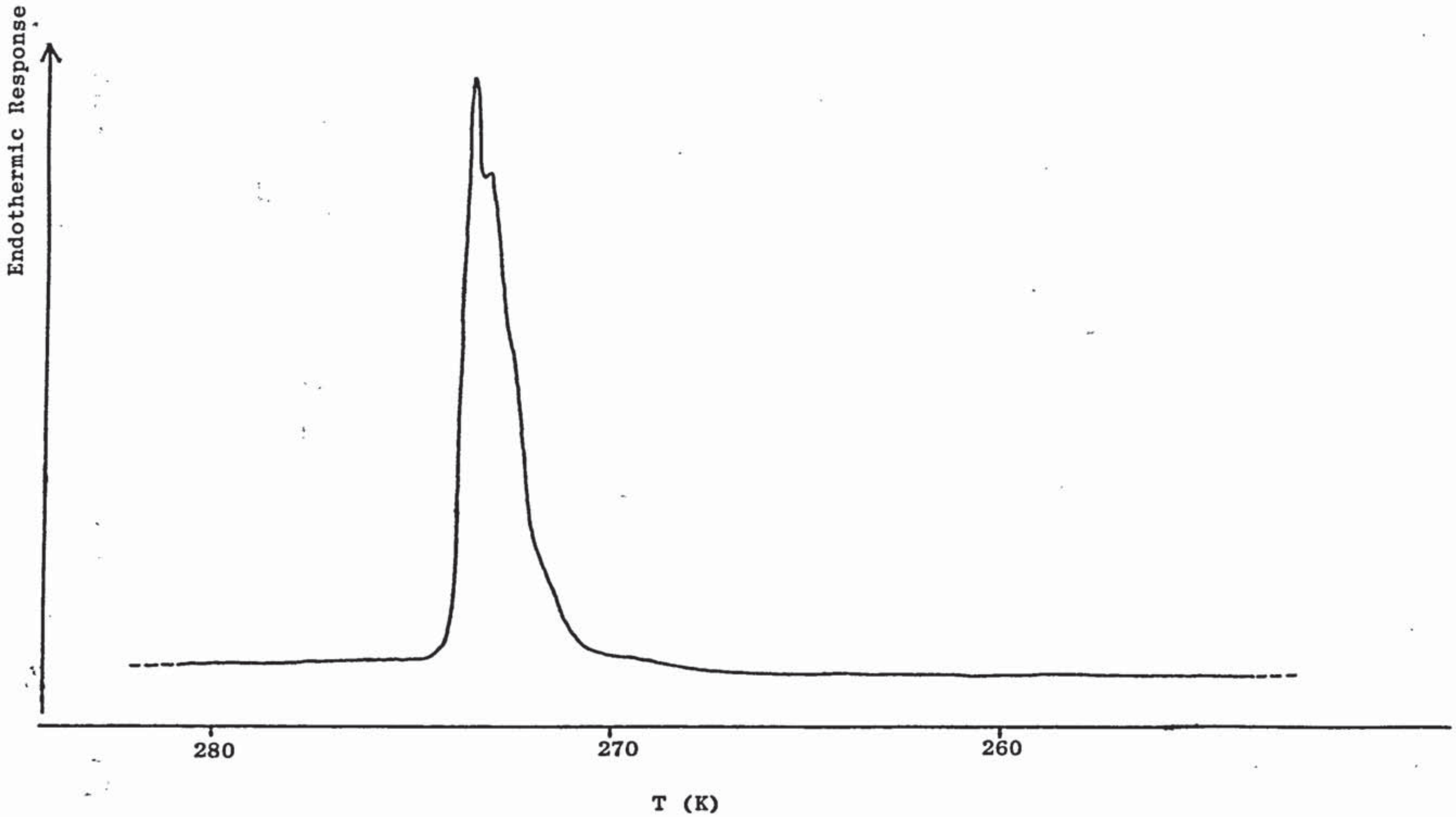


PLATE 8

Perkin Elmer DSC - 2 Differential Scanning Calorimeter

FIGURE II.7

A Typical Melting Endotherm of a Hydrogel Material as seen by Differential Scanning Calorimetry



2 mcal sec^{-1} or 5 mcal sec^{-1}, up to 20°C. An example of a typical trace obtained for a hydrogel can be seen in Figure II.7. The area under the peak would be determined by DISC integration and the amount of freezing water in the sample computed using a calibration constant. The calibration constant was the slope of a plot of the peak areas of various weights of distilled water samples. Since it has been shown that the heat of fusion of freezing water contained in water swollen polymers is virtually identical to the heat of fusion of pure water,[85,86] the freezing water content of a sample can be expressed as a percentage of the weight of hydrated polymer. The amount of non-freezing water being taken as the difference between the total water content of the sample and the amount of freezing water.

It is interesting to note here that many of the melting endotherms obtained previously[10] and during the course of this research (and indeed freezing exotherms, where carried out) did not show the simple melting behaviour associated with pure water, but were more complex. These "fine" structures were considered to be indicative of the various states of water that have been envisaged (Chapter I) and their significance will be described in detail in Chapter IV.

II.5.iii Vapour Pressure Osmometry (V.P.O.)

The technique of vapour pressure osmometry was employed to investigate the possible existence of intermolecular associations in solutions of the various antibiotics studied. The instrument used was a Mechrolab Vapour Pressure Osmometer Model 301A fitted with a 37°C probe and 37°C thermostat (Plate 9).

The methods of equilibration (using solvent appropriate to the intended experiment) and calibration of the instrument as well as experimental

PLATE 9

Mechrolab model 301A Vapour Pressure Osmometer procedure, were carried out in accordance with the instruction manual for the instrument.[87]

The operating principle of the instrument was that of vapour pressure lowering. A solution in a given solvent will always have a lower vapour pressure than the pure solvent. In this instrument, a drop of solvent and a drop of solution were suspended, side by side, in a closed chamber saturated with solvent vapour.

The difference in vapour pressure of the two drops results in differential mass transfer between the two drops and the solvent vapour phase resulting in lower evaporation from the solution drop than from the solvent drop. This transfer causes a temperature difference between the two drops (because of the heat of vapourisation) which is proportional to the vapour pressure lowering and hence to the solute concentration.

The instrument itself consisted of an insulated, thermostatically controlled chamber into which a probe comprised of two thermistors was placed. By means of syringes it was possible to suspend droplets of solvent or solution on the probe. The probe had been already positioned above a small reservoir of pure solvent containing a solvent wick. This arrangement ensured that the chamber contained a saturated atmosphere of pure solvent vapour. The chamber was allowed to reach temperature and vapour equilibria before any measurements were made, the thermistor probes were connected into a wheatstone bridge circuit and hence any changes in temperature caused a change in resistance (ΔR) which could be found by balancing the circuit using a dekostat.

Any temperature change was a colligative effect, dependant solely upon the number of dissolved molecules, being independant of their chemical characteristics. The instrument may therefore be calibrated with a series of concentrations of a "known" solute in the solvent to be used. Data relating to the "unknown" parameters of the substances under investigation can be derived from such a calibration.

II.5.iv Determination of "Apparent" Molecular Weight Using Vapour Pressure Osmometry

Calculations and the assessment of data were carried out according to the "constant K" method[88] which was developed from the "variable K" method described in the manual[87]. A brief account is presented here.

The small temperature changes observed during V.P.O. studies cause changes in resistance which can be represented by Equation II.4

$$\Delta R = a_0 + a_1c + a_2c^2 + a_3c^3 \qquad \text{Equation II.4}$$

where ΔR was the dekostat reading for a given concentration c (gl^{-1}). The coefficient a_0 represents a zero point displacement (ie ΔR not zero at zero concentration). This arises if the balance control was accidentally altered while altering the dekostat or by using solvents from different sources. Generally a_0 should be zero.

Coefficient a_1 can be expressed as K/M, where K is a calibration constant and M the molecular weight of the solute. The term a_2 is large enough to necessitate the inclusion of a_2c^2 but a_3c^3 can be neglected.

Thus Equation II.4 reduces to Equation II.5,

$$\Delta R = a_1c + a_2c^2 \qquad \text{Equation II.5}$$

which can be rewritten as Equation II.6,

$$\frac{\Delta R}{c} = a_1 + a_2c \qquad \text{Equation II.6}$$

Therefore a plot of $\Delta R/c$ versus c should yield a straight line of intercept a_1 and slope a_2.

To calibrate the instrument ΔR measurements were made on a range of solution concentrations of a solute of known molecular weight. The intercept a_1 was found and since $a_1 = K/M$, the calibration constant K, could be found.

To determine an "unknown" solute's molecular weight, a range of solution concentrations of the unknown were made in the same solvent used for instrument calibration. The ΔR reading of each solution was determined and plotted in the form above. Since K was now known, M could then be derived from the value of the intercept.

An alternative method, when using high concentration solutions and especially if the solute is of high molecular weight, is to plot $\sqrt{\Delta R/c}$ against c.[89]

Using the vapour pressure osmometry technique it is possible to follow intermolecular association since association leads to fewer particles in solution. This modification of the colligative effect of the solution results in the "apparent" molecular weight of the solute being higher than its known molecular weight.

II.5.v Permeability Apparatus - Introduction

The assessment of various permeability parameters of hydrogel/antibiotic systems was possible using a permeability cell made available to this research group by D.C. Sammon of A.E.R.E. Harwell. Only the assembly and operation of the apparatus has been described here. A full account of the characterisation of the apparatus and its subsequent use, together with details of the systems studied and the results obtained, can be found in Chapter V. Permeability studies on more complex systems have been presented in Chapter VI.

II.5.vi Permeability Apparatus - Design and Assembly

The permeability cell can be seen in Plate 10. The unit was mainly constructed in perspex and was comprised of two chambers which were designed so that a membrane barrier, held between two silicon rubber gaskets, could be clamped firmly between the two compartments using a number of bolts. Extreme care was required when clamping membranes into position. Insufficiently tightened cells would allow the fluid contents of the chambers to leak out of the unit. However, overtightened cells could result in the membrane being damaged.

The average membrane thickness was determined for at least 10 to 15 different sites, on the region of the membrane which had been exposed within the cell, at the end of the experiment using a thickness gauge.

Provision was made for the circulation of fluid into and out of each compartment. The solutions in contact with each side of the membrane were continually stirred by stirrer paddles positioned close to the membrane which were driven, via magnetic couples, by stirrer motors external to the system. The cell was positioned so that the fluid outlet of each compartment was directly above the fluid inlet in order to facilitate the removal of air bubbles. The arrangement of the assembled permeability cell can be seen in Figure II (8). The assembled permeability cell was then immersed in a water bath specially constructed to enable the proper functioning of the magnetically coupled stirrers. The use of a water bath also prevented exposed edges of membranes clamped into the permeability cell from dehydrating, a process that could lead to damage to the rest of the membrane.

The inlet and outlet of each compartment of the permeability cell were connected, using silicon rubber tubing to fluid reservoirs.

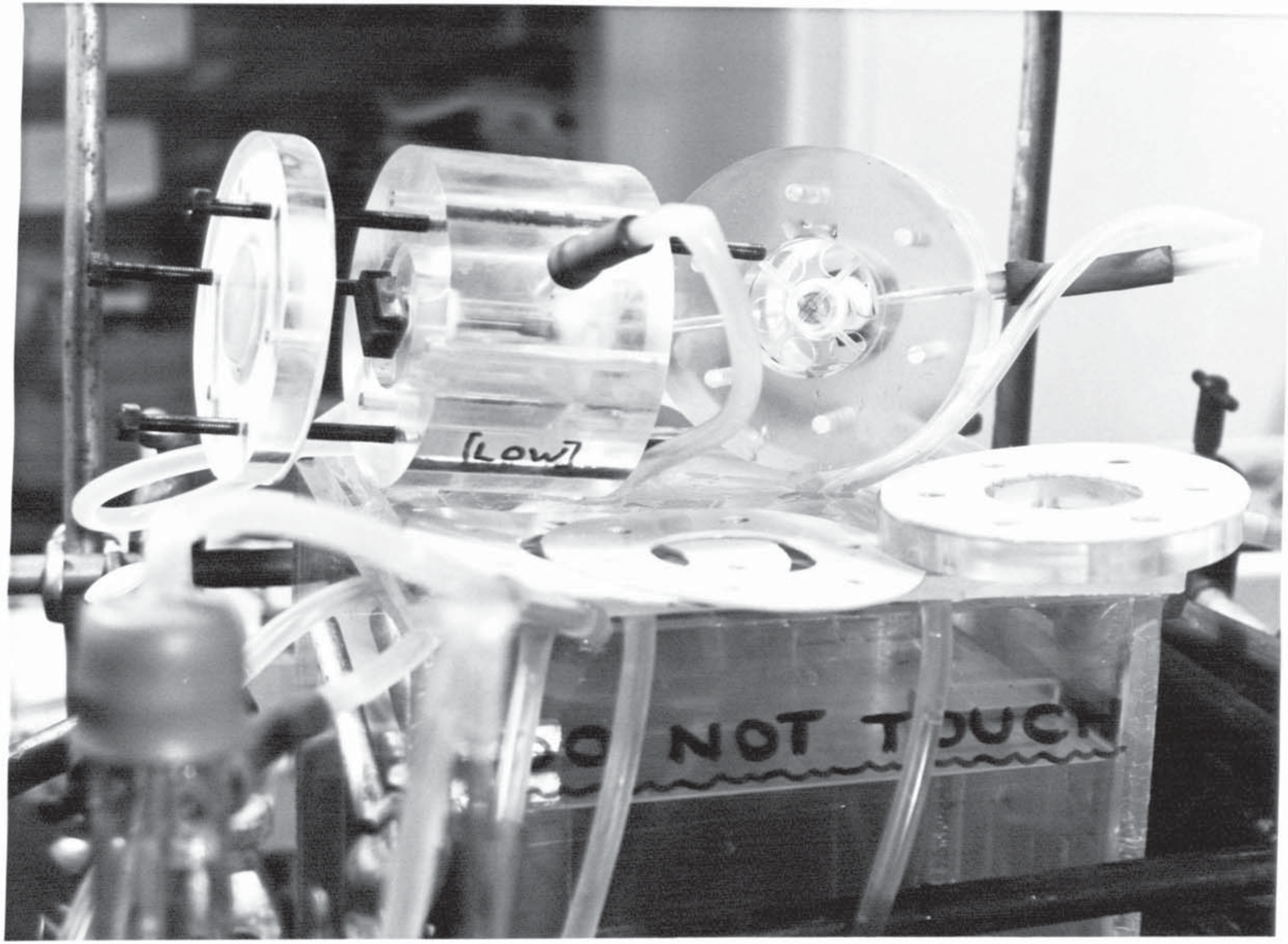


PLATE 10

Permeability Cell

FIGURE II(8)

Diagrammatic representation of permeability cell

The closed fluid loops of each side of the permeability cell were circulated using a Watson and Marlow flow inducer. Thus the fluid in each compartment was stirred and circulated. Each fluid circuit was designed to hold 130 ml of fluid and the peristaltic pump circulated the fluid in each circuit at the rate of 90 ml min^{-1}. The stirring rate for each compartment was approximately 250 rpm. The complete apparatus can be seen in Plate 11. The water bath was maintained at the desired temperature using a Churchill heater/circulating pump unit connected to the inlet and outlet of the water bath.

II.5.vii Permeability Apparatus-Experimental Procedure

Once a membrane had been fitted to the permeability cell and the whole apparatus assembled, the system was left for at least 1.5 hours at the desired temperature to allow temperature equilibration of the system. The solutions with which each compartment was to be filled were also equilibrated, at the temperature to be used for the experiment, prior to their use.

The permeability cell was filled via the two fluid reservoirs (one for each fluid circuit) once the peristaltic pump was in operation, a process that usually took about 2 minutes. The solution of the component to be studied was on one side of the membrane and an equal volume of distilled water was on the other side of the membrane. Zero time was taken as the commencement of the filling process. Any air bubbles in the fluid circuits were eliminated by the debubbling action of the fluid reservoirs. Samples of the solutions in contact with both sides of the membrane could be removed, by syringe, from the fluid reservoirs. Sample sizes of 1 ml were generally used and were taken from the low concentration (receptor) solution side of the membrane.

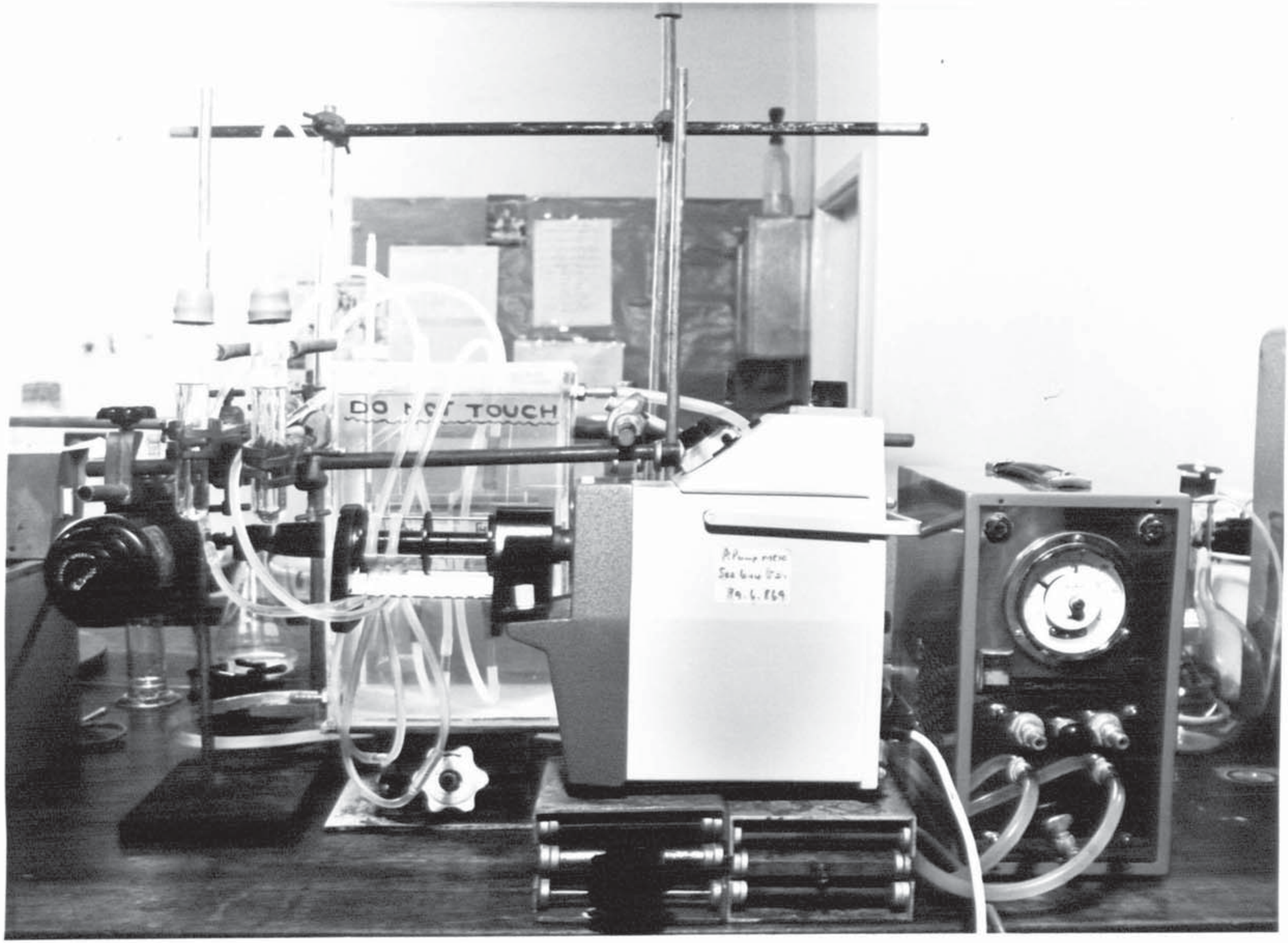


PLATE 11

Completely Assembled Permeability Apparatus

Once the permeability cell was filled the hydrostatic pressure gradient across the membrane could be equalised. Coarse adjustment was carried out using an adjustable clip which, by narrowing the bore of the outlet tube from one side of the permeability cell, could increase the pressure exerted on that side of the membrane. Fine adjustment of hydrostatic balance was carried out by either raising or lowering the height of one of the fluid reservoirs. The state of the hydrostatic balance could be assessed from observations of the fluid height in each fluid reservoir. In cases of poorly adjusted hydrostatic pressure the state of hydrostatic balance could, when very thin or fragile membranes were used, be seen from distortion of the membrane. Hydrostatic balance was generally achieved before 5 minutes of experimental time had elapsed and the balance was maintained throughout the course of the experiment. Stirring of each chamber would be commenced after hydrostatic balance was achieved since the stirrer rotor might otherwise damage a membrane which had distorted slightly before the hydrostatic pressure was equalised.

The use of gauzes to support thin or fragile membranes has been reported. Flynn and Smith[90] observed no significant change in the transport characteristics of p-amino acetophenone through polymer membranes whether or not a membrane support screen was fitted. However, previous work on membranes using gauze supports, with this permeability cell, had been abandoned in favour of a hydrostatic pressure control system.[91]

II.5.viii Some Advantages and Disadvantages of Equicompartment Horizontal Permeability Cells

The permeability cell used during these studies can be described as an equicompartment horizontal unit. There are many advantages of units constructed in such a way over other types of system. Earlier systems possessed many drawbacks, for example, fluid compartments mounted one above the other resulted in a hydrostatic head being produced. Stirring of fluid layers at the membrane, especially the underside was difficult, air bubbles may collect at the underside of the membrane, and assembly of the apparatus was generally complicated and temperature control may not be possible.

It is important to remember that while trying to improve the design of a cell in the above areas, the sensitivity of the unit might be compromised. Good sensitivity can be achieved from a unit with a high membrane surface area:fluid volume ratio.

The permeability apparatus used here eliminated the problems associated with a hydrostatic head, allowed removal of air bubbles via debubblers and had good temperature control. The removal of samples was also straightforward.

Systems similar to that used here have appeared in the literature, however these have often failed to adequately overcome the main disadvantage of the design of this type of system, namely, the stirring of fluid in each compartment. The stirring of each compartment must be adequate and uniform so as to minimise the diffusional layer thickness and maintain diffusional layer uniformity. However, adequate stirring in the plane of diffusion in horizontal systems is difficult to achieve.

Shenouda and Mattocks[92] resorted to a system which used stirring perpendicular to the plane of diffusion. Misra et al[93] and Olsen et al[94] succeeded in producing a fairly complex method of achieving diffusional plane stirring. However, the system used during these studies was considered to be superior in assembly and operation to the above units.

Unfortunately sensitivity was not as high as could have been expected mainly because of the assay used for the analysis of samples (in the case of antibiotic solutions). Since 1 ml samples were required for the antibiotic assay and in order to provide a reasonable number of samples throughout the course of the experiment whilst trying to avoid significant depletion of fluid volumes as a result, it was necessary, to use fluid volumes of 130 ml on each side of the membrane. This inevitably lowered the surface area:fluid volume ratio and so lowered sensitivity. Fluid volumes could have been reduced for later experiments on solutes which could be assayed with smaller sample volumes but it was decided to keep as many of the parameters of the permeability system constant as was possible throughout the course of this research.

II.5.ix Antibiotic Assay Development - Introduction

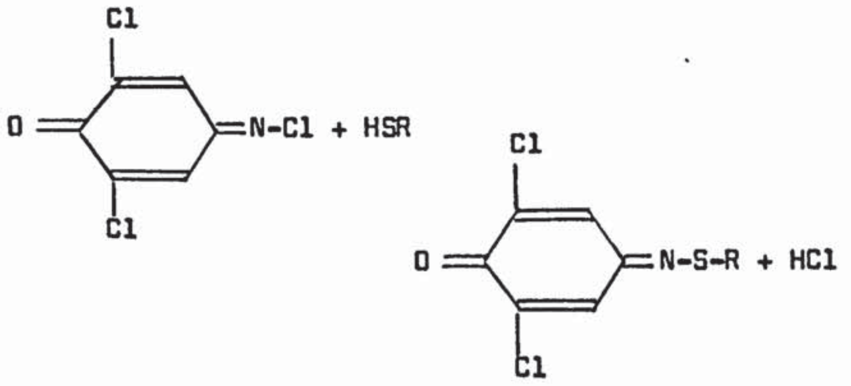
The development of an assay for the Lincomycin group of antibiotics was essential to the course of the research to be undertaken. Permeability experiments (Chapter V) required an assay that could cope with a large number of samples of aqueous antibiotic solutions while maintaining a reasonable level of sensitivity.

A number of techniques for determining antibiotics of the Lincomycin group have been reported previously. Examples of these include G.L.C.-mass spectroscopy,[95] high pressure liquid chromatography[96] and bacteriological methods.[97] These techniques, although very sensitive, were not considered to be able to cope with the large number of samples involved within a reasonable time.

II.5.x Development of a 2,6-Dichloro-p-benzoquinone-4-chlorimine (D.C.Q.C.) Assay for Lincomycins

The Assay described here was based on a procedure developed by Baudet.[98] This was itself a simplification of a procedure reported by Prescott,[99] a method used later to try to improve the sensitivity of the assay. Both of these assays were based on the colorimetric determination of methanethiol generated from the acid hydrolysis of the methylthioglycosido group of the antibiotics. The D.C.Q.C. assay described in this section was based on the formation of a quinonesulphonimine according to the reaction shown in Figure II (9).

FIGURE II (9)



The conditions of the assay were adjusted until optimum conditions for colour development and sensitivity were achieved. The assay procedure used was as follows:

Reagents : D.C.Q.C. Reagent, 2,6-Dichloro benzoquinone-4-chlorimine (D.C.Q.C.) 0.5% in 1,4 Dioxan

0.1 M HCl

Standard solutions of lincomycin hydrochloride, clindamycin hydrochloride and clindamycin phosphate of 0.001 to 0.1M were prepared in distilled water.

1 ml aliquots of samples and standards were placed into test-tubes which were then closed with subaseals. 1 ml of 0.1M HCl was then injected into each tube through the subaseals. Each tube was shaken and then placed in a water bath at 70°C for 30 minutes (with occasional shaking). At

the end of this incubation period the tubes were removed from the water bath and rapidly cooled to room temperature (23°C). 0.25 ml aliquots of D.C.Q.C. reagent were injected into each tube through the subaseals and the tubes shaken well to allow the colour reaction between the D.C.Q.C. reagent and the methanethiol gas to take place. The tubes were allowed to stand for 5 minutes to enable full colour development and their absorbance at 450 nm was then determined using a Brinkman PC/600 probe colorimeter. Blank samples of distilled water were also run and these were used to zero the colorimeter absorbance. Calibration plots were constructed using the prepared standards for each antibiotic type assayed. The concentration of a sample solution was then found by reading off the concentration corresponding to its absorbance on a plot of standard concentration versus absorbance.

The D.C.Q.C. reagent was found to be stable for at least one month after which time it slowly changed from a bright, clear yellow solution to a darker orange solution, an associated drift in calibration curves was also noted. Fresh D.C.Q.C. reagent was therefore frequently prepared.

The Lincomycins are known to be stable in solution for long periods of time, liquid pharmaceutical preparations of the antibiotics are stable for at least three years at temperatures of about 20°C.[67] Experiments carried out during the course of this research indicated that there was no significant change in the amount of antibiotic detectable in 1.0M aqueous solutions when samples were stored for periods of 1 to 8 days before analysis.

The above assay technique was used for the determination of results involving lincomycin hydrochloride and clindamycin hydrochloride throughout the course of this research. In each case the application of the assay to a series of standard solutions resulted in an error limit better than ± 5%. Unfortunately, while the absorbances of equal concentration solutions of lincomycin hydrochloride and clindamycin hydrochloride were comparable, the absorbance of clindamycin phosphate solution of the same concentration was found to be much lower. Despite attempts to adapt the conditions of the assay so as to increase sensitivity for clindamycin phosphate, the assay for this antibiotic was too irreproducible and inaccurate to be used. The reason for this was not clear but would probably include interference of the phosphate group with the acid hydrolysis or hydrolysis of the phosphate group itself which then reacted with the methanethiol liberated or the D.C.Q.C. reagent before the colour reaction between methanethiol and D.C.Q.C. reagent was complete.

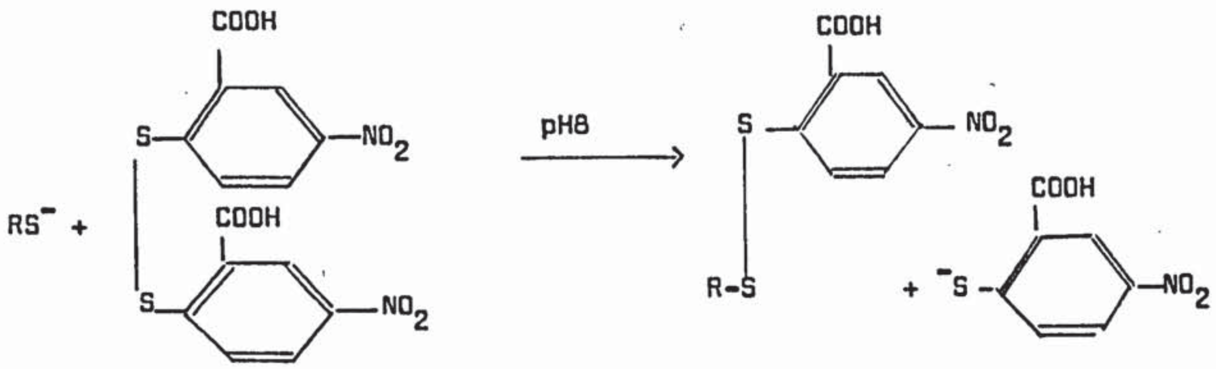
Because of these difficulties the study of the permeability characteristics of clindamycin phosphate/hydrogel systems was eventually abandoned and studies confined to the behaviour of lincomycin hydrochloride and clindamycin hydrochloride in such systems.

II.5.xi Development of a 5,5'-Dithiobis (2-nitrobenzoic acid) (D.T.N.B.) Assay for Lincomycins

Whilst the D.C.Q.C. assay, described above, was considered adequate, any possibility of increasing the sensitivity of antibiotic assay was important. Such an improvement would increase the accuracy of the determination of the permeability parameters of the antibiotics.

In work already mentioned Prescott[99] developed an automated assay for the determination of lincomycin hydrochloride at levels as low as 0.065×10^{-3}M, a greater than ten-fold improvement on the D.C.Q.C. assay. The principle involved was the same as for the D.C.Q.C. assay, namely the colorimetric determination of methanethiol generated from the acid hydrolysis of the methyglycosido group of the antibiotic. In this case the reaction of the thiol with 5,5'-Dithiobis (2-nitrobenzoic acid), or D.T.N.B., was followed. The reaction scheme can be seen in Figure II (10).

FIGURE II (10)



Following previous work by Ellman,[100,101] this reagent was used by Prescott because of its high sensitivity, stability and water solubility. Ellman had initially worked with water insoluble disulphides[100] but later synthesised 5,5'-Dithiobis (2-nitrobenzoic acid), known as Ellman's reagent (D.T.N.B.) as a water soluble derivative.[101] The introduction of the carboxyl groups provides the necessary solubilising effect. Ellman reported >99% ionisation of previously used disulphides at pH 8.0 and the highly coloured anions produced were used as a measure of the thiol concentration.

The assay procedure developed from the automated assay of Prescott was as follows:

Reagents: D.T.N.B. Reagent

0.01% 5,5'-Dithiobis (2-nitrobenzoic acid)
(100 mg dissolved in 5 ml of ethanol made up to 1l with distilled water. The reagent has been reported to be stable for over a month.)

0.1M.HCl

Tris buffer 0.1M pH 8.0

Sigma 7-9, Tris(hydroxymethyl) aminomethane

1 ml aliquots of standards of the antibiotics (0.0001M to 0.008M) were placed in test tubes which were then sealed with subaseals. 1 ml of 0.1M.HCl was injected into each tube through the subaseals, and the tubes were shaken before being incubated in a water bath at 70°C for 30 minutes. The tubes were then cooled to room temperature (23°C) and 2.0 ml of 0.5M NaOH followed by 2.0 ml of tris buffer were injected into each tube. After shaking, 2.0 ml of D.T.N.B. reagent was also added to each tube. The colour reactions occurred almost immediately. Distilled water blanks were also run.

The above scheme allowed the detection of levels of lincomycin hydrochloride and clindamycin hydrochloride of about 0.0005M. This procedure was not considered to be the optimum attainable but it was apparent that the increase in sensitivity achieved was not as great as had been expected. It was decided to abandon the further development of this assay in favour of the by now proven D.C.Q.C. assay method.

II.5.xii Development of an Arsenomolybdate Reagent Assay for Lincomycins

At an early stage in the course of this research and before the previously discussed D.C.Q.C. and D.T.N.B. assays had been assessed, an attempt was made to modify an assay which had been used for the determination of Erythromycin by Upjohn Limited.[9]

The method of this general sugar assay was as follows:

Reagents: Arsenomolybdate reagent

0.04M Ammonium molybdate

0.019M Sodium arsenate

0.75M (approx) Sulphuric acid

Sulphuric Acid (6M)

Arsenomolybdate reagent and sulphuric acid were mixed in the ratio 1:2 ($^{v}/v$) immediately prior to use. 1 ml aliquots of each test solution of antibiotic were mixed with 5 ml of Arsenomolybdate/Sulphuric acid reagent and the mixtures were heated at 100°C for 17 minutes. At the end of this time the samples were cooled to room temperature (23°C) and their absorbance at 600 nm recorded. Limited success was achieved with this method when applied to the Lincomycins, the blue colouration found for lincomycin hydrochloride and clindamycin hydrochloride solutions of concentration >0.05M indicated that the method was capable of detecting these antibiotics. Slight modifications to the method resulted in some improvement in sensitivity but calibrations were often unreliable. The method was abandoned at an early stage in its development when the more promising D.C.Q.C. and D.T.N.B. assay methods showed success.

II.5.xiii Automated Analysis of Nitrogen Containing Compounds

A Technicon Autoanalyser was used in the determination of the amounts of glycine, phenylalanine and creatine phosphate in samples collected during permeability experiments presented in Chapter VI. The method used was that of Chapman, Cooke and Whitehead[102] for the determination of ammoniacal nitrogen. Samples to be determined by this method were kindly assayed by P.A. Caesar of this research group. The method depended on the reaction of alkaline phenol and sodium hypochlorite with ammonia to form a blue colouration, the optical density of which was measured at 625 nm. Concentrations as low as 0.1 mg/l (nitrogen) may be measured.

II.5.xiv Flame Photometry

The technique of flame photometry was used to determine the levels of sodium, potassium and calcium in samples taken during permeability studies described in Chapter VI. The instrument used was an EEL Flame Photometer Model A made by Evans Electroselenium Limited. Calibration was carried out, using an appropriate filter, on a series of standard solutions in distilled water over the concentration range 0.025 mM to 6.0mM for sodium chloride and potassium chloride and 0.1mM to 6.0mM for calcium chloride. The reading obtained for a sample could be read off as a concentration value from calibration curves constructed from the readings found for standard solutions of known concentrations of either sodium chloride, potassium chloride or calcium chloride, as appropriate.

CHAPTER III

CHARACTERISATION STUDIES

III.1 Introduction

This chapter concerns the study of various properties of the antibiotics used, (in addition to the data available in the literature) which was considered necessary for the interpretation of results from experiments involving the antibiotics. A brief account of the factors affecting the properties of hydrogels can also be found together with results obtained from various determinations on the hydrogels used during this research. These results have been discussed in terms of the characterisation of such systems.

III.2 Studies on the Antibiotics and Their Aqueous Solutions

III.2.i Data Previously Determined for Antibiotics Used During This Research

The amount of published data concerning the physical-chemical properties of the antibiotics was surprisingly limited. For a number of parameters such as pH and solubility unspecified ranges of values were reported at best. The information presented here was assembled from various sources.[67,64,65,66,71,9]

The hydrochloride salts of Lincomycin and Clindamycin as supplied were white and off-white crystalline powders respectively. Both had a characteristic odour and bitter taste. The molecular weight of clindamycin hydrochloride was 461.4 and that of lincomycin hydrochloride monohydrate was 461.0. The molecular weight of clindamycin phosphate was 504.9. However, the compounds are hygroscopic and different crystal forms have been observed according to the degree of hydration. Clindamycin hydrochloride was generally found to be mentioned without any water of crystallisation. The crystalline hydrochloride salt of Lincomycin has been studied at greater depth and has been more frequently reported to form hydrates,[71] when isolated it was found to have 0.5 H_2O associated with it. However, in later publications concerning its pharmaceutical properties it was generally referred to as a monohydrate, although a range of 3% to 6% water was frequently quoted (approximately 0.8 to 1.5 H_2O). The amount of water found to be associated with Lincomycin free base was 1.5% (approximately 0.4 H_2O).

Throughout this work the term "Clindamycin" is synonymous with clindamycin hydrochloride and "Lincomycin" is synonymous with lincomycin hydrochloride monohydrate except where otherwise stated.

Solubility data for the antibiotics was not specific. Figures for the solubility of Clindamycin in water were of the range < 1000 and > 500 mg/ml.[73] However these were based on a personal communication and a temperature for the determination was not quoted. Similar solubility ranges were reported for Clindamycin in methanol (< 200, > 100 mg/ml), D.M.F (< 500, > 250 mg/ml) and ethanol (< 10, > 5 mg/ml). Clindamycin solubility in ethyl acetate, acetone, chloroform, benzene and cyclohexane was less than 1 mg/ml.

Data from sources listed earlier, for Lincomycin solubility at 20°C, report that Lincomycin was soluble in 1 part water, 40 parts alcohol, 20 parts D.M.F, and practically insoluble in ether and chloroform.

More specific figures for the solubility of both Lincomycin and Clindamycin in water over a range of temperatures have been determined during this research and are presented later in this chapter. The aim of such work was to establish a more acceptable evaluation of the aqueous solubilities of these compounds.

The only pH data published (to the author's knowledge) report the pH of a 10% ($^w/v$) aqueous Lincomycin solution as 3.0 to 5.5.

Data pertaining to the Erythromycins used in this work, above what has already been presented in Chapter I, was not required for interpretation of the results presented in this thesis.

Whilst not wishing to be sidetracked, at an early stage in the research, into the assimilation of physical-chemical data on the antibiotics, a greater understanding of some of the more fundamental properties of the antibiotics was considered essential.

III.2.ii Change of pH with Concentration for Aqueous Antibiotic Solutions

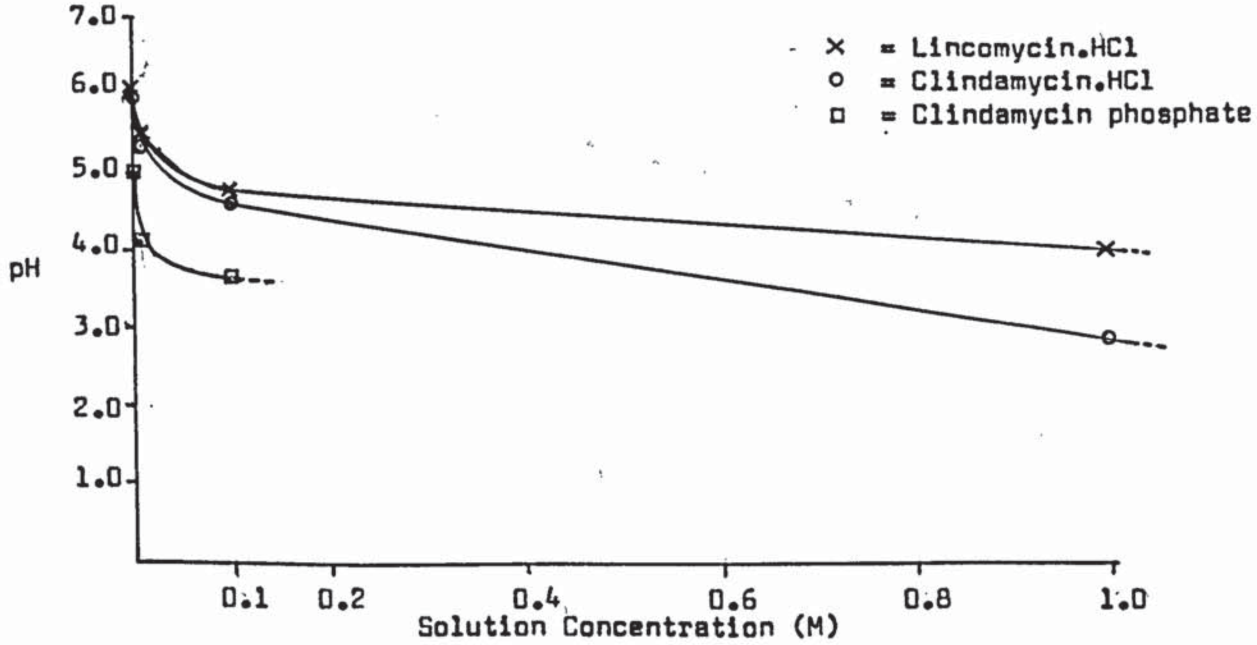
The pH of aqueous solutions of Lincomycin and Clindamycin, over the concentration range 0.001M to 1.0M were determined. Calibration of the pH meter was carried out using buffer solutions of pH 4 and pH 9. The determinations were made at room temperature (23°C) with a temperature compensation probe attached. Plots of the pH values of a range of concentrations of antibiotic solutions are shown in Figure III (1).

Figure III (1) shows a substantial difference in the pH of Lincomycin and Clindamycin at the 1.0M concentration level, 1.0M Clindamycin solution being approximately 1 pH unit more acid than a 1.0M Lincomycin solution. The significance of this observation has been discussed in conjunction with other findings on aqueous antibiotic solutions later in this chapter. However, changes in intracellular pH can be shown to correlate with the initiation of certain cell activities,[103] these systems seeming to depend on the cell controlling its own pH rather than responding to external influences. As a possible route to irritation this method was unlikely since many other antibiotics have similar pH, the pH of clindamycin phosphate solutions were of lower pH than the same solution concentrations of clindamycin hydrochloride.

The difference in pH between Lincomycin and Clindamycin solution was considered likely to reflect something of an alteration in their "activity" in an indirect manner as a result of factors producing the difference in irritancy.

FIGURE III (1)

CONCENTRATION VERSUS pH FOR AQUEOUS SOLUTIONS



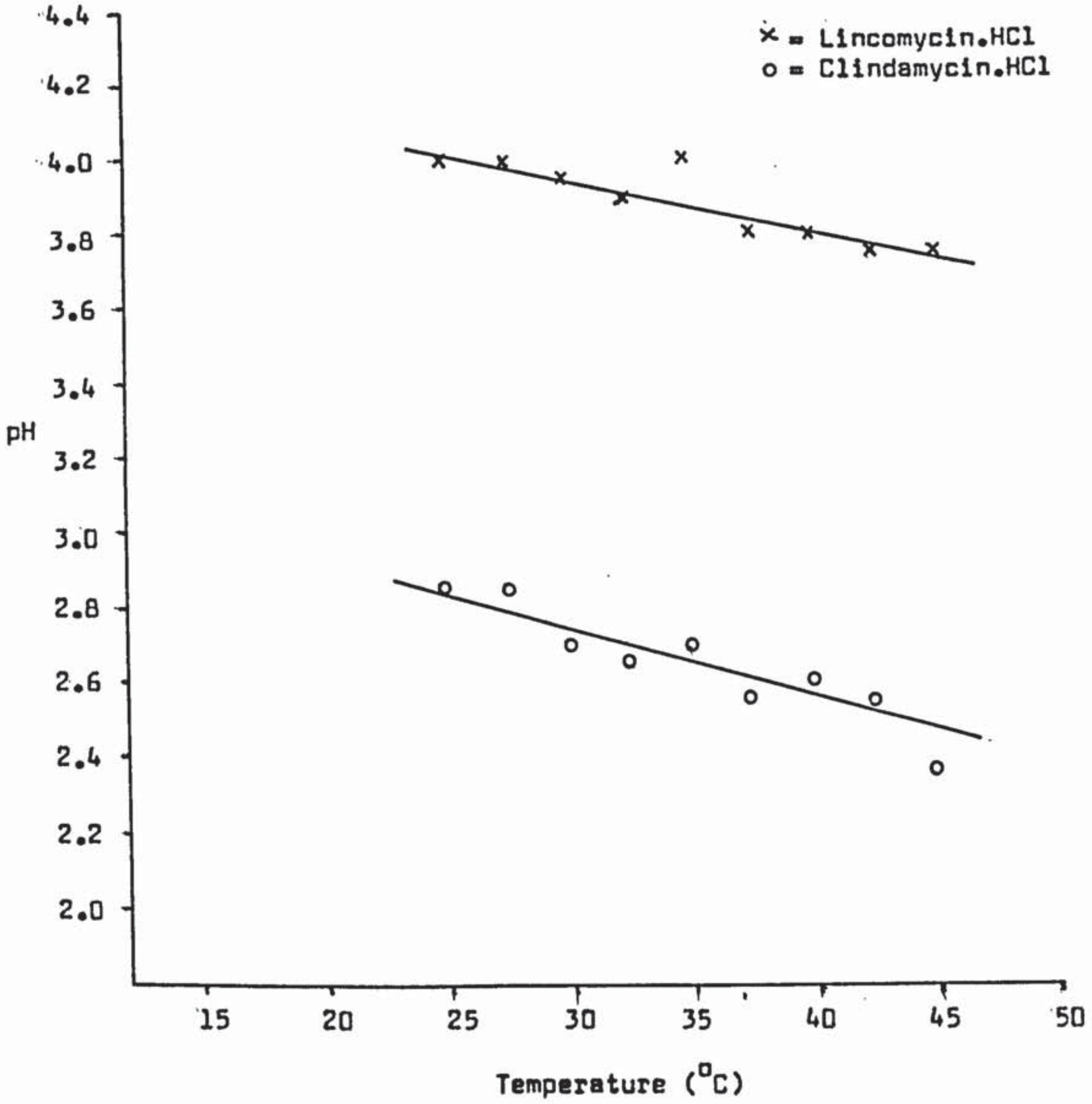
III.2.iii Variation of the pH of Aqueous Antibiotic Solutions with Temperature

The determination of the effect of temperature on the pH of the antibiotic solutions was necessary because of the permeability studies on the antibiotics at various temperatures reported in Chapter V.

The pH meter and temperature compensation probe described before were used. Buffer solutions for calibration of the instrument were incubated at the same temperature as the solutions under investigation (approximately ± 0.1 degree). This procedure prevented errors due to inaccurate calibration and also changes in pH scale position and pH scale dimensions due to the variation of $E^{\ominus}$ and 2.303 RT/F respectively.

FIGURE III (2)

VARIATION OF pH OF 1.0M AQUEOUS ANTIBIOTIC SOLUTION WITH TEMPERATURE



The variations of the pH of 1.0M aqueous solutions of lincomycin hydrochloride and clindamycin hydrochloride with temperature are shown in Figure III (2). From Figure III (2) it was apparent for both antibiotics that increasing solution temperature resulted in slightly increased acidity. The extent of this process was similar for both compounds.

III.2.iv Solubility Determinations

The aqueous solubilities of both lincomycin hydrochloride and clindamycin hydrochloride were determined at a number of temperatures. A saturated aqueous solution of an antibiotic was placed in a split resin flask fitted with a stirrer. The assembly was incubated in a water bath at the desired temperature (approximately ± 0.1 degree) for at least 24 hours whilst ensuring that an excess of the antibiotic was present. After this equilibration period a sample could be extracted, placed in a pre-weighed sample bottle and re-weighed. The sample was then evaporated to dryness under vacuum at 60°C until the sample reached constant weight. The difference between the wet sample weight and dry sample weight corresponded to the volume of water needed to fully solvate the dry weight of antibiotic. The results of the studies on the effect of incubation temperature on the aqueous antibiotic solubilities are shown in Figure III (3). From Figure III (3) it can be seen that clindamycin hydrochloride was far more soluble in water than lincomycin hydrochloride and that the extent of the difference appeared to increase with increasing temperature at least over the temperature range studied. The rate at which the solubility increased was greater for clindamycin hydrochloride than lincomycin hydrochloride.

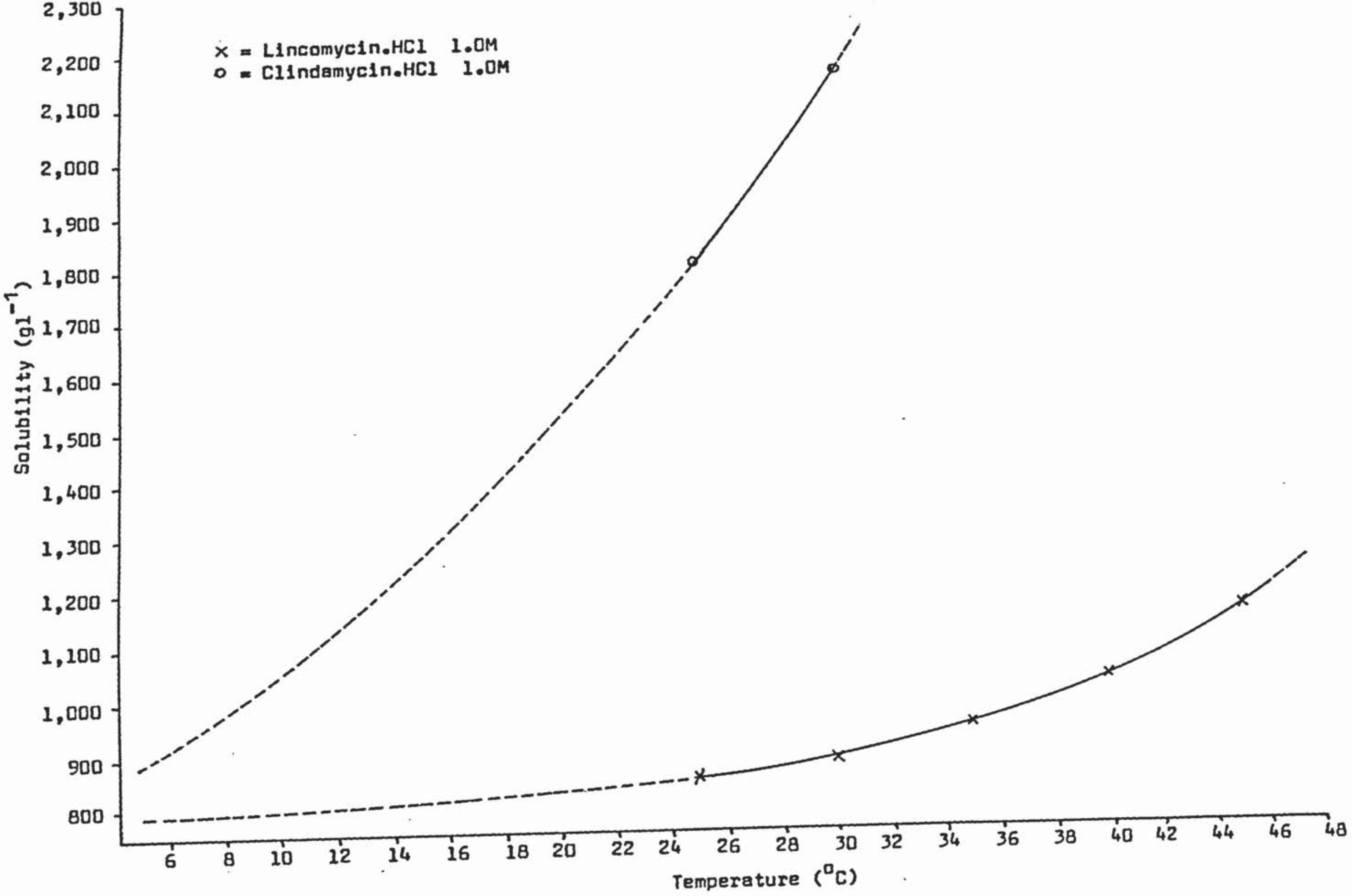
Extrapolations for both Clindamycin and Lincomycin data can be seen in Figure III (3). These were rather speculative but seemed to suggest that even with a slight curvature of the Clindamycin extrapolation the two compounds would reach comparable values of solubility at solution temperatures of about 0°C.

III.3 Vapour Pressure Osmometry Studies of Antibiotic Solutions

Vapour pressure osmometry studies of the antibiotics in solution provided a means of observing possible intermolecular interactions in

FIGURE III (3)

EFFECT OF TEMPERATURE ON AQUEOUS ANTIBIOTIC SOLUBILITY



solution. The occurrence of such interactions was a possible explanation for the differing extents of irritancy of the antibiotics and could be implicated in observations on the water structuring effects of the two compounds (Chapter IV). The vapour pressure osmometer and its use has already been described in Chapter II.

III.3.i Determination of the Calibration Constant

The instrument must be calibrated using a solute of known molecular weight for each solvent to be used. Since the solvent to be used in the majority of these studies was water, d-Glucose was used as the standard solute for the determination of the calibration constant, K. Solutions of d-Glucose in distilled water of concentrations 0.1M, 0.06M, 0.03M and 0.01M were prepared and the calibration constant determined in the manner outlined in Chapter II. The calibration was checked each time the instrument was used but was not found to vary significantly. The value of K found was generally about 78.0, the precise value obtained prior to sample studies was used in each case.

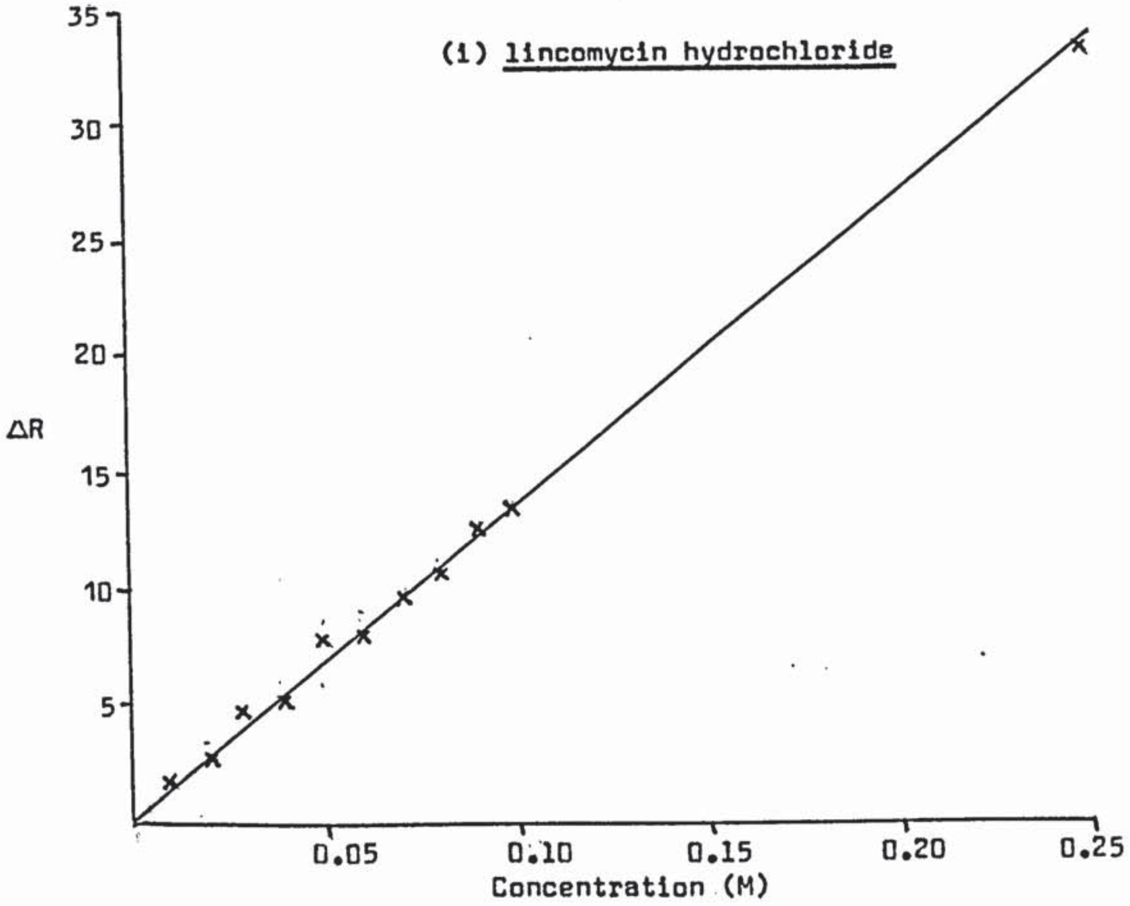
III.3.ii Low Concentration Aqueous Antibiotic Solution V.P.O. Studies

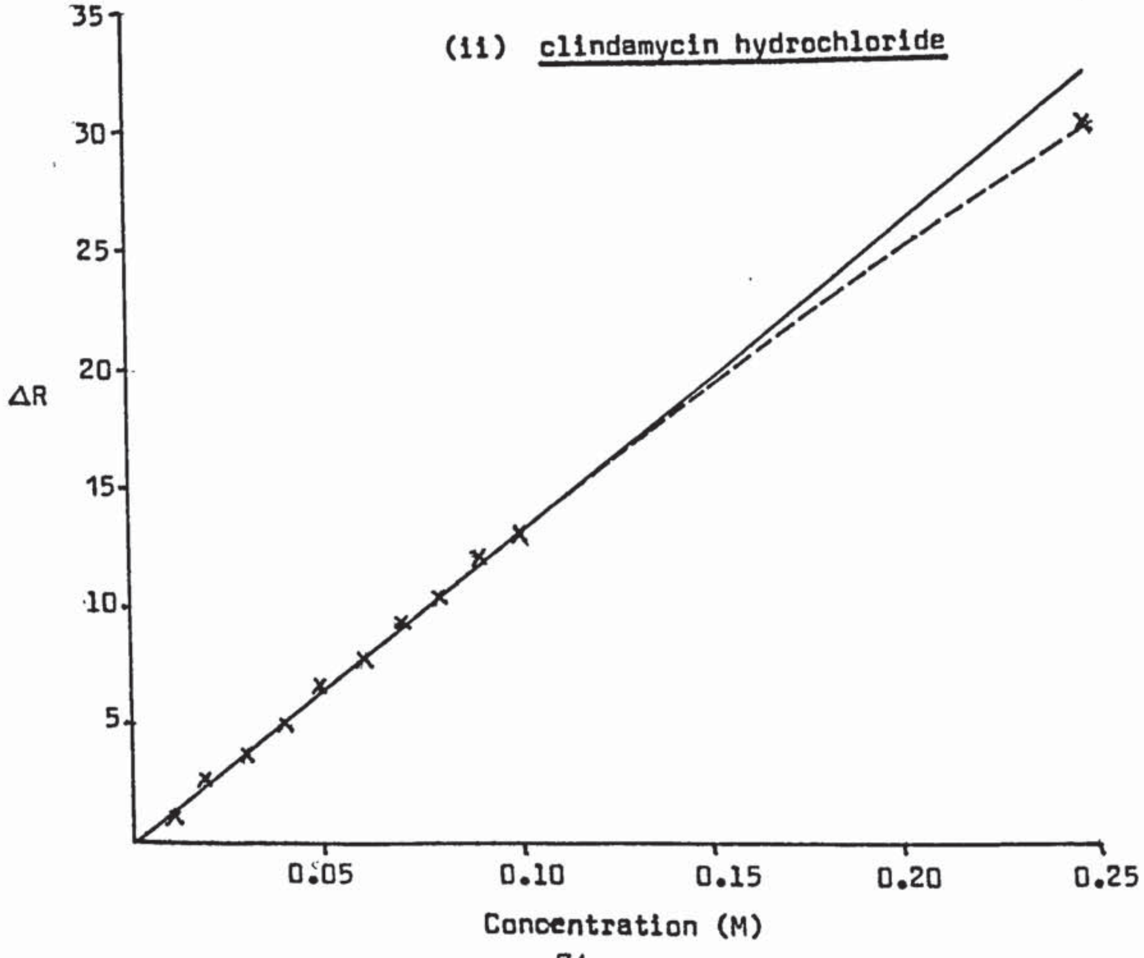
Initial vapour pressure osmometry (V.P.O.) studies were carried out on low concentration aqueous solutions of the antibiotics. The simplest way of presenting the data obtained was to plot the value of ΔR obtained directly against the concentration (in moles) of the solution studied. The ΔR versus M plots for aqueous solutions of lincomycin hydrochloride, clindamycin hydrochloride and clindamycin phosphate are shown in Figure III (4) (i), (ii) and (iii) respectively.

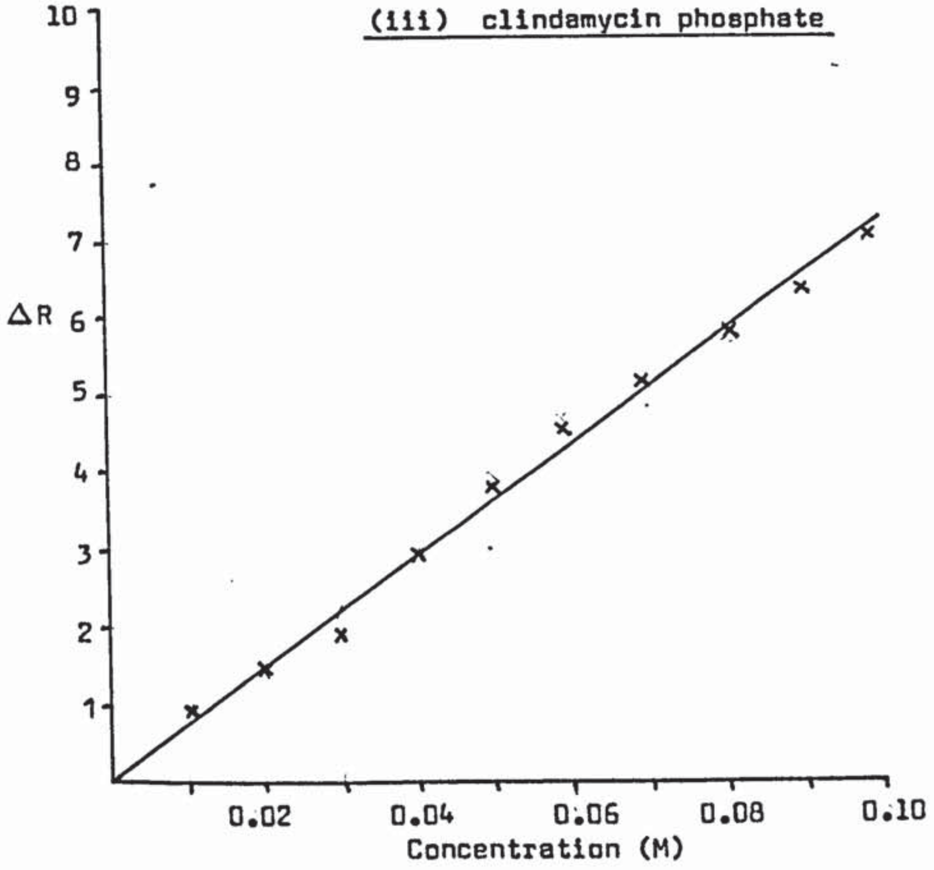
Figure III (4) clearly showed a substantial difference between the ΔR values found for clindamycin phosphate and those found for the same concentration solutions of clindamycin hydrochloride and lincomycin

FIGURE III (4)

ΔR vs M PLOTS FOR V.P.O STUDIES ON LOW CONCENTRATION AQUEOUS SOLUTIONS







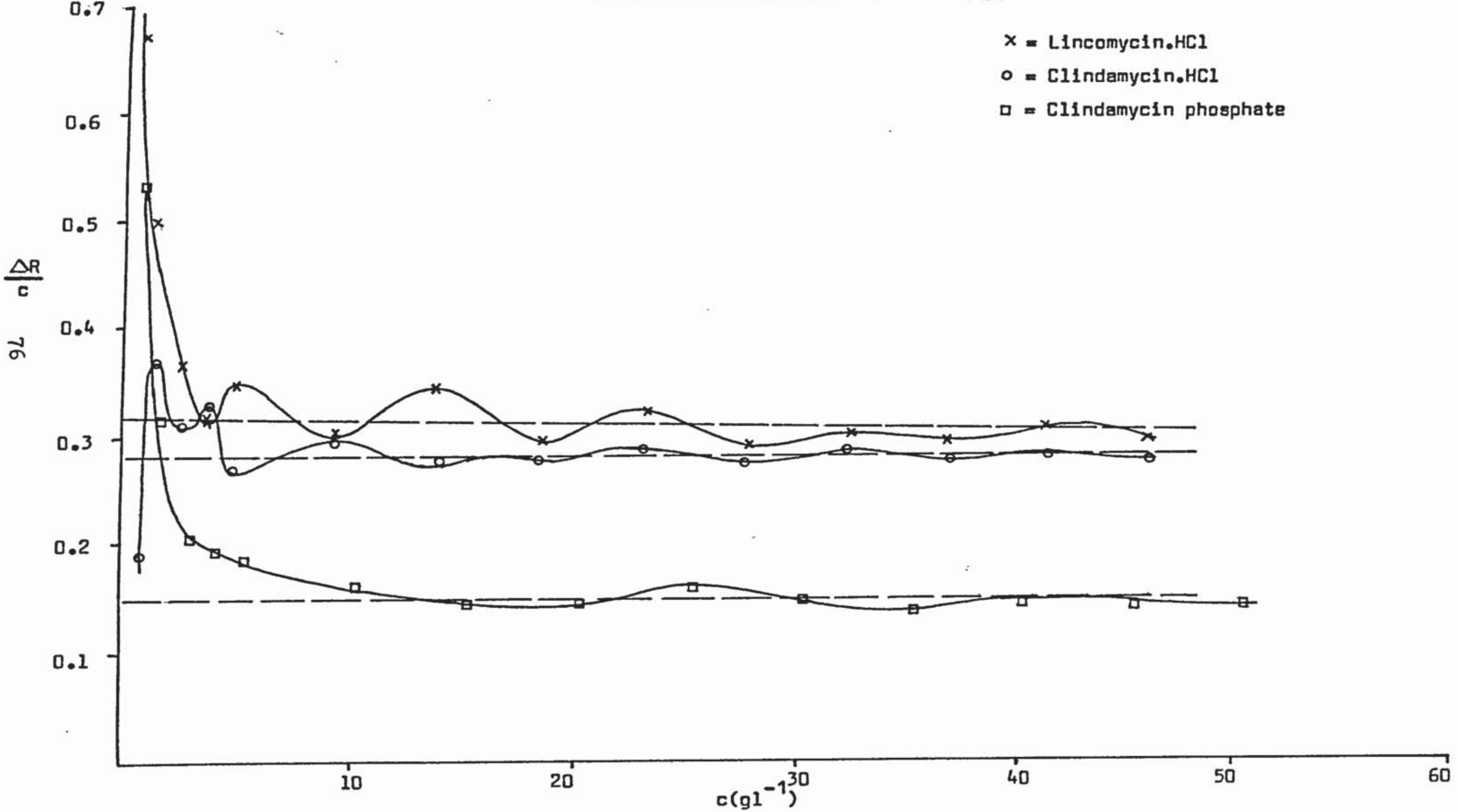
hydrochloride. A slight tailing off was observed for the high concentration solutions of clindamycin hydrochloride but otherwise the values of ΔR found were very similar to those found for lincomycin hydrochloride.

An alternative way of presenting this data was to plot ΔR/c against c, where c was the solution concentration in grams per litre (gl^{-1}). These plots can be seen in Figure III (5).

Extrapolation of the plots shown in Figure III (5), in the manner discussed in Chapter II, to zero concentration yields the intercept $a_1 = K/M$. Since K was known from calibration, the molecular weights of the compounds (M), as determined by V.P.O, could be calculated. The extrapolation was based on the ΔR/c values for solution concentrations higher than 10 gl^{-1}

FIGURE III (5)

$\Delta R/c$ vs c PLOTS FOR V.P.O. STUDIES ON LOW CONCENTRATION AQUEOUS SOLUTIONS



since the variation of $\Delta R/c$ values below this concentration was large, a general feature of low concentration solution V.P.O. studies.

The determined molecular weight of clindamycin phosphate (520) was in good agreement with its known value (505) being within an error limit of ± 3%.

The determined molecular weights of Lincomycin and Clindamycin were 0.53 and 0.59 of their known values respectively. This observation was readily explained in terms of dissociation of the hydrochloride salts. Since the technique depended on "counting heads" of solute particles then a decrease in the determined molecular weight of a compound relative to its known molecular weight was indicative of dissociation. In the case of Lincomycin and Clindamycin the results were adequately explained in terms of the dissociation of the hydrochloride and free base constituents of the compounds.

An interesting feature of the plots shown in Figure III (5) was their apparently slightly cyclical nature. This behaviour was rather tentatively drawn into Figure III (5) since the behaviour could well be imaginary. However, the observation that the cyclical behaviour for Lincomycin and Clindamycin seemed to be "in phase", was considered worthy of note.

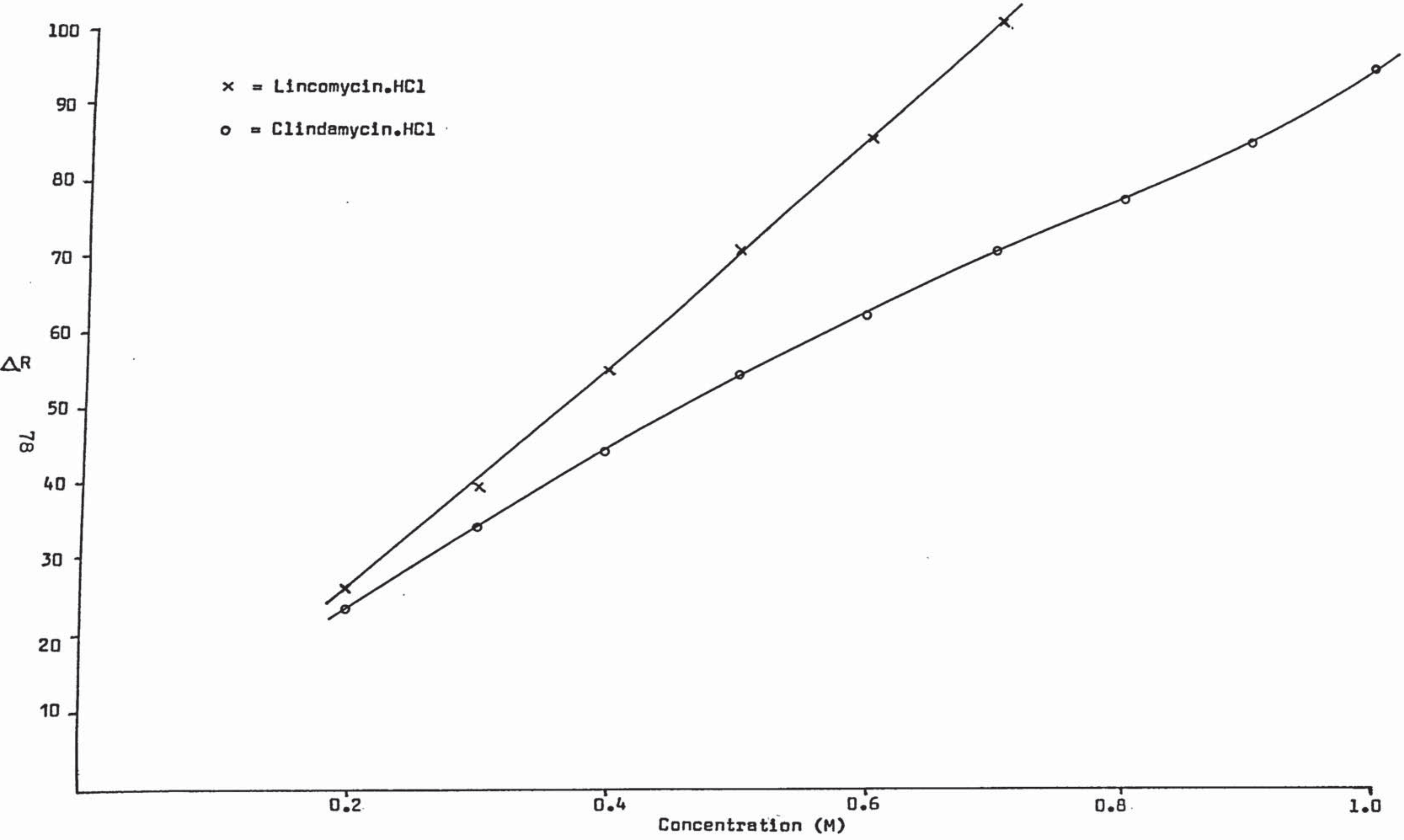
III.3.iii <u>High Concentration Aqueous Antibiotic Solution V.P.O. Studies</u>

Similar studies to those described above were carried out on an extended concentration range of aqueous solutions of Lincomycin and Clindamycin. The variation of ΔR with solution molarity (M) can be seen in Figure III (6).

From Figure III (6) it can be seen that for aqueous solutions of high concentration the behaviour of Lincomycin and Clindamycin was significantly

FIGURE III (6)

ΔR vs M PLOTS FOR V.P.O. STUDIES ON HIGH CONCENTRATION AQUEOUS SOLUTIONS

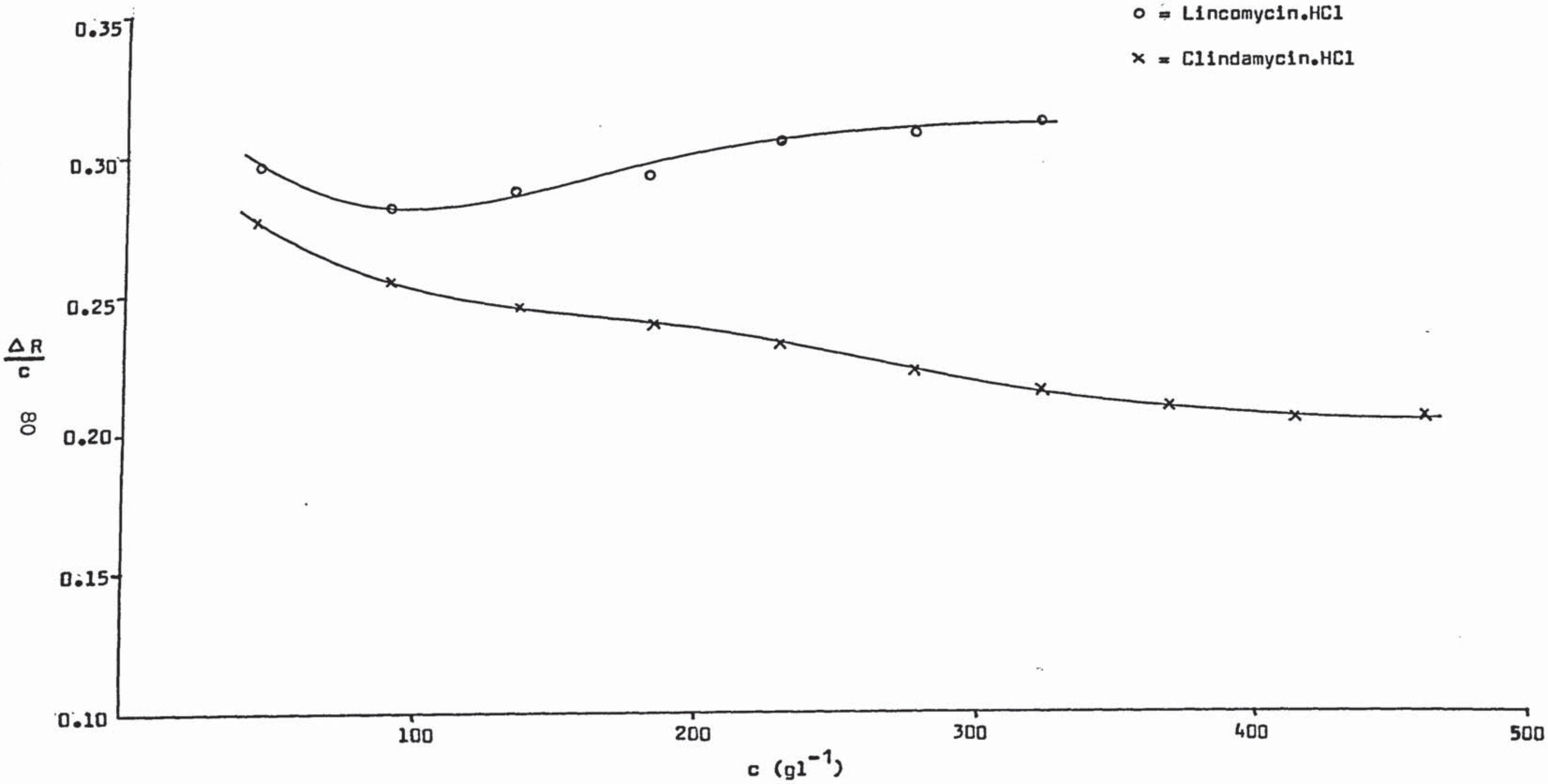


different. Interpretation of this behaviour was clear when $\Delta R/c$ against c was plotted, as shown in Figure III (7). The results shown in Figure III (7) for Lincomycin would indicate a slight fluctuation in the extent of the dissociation of the free base and hydrochloride although the effect was not marked. The results shown for Clindamycin showed a more significant trend in that the value of $\Delta R/c$ steadily dropped as the solution concentration increased, this in effect meant that the "apparent" molecular weight of Clindamycin began to approach the value of its known molecular weight. This process was indicative of an associative effect occurring in solution. The association could possibly have been a reassociation of the free base and the hydrochloride but such an interpretation was considered unlikely since Lincomycin would also be expected to undergo the same process which it apparently did not do. The more likely interpretation was that the increase in "apparent" molecular weight was the result of intermolecular association between Clindamycin free base units. Such a process would display the concentration dependant behaviour observed. The existence of associative behaviour for Clindamycin but not Lincomycin goes a long way in providing a basis for explanations of the differing extents of irritancy of the antibiotics. The pH data presented earlier in this chapter correlated with such an observation in that the greater acidity of Clindamycin solutions than Lincomycin solutions might be due to increased ionisation of Clindamycin favoured by the stabilising influence of intermolecular association of units of Clindamycin free base.

The actual processes occurring in aqueous solutions of the antibiotics seemed to be quite complex. The results of the solubility studies could be explained in a number of ways. It was possible that the extent of association of Clindamycin could increase with increasing temperature.

FIGURE III (7)

$\Delta R/c$ vs c PLOTS FOR V.P.O. STUDIES ON HIGH CONCENTRATION AQUEOUS ANTIBIOTIC SOLUTIONS



This type of entropically unfavourable process has been found in studies of hydrophobic bonding. The association of Clindamycin free base units, if utilising such a mechanism, would then provide a larger number of accessible hydrogen bonding groups per unit size than Lincomycin, the compact associate with its hydrophilic exterior might then be more soluble than monomeric Lincomycin units. This process would certainly agree with the observations on solubility presented in this chapter.

Alternatively the rapid increase in Clindamycin solubility with increasing temperature could be considered as associated units breaking up into smaller more soluble species. However, if this were the case then the solubility of the associated Clindamycin at low temperatures might be expected to be significantly lower than that of non-associated Lincomycin. The limited data do not seem to support such a possibility.

The former explanation would seem to agree with observations of increases in hydrophobic bonding up to 45°C. After this temperature entropic forces would be expected to prevail resulting in a tailing off in the solubility increase with increasing temperature as Clindamycin association breaks down. Precedents for such behaviour do exist.

The anomolous solubilities of calcium sulphate and calcium hydroxide are such examples where the compounds are less soluble in hot water than cold, a gradual tailing off of solubility occurring with increasing temperature. In other cases the solubility change can be far more dramatic. Crystalline sodium sulphate, $Na_2SO_4.10H_2O$ displays an increasing aqueous solubility up to 32°C at which point a sharp break occurs and the solubility gradually decreases. The transition represents the breakdown of the hydrate into anhydrous sodium sulphate and water. Such behaviour is not unimaginable for Clindamycin. Evidence has been presented in Chapter IV suggesting that Clindamycin has about 6 to 12 molecules of non-freezing water associated with it (depending on concentration), not seen for Lincomycin. It was therefore considered possible that at temperatures higher than 30°C the solubility gradually tailed off rather than increasing rapidly as might otherwise be assumed. This process either being due to breakdown of association or formation of the anhydrous form. It would certainly be interesting to study the extent of the association seen for Clindamycin at higher temperatures.

As previously stated, the processes occurring in aqueous solutions of these antibiotics seemed to be quite complex and a more extensive study of this aspect of the work was required. The processes also seemed to be to some extent competitive, the balance of the interactions between antibiotic and water and antibiotic and antibiotic appearing to be dependant on solution concentration and temperature.

Having substantially improved the standard of data relating to the properties of the antibiotics in aqueous solutions, a discussion of properties of the hydrogel systems in which they will next be studied was appropriate.

III.4 Studies on Hydrogels and Their General Properties

III.4.1 General Factors Affecting the Nature of Hydrogels

The nature of hydrogels can be influenced by a number of controllable factors. The hydrogels prepared and used during the course of this work were selected from a variety of compositions and polymerisation conditions studied in the past by members of this research group. The compositions used were chosen because of the extent and type of water structure they possessed and so the factors affecting these hydrogel properties need to be discussed.

The investigation of the factors affecting a wide range of hydrogels has been carried out in some depth.[11,13,14,15,16] Before summarising the nature of the influences on the properties of hydrogels used during this research, the preparations synthesised have been listed with appropriate information relating to their synthesis. The different ways in which hydrogels may be swollen and soaked and the incorporation of solutes into the preparations by dilution of the monomer mixtures with solutions have been discussed in Chapter II.

Throughout the course of this work it has been necessary to keep the number of variables minimal in order to evaluate differences due to the effects of the antibiotics used. To this end the hydrogel preparations were limited to just five types. The ratios of the monomers were held constant and polymerisation conditions never altered throughout the research. The details corresponding to these preparations can be seen in Table III A. The only exceptions to these preparations were those in which the initial monomer solution was diluted with antibiotic solution in order to incorporate antibiotic into the hydrogel during polymerisation, and a range of pAcrylamide preparations in which different amounts of cross-linking agent were used. Apart from these adjustments the polymerisation parameters were as stated in Table III A.

The preparations listed were chosen so as to provide a series of hydrogels ranging from high E.W.C. to low E.W.C. and also covering a range of ratios of freezing:non-freezing water components from mainly freezing water to mainly non-freezing water.

Throughout this work where hydrogel compositions have been mentioned the monomer ratios were as shown in Table III A. For example, mention of MAA/ACM/STY is synonymous with a monomer ratio of 43.75:43.75:12.5 (mole %) and HEMA/STY is synonymous with a monomer ratio of 90:10 (mole %).

TABLE III A

Monomer Mixture	Feed Ratio	Cross-linking Agent (% wt)	Initiator (% wt)	Polymerisation Conditions	Postcure Conditions
ACM/H_2O	25:75*	MBACM (1%)	Am.P.S. (0.1%)	$65^{0}C$/4 hours	-
HEMA	100*	EDMA (1%)	AZBN (0.1%)	$65^{0}C$/70 hours	$90^{0}C$/2 hours
HEMA/STY	$90{:}10^{\ddagger}$	EDMA (1%)	AZBN (0.1%)	$65^{0}C$/70 hours	$90^{0}C$/2 hours
MAA/ACM	$50{:}50^{\ddagger}$	MBACM (1%)	AZBN (0.1%)	$65^{0}C$/70 hours	$90^{0}C$/2 hours
MAA/ACM/STY	$43.75{:}43.75{:}12.5^{\ddagger}$	MBACM (1%)	AZBN (0.1%)	$65^{0}C$/70 hours	$90^{0}C$/2 hours

* = % wt

‡ = mole%

It has already been mentioned that the presence of hydrophilic groups within the polymer matrix gives rise to the uptake of water from an aqueous solution. The uptake is offset by the physical restriction of the three-dimensional polymer network. The point at which these forces stabilise being known as the equilibrium water content (E.W.C.) of the system.

The swelling properties of hydrogels can be compared with those of hydrophobic networks in organic solvents[89] whose swelling depends mainly

on a) the interaction parameter between polymer and solvent,

b) the extent of cross-linking,

and c) the relative swelling at the time of cross-linking (ie initial dilution of monomers).

The water present in a swollen hydrogel may be of the different types already mentioned. Water molecules may be polarised around charged ionic groups or orientated around hydrogen bonding groups or other dipoles. Water in "ice-like" configurations can exist around hydrophobic groups and completely free or "bulk" water can be imbibed into large pores. The presence of such interactions results in the "bound", "free" and "intermediate" forms of water present in hydrogels.

Hydrogels of two distinct types can be prepared, these are the homogeneous and heterogeneous forms. The homogeneous type were of primary importance during this research. In homogeneous hydrogel preparations the water present is dispersed evenly throughout the hydrogel and therefore no true discrete pores exist, only a randomly fluctuating system of hydrated polymer chains. Therefore, for homogeneous membranes, pore size is only a theoretical concept but, as will be seen later, it is still useful since it enables the prediction of the size of solute molecules able to permeate the matrix.

When such an even dispersion of water throughout the hydrogel does not occur, some of the water in the hydrogel will be immediately associated with polymer molecules and some water will be found in pockets of bulk water randomly apportioned within the matrix.

This type of water distribution in which various regions will have different refractive indices produces light scattering characteristics that result in the translucency or opacity of the preparation.

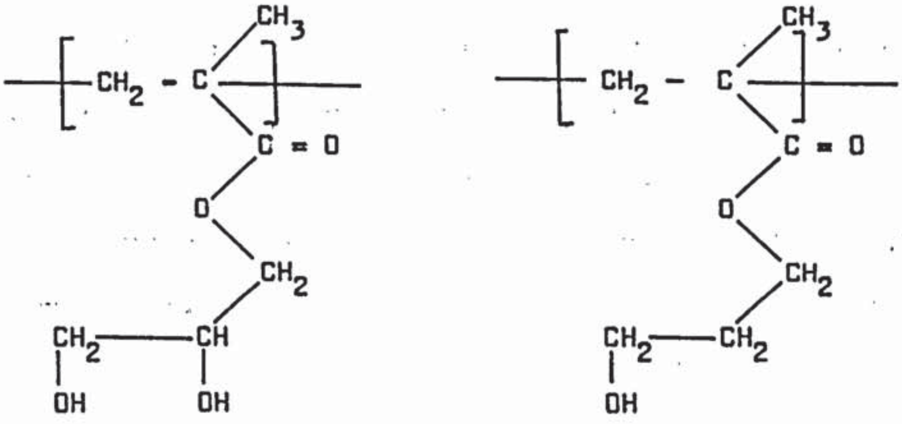
III.4.ii The Effect of the Polymer/Solvent Interaction Parameter on the E.W.C. of Hydrogels

The solvent used in the preparation of the hydrogels of concern here was water. If the polymer/solvent interaction parameter is low then this means that water is a poor solvent for the polymer and the degree of hydrogel swelling will not be great. However, if the interaction parameter is high then the polymer will dissolve unless factors such as cross-links prevent such an occurrence. An example of the first case would be pHEMA and the second case pV.P.

The reasons for the differences in the interaction parameters of various polymers are the number and nature of the hydrophilic groups on the polymer backbone. This effect is well demonstrated by reference to pGMA (poly(Glycerol methacrylate)) and pHPMA (poly(hydroxypropylmethacrylate)) as shown in Figure III (8).

The small change in chemical structure results in a large change in water content. E.W.C. = 75% for pGMA, E.W.C. = 23% for pHPMA prepared with equal amounts of the same cross-linking agent.[11,78]

FIGURE III (8)


pGMA pHPMA

III.4.iii The Effect of Cross-linking Agents on the E.W.C. of Hydrogels

There are two factors to be considered here since the E.W.C. of hydrogels can be shown to be affected not only by the amount of cross-linking agent used but also by the chemical structure of the cross-linking agent.

Some preparations seem relatively unaffected by changes in the amount of cross-linking agent used. Refojo and Yasuda[78] reported that the water content of pHEMA remained unaffected by an increase from 0.3% to 3.0% (by wt) in the amount of TEGDA cross-linking agent used. However, several workers have shown that hydrogels of higher water content show a far greater E.W.C. dependance on the amount of cross-linking agent used.[104,105] Refojo[78] showed that a similar variation in the amount of cross-linking agent used in the studies of pHEMA above, reduced the E.W.C. of pHPA from 53% to 49%.

A study of the E.W.C's of pGMA preparations cross-linked with between 1% and 10% (by wt) TEGDA[106] showed a decrease in E.W.C. from 76% to 61% with increasing amounts of cross-linking agent. Ng[11] found similar effects of cross-linking agent on E.W.C's. Jolly[16] reported the effect on the E.W.C's of homogeneous pHEMA preparations of higher amounts of EDMA cross-linker than had been used by Refojo and Yasuda. Figure III (9) shows the affect of increasing amounts of EDMA up to 20 mole% on the E.W.C. of pHEMA. The freezing and non-freezing water component contributions to these preparations are shown in Figure III (10). The overall effect of increasing levels of EDMA was to decrease the E.W.C's of the preparations. Figure III (10) shows that this occurred in two ways. Over the 0 to 10 mole% EDMA range the amount of freezing water was markedly affected since the increasing level of cross-linking "tightened" up the matrix thus decreasing the space available for freezing water. Between 10 and 20 mole% EDMA the "tightening" effect became less important and the non-freezing water component gradually became affected. The cross-linking agent was now acting as a hydrophobic monomer and reduced the hydrophilicity of the system. In this way the layer of water molecules most closely associated with hydrophilic groups in the matrix could be influenced.

Middleton[15] showed that for preparations of ACM/NVP/HPA/STY (33:47:10:10) (mole%) with varying amounts of either MBACM or EDMA as cross-linking agents up to levels of 4.5 mole%, that the E.W.C's of preparations cross-linked with EDMA dropped from about 82% to 60% whereas those cross-linked with MBACM dropped from about 90% to 68%. This can be seen from Figure III (11). The lower E.W.C's of the preparations cross-linked with EDMA were interpreted in terms of the absence of the extremely hydrophilic amide groups present when MBACM was used.

III.4.iv The Effect of Initiator Concentration on the E.W.C. of Hydrogels

Yasuda[107] reported that increasing the concentration of initiator used resulted in an increase in E.W.C. It was suggested that an increase in the number of growing free radicals was the cause. Ng[11] also reported

FIGURE III (9)

EFFECT OF CROSS-LINKING AGENT ON THE E.W.C. OF HOMOGENEOUS pHEMA HYDROGELS

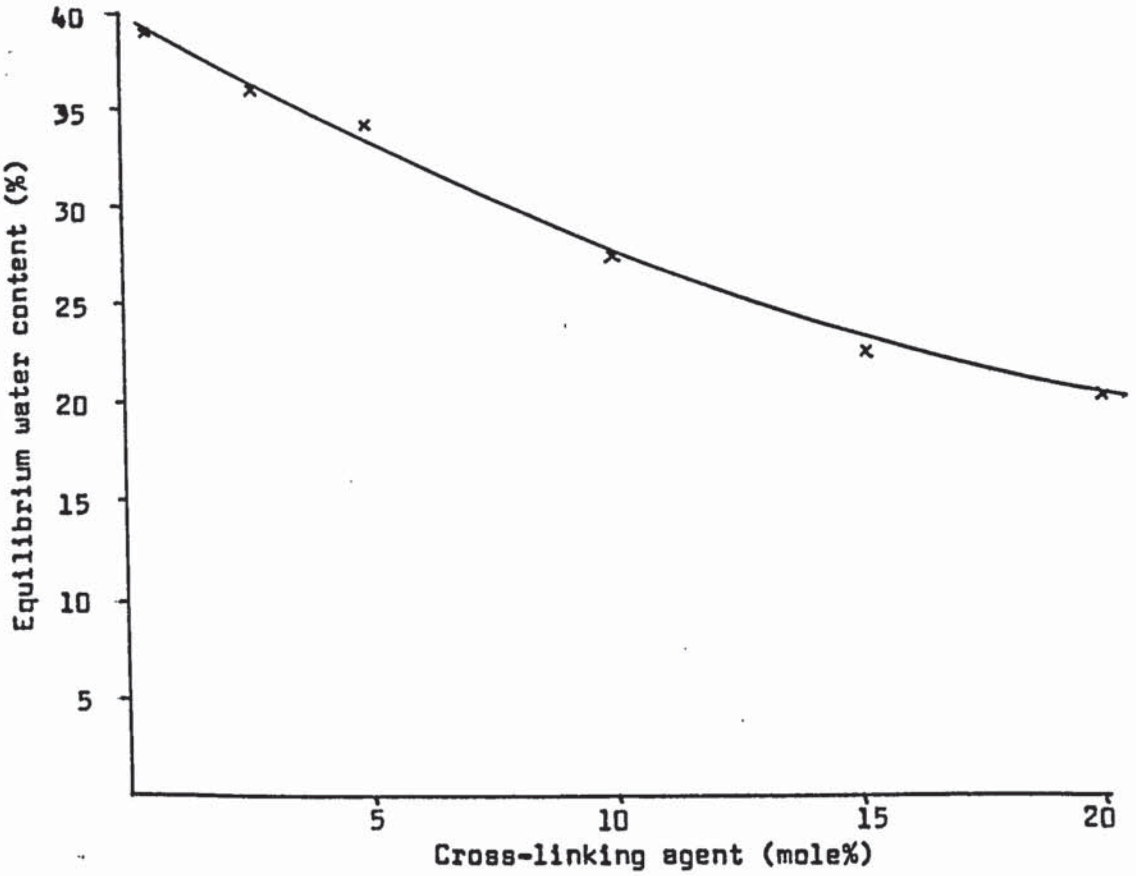
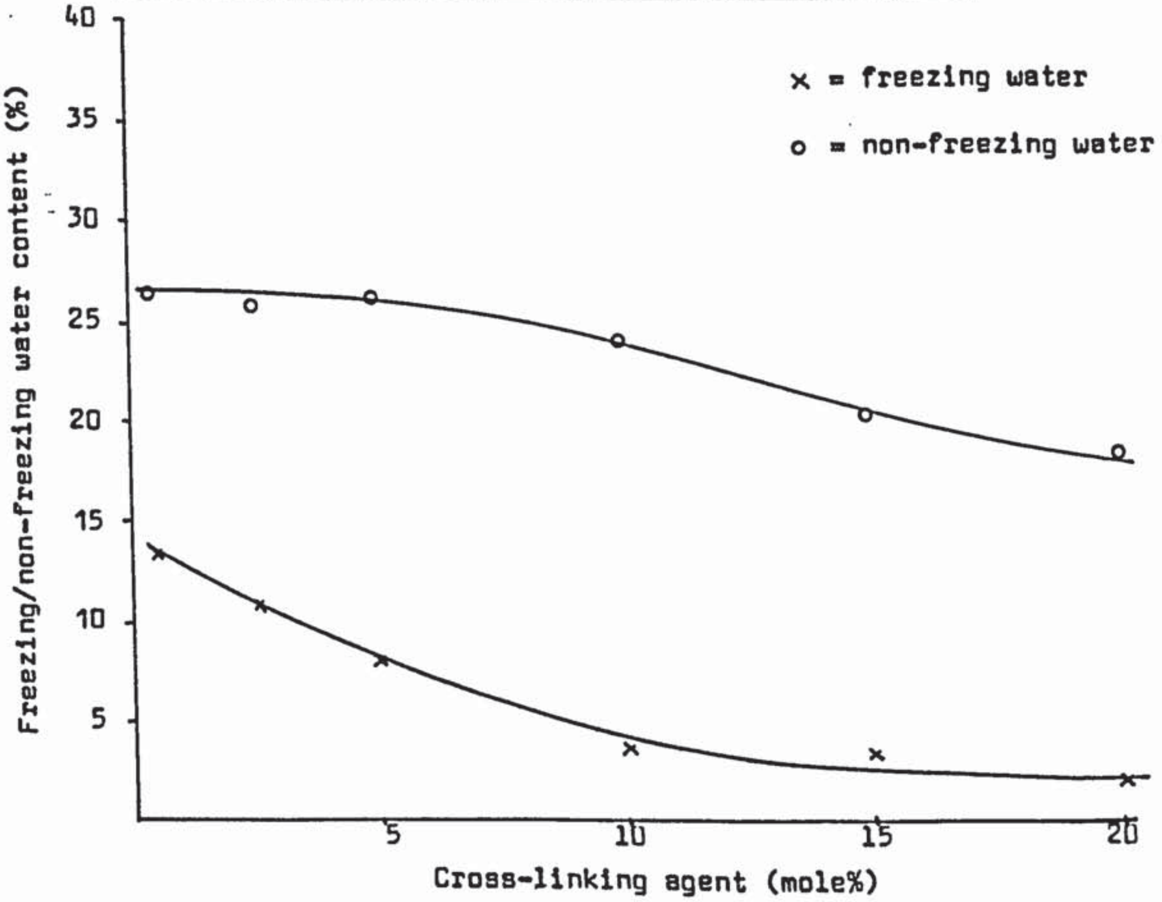


FIGURE III (10)

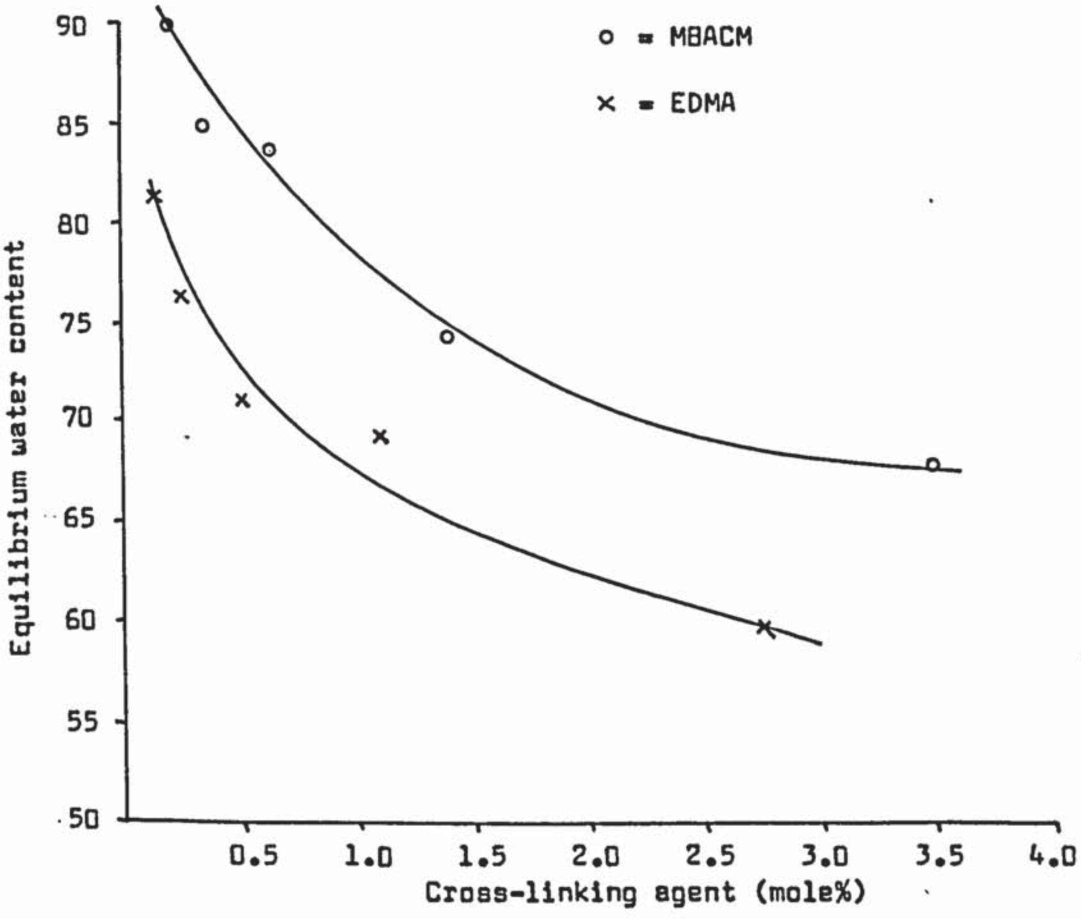
EFFECT OF CROSS-LINKING AGENT ON THE FREEZING WATER AND NON-FREEZING WATER OF HOMOGENEOUS pHEMA HYDROGELS



such observations suggesting that the presence of more chain ends facilitated the swelling processes of the hydrogels. The molecular weight distribution and average chain length of the polymers affected the E.W.C. since a greater concentration of initiator produces a larger number of initiating species and so affects the rate of termination resulting in a higher number of shorter chains. The shorter chains being able to imbibe greater amounts of water.

FIGURE III (11)

EFFECT OF CROSS-LINKING AGENT TYPE ON THE E.W.C. OF ACM/NVP/HPA/STY (33:47:10:10) HYDROGELS



III.4.v The Effect of Initial Dilution of Monomers on the E.W.C. of Hydrogels

For hydrogels such as pHEMA where water is a thermodynamically poor solvent for the polymer, the E.W.C. is relatively unaffected by the number of cross-links. The same independance of E.W.C. on the initial dilution of the starting mixture has also been observed for pHEMA[78,107], the E.W.C. remaining fairly constant at about 40%.

For hydrogels for which water has a higher solvent power, such as pHPA and pGMA, the dependence of swelling on the initial dilution and extent of cross-linking was more evident[106]. The E.W.C. of pGMA hydrogels increased linearly with increasing water content in the starting mixture. Preparation of pHEMA with E.W.C's exceeding 40% were generally found to be heterogeneous.

III.4.vi The Effect of Polymerisation Temperature and Time on the E.W.C. of Hydrogels

Middleton[15] has shown that since temperature changes affect initiation, propagation and termination, the resultant change in E.W.C. is less predictable than, for example, changes in the amount of initiator used. It was generally found that a higher polymerisation temperature resulted in a higher E.W.C. perhaps due to an increase in the rate of termination due to disproportionation which led to a reduction in the average chain length of the polymer and hence an increase in the E.W.C. of the hydrogel. (An increase in radical recombination would result in a polymer of higher molecular weight distribution and hence lower E.W.C.).

Although the systems studied were different to those used here, Middleton also found that the E.W.C's of a variety of preparations did not show any appreciable variation for polymerisation periods up to 7 days.

III.4.vii The Effects of Postcuring Polymers on the E.W.C. of Hydrogels

The postcuring of polymers at elevated temperatures can be carried out to ensure that any fraction of the initiator remaining unreacted after polymerisation is converted to free radicals, giving rise to the polymerisation of any residual monomer units.

Postcured samples have been generally found to have higher E.W.C's than unpostcured samples,[15] the increase becoming less marked as the temperature of the initial polymerisation was increased.

The use of a postcure technique also decreases the percentage of the residual fraction, the affect of the residual fraction on E.W.C. being quite variable and dependant on factors controlling the various rates of the polymerisation processes.

III.4.viii Hydrophilic Systems

Having considered the factors affecting the nature of hydrogels during polymerisation, the nature of polymer/polymer and polymer/solvent interactions can now be discussed.

Perhaps the most straightforward of the preparations studied were the hydrophilic poly(acrylamide) hydrogels in general, an increase in the polarity of the monomer units in a hydrogel leads to increased hydrophilicity and increased solvation. Thus pACM, which contains a highly polar amide group, is water soluble in its uncross-linked state whereas pHEMA with its less hydrophilic hydroxyl group only absorbs water up to 40% of the total weight.

Acrylamide is known to form "plurimolecular" aggregates in various solvents[108] and the strong hydrogen bonding of amides is also responsible for the high boiling points of many of them.

The pACM preparations used during this research were in part "preswollen" since the initial monomer solution was 75% (by wt) water. The effect of this initial dilution on the hydrogel was such as to prevent extensive interchain hydrogen bond formation by solvation of the hydrophilic regions of the monomer before polymerisation occurred.

III.4.ix Hydrophilic/Hydrophobic Systems

The HEMA/STY hydrogel preparations used during this work were examples of hydrophilic/hydrophobic systems. The addition of an hydrophobic monomer to an otherwise hydrophilic preparation has been widely used to produce hydrogels with specific properties. The effect of the incorporation of various amounts of styrene into HEMA/Styrene copolymers can be seen in Figure III (12).[10]

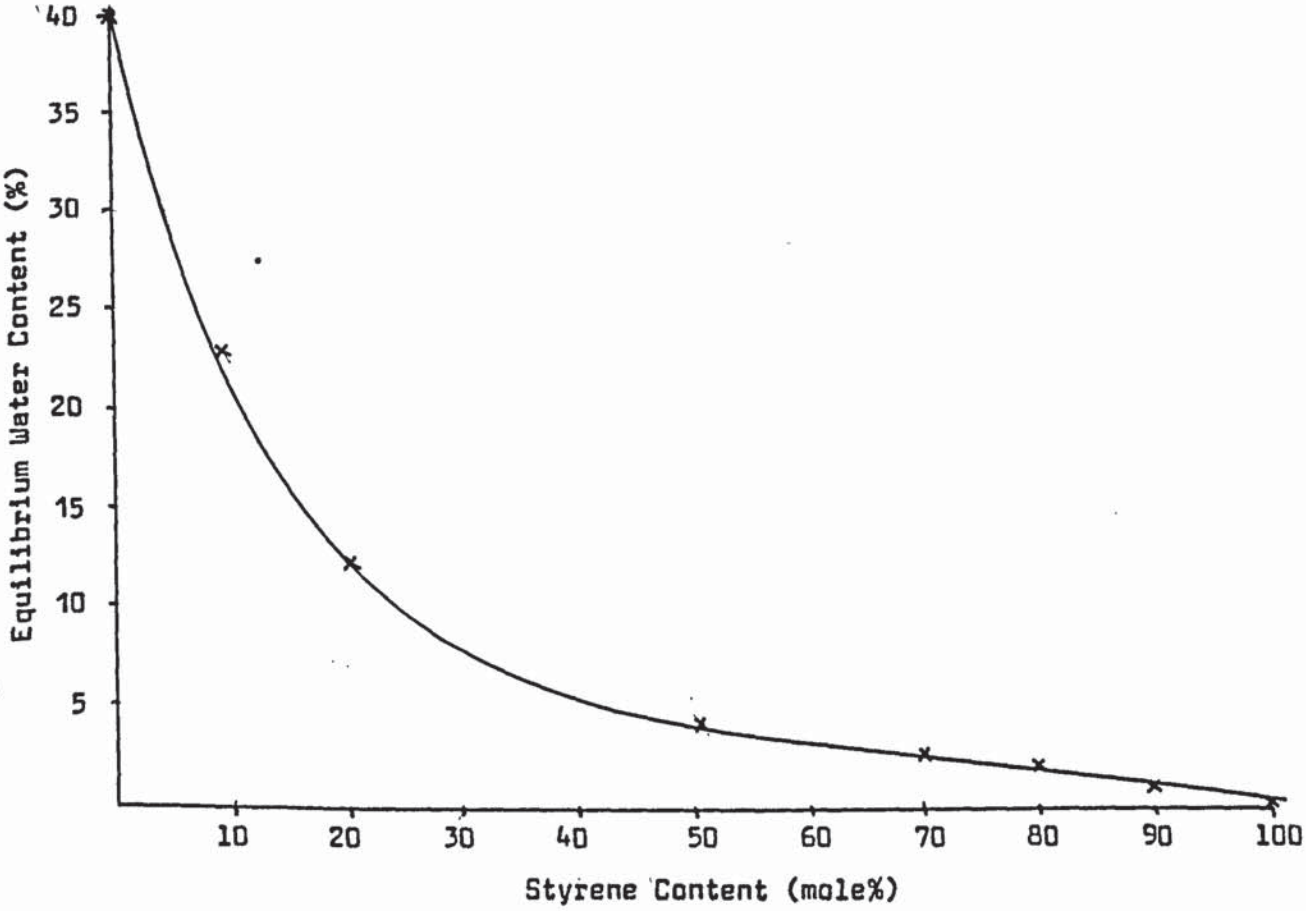
The effect of styrene incorporation on the freezing water content of HEMA/STY copolymers is perhaps of more interest than the reduction of E.W.C. as styrene content was increased. Figure III (13) shows both the freezing water contents and the E.W.C's of HEMA/STY copolymers of a wide range of compositions.[10] The E.W.C. of pHEMA was about 40% of which previous work has shown that 23% was freezing water and 17% was non-freezing water.[10,16] The incorporation of 10 mole% styrene reduced the E.W.C. to 23%, of which only 4% was freezing water, the remaining 19% being non-freezing water. HEMA/STY copolymers with greater than 20 mole% styrene incorporated had no observable freezing water. This information could also be presented in terms of the numbers of water molecules associated with each HEMA unit in the HEMA/STY copolymer as shown in Figure III (14).[10]

Styrene is a fairly bulky hydrophobic monomer and the above results demonstrate the strong influence that relatively small amounts of styrene have on both the E.W.C. and the constituent amounts of freezing and non-freezing water.

Observations such as these are very important since the freezing water/non-freezing water ratio controls a number of the exploitable properties

FIGURE III (12)

EFFECT OF STYRENE INCORPORATION ON THE E.W.C. OF HEMA/STY COPOLYMERS



of hydrogels. The nature of the water in a hydrogel membrane and the physical construction of the membrane are generally accepted as being the major factors controlling permselectivity.

Another hydrophobic monomer which has been widely studied is methylmethacrylate (MMA). The affect of increasing the hydrophobic monomer components of both HEMA/STY and HEMA/MMA copolymers on E.W.C. can be seen in Figure III (15).[15] From this graph it can be seen that styrene incorporation resulted in a greater reduction in E.W.C. than did an equal amount of MMA. Whereas the bulky phenyl group of styrene when attached to the polymer backbone limited the accumulation of water

FIGURE III (13)

E.W.C'S AND FREEZING WATER CONTENTS OF HEMA/STY COPOLYMERS

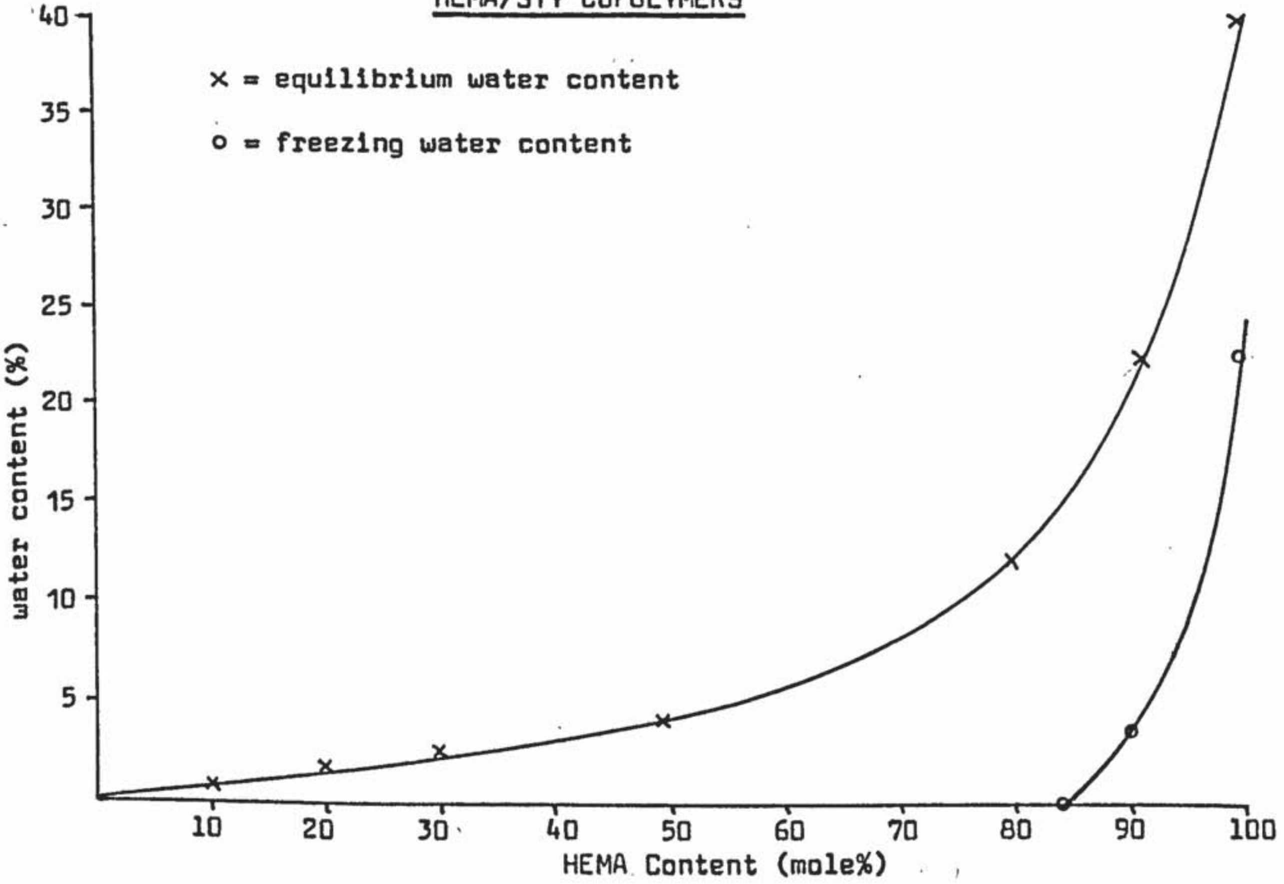


FIGURE III (14)

NUMBERS OF WATER MOLECULES ASSOCIATED WITH EACH HEMA UNIT IN HEMA/STY COPOLYMERS

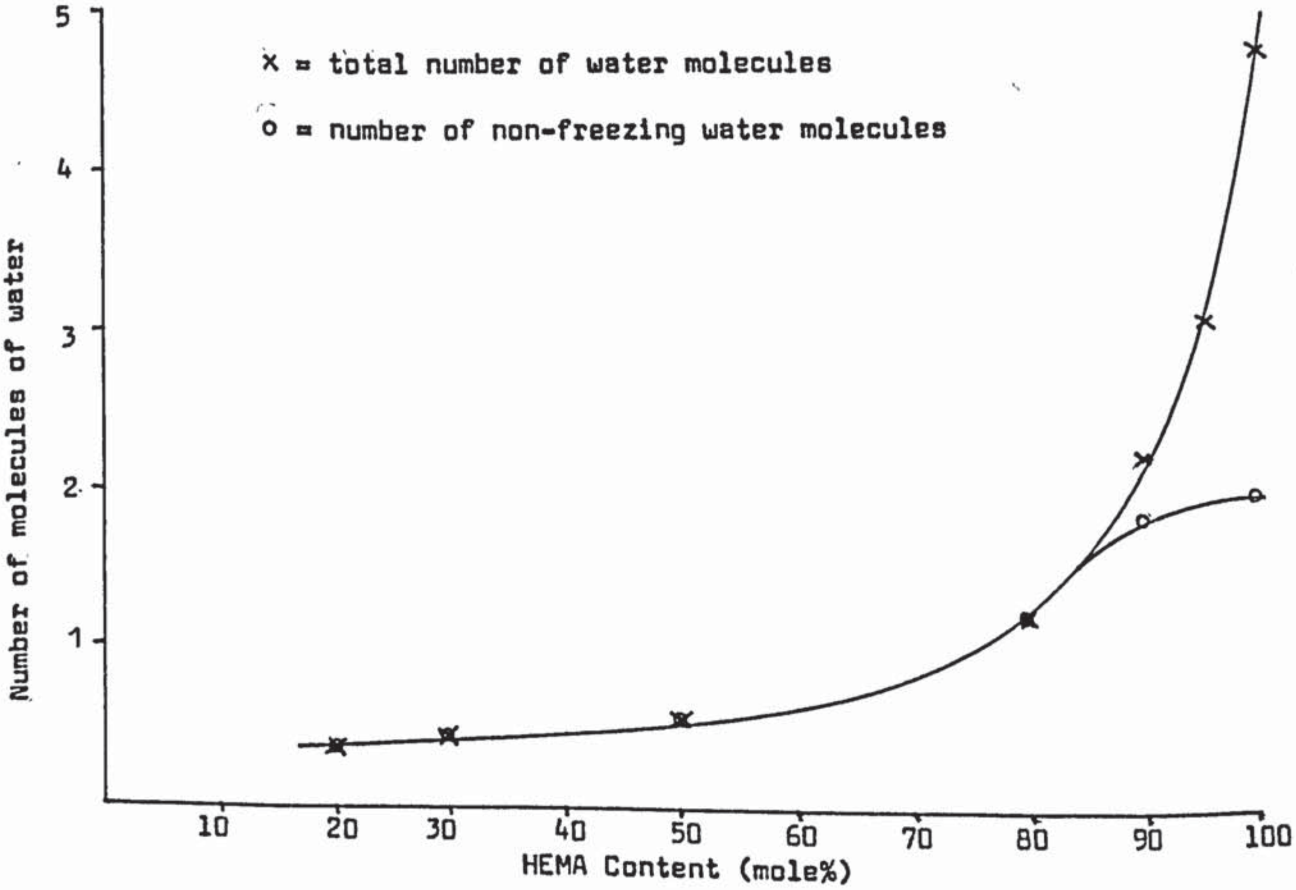
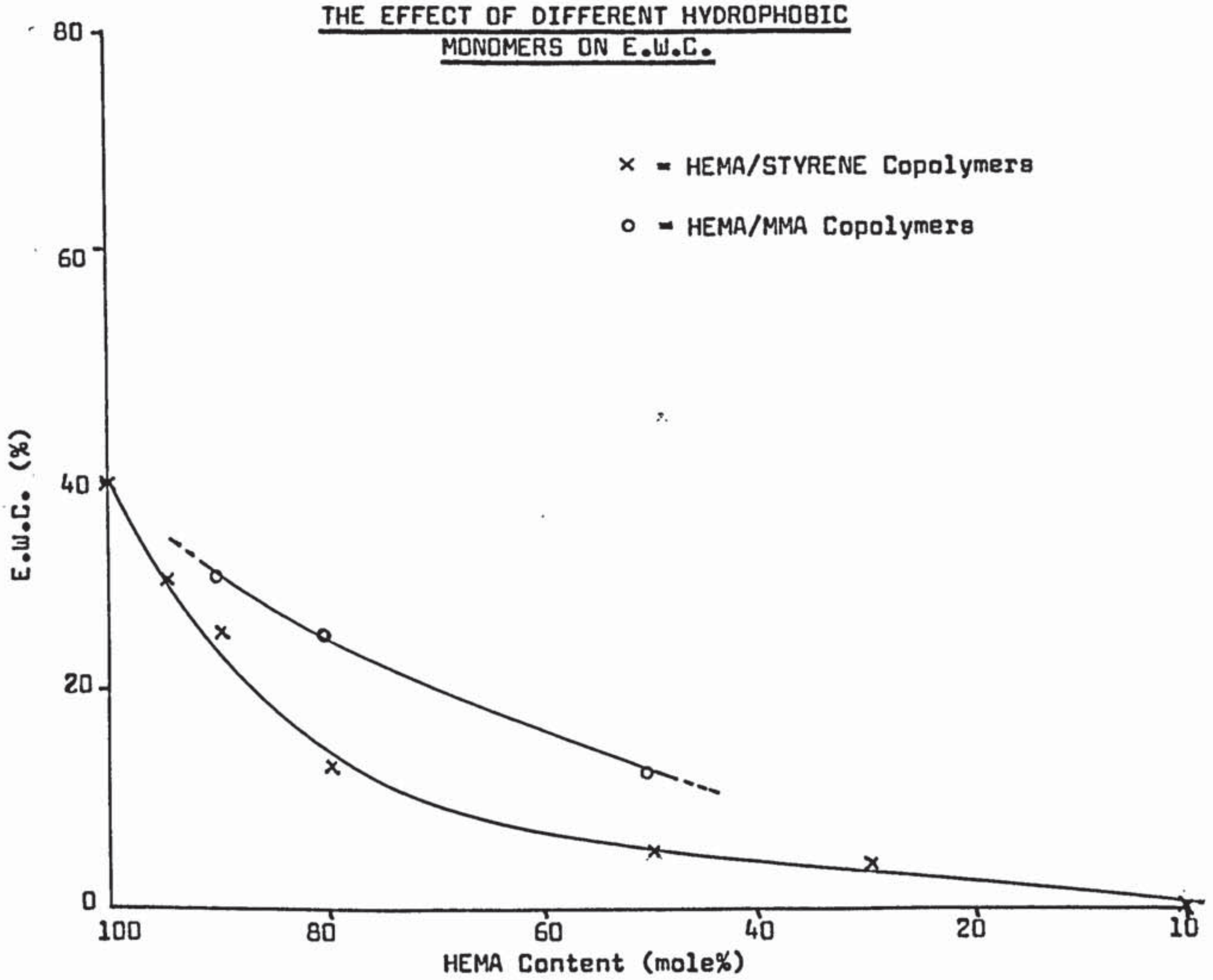


FIGURE III (15)

THE EFFECT OF DIFFERENT HYDROPHOBIC MONOMERS ON E.W.C.



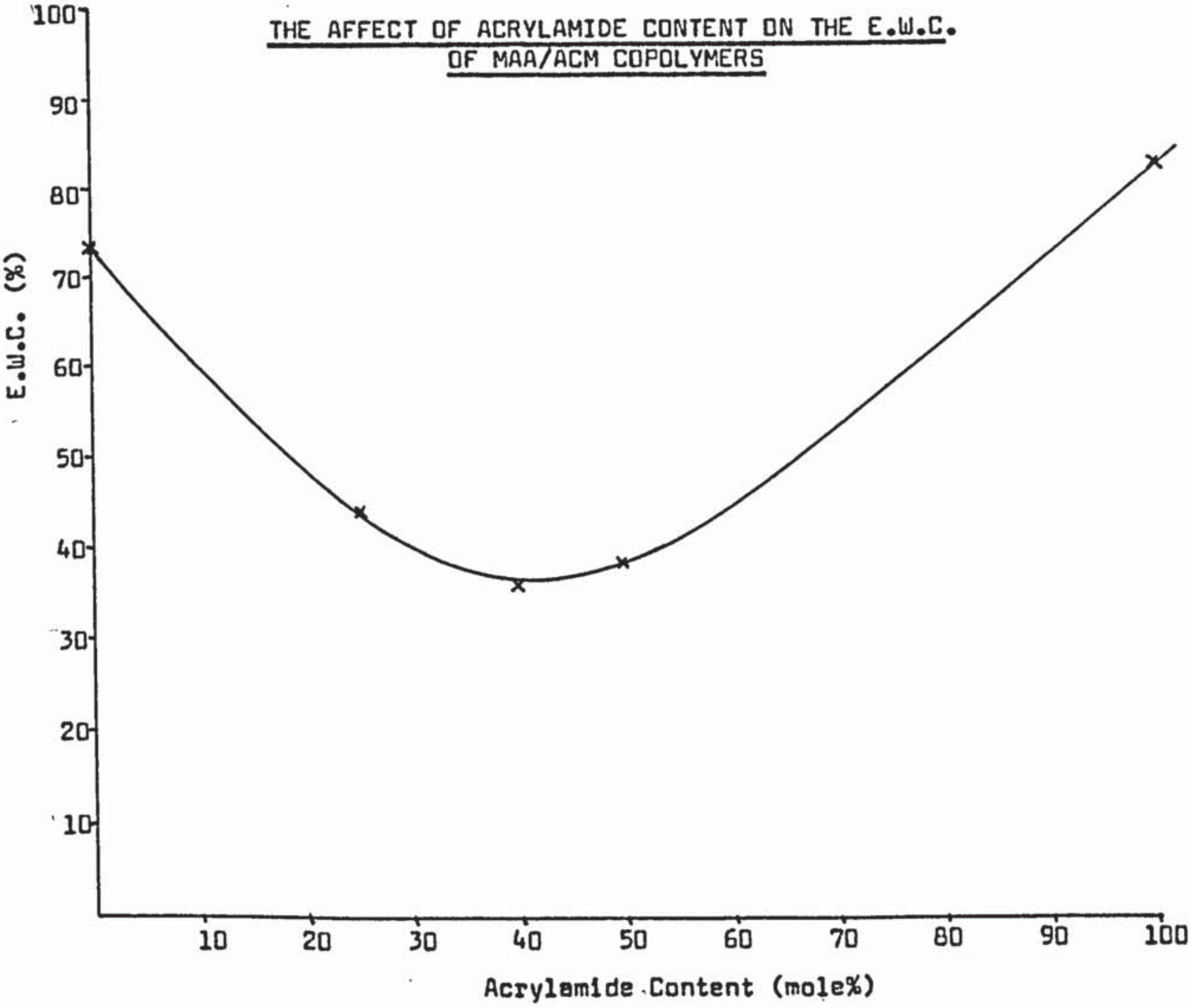
by steric hindrance, MMA had a smaller steric effect and was also slightly polar due to the carboxylate group in the side chain ester linkage. Both these factors contributed to the higher E.W.C. of HEMA/MMA copolymer compared to HEMA/STY copolymer.

III.4.x Hydrophilic/Hydrophilic Systems

Hydrogel preparations of copolymers of two hydrophilic monomers represent quite a complex system since the monomers can either participate in bonds with each other or with water molecules. Problems such as these cast doubt on the validity of the establishment of a hydrophilicity series for monomers. Work by Anderton[109], Berry[110] and Middleton[15] concluded that a knowledge of the hydrophilicity of monomers could only be useful

FIGURE III (16)

THE AFFECT OF ACRYLAMIDE CONTENT ON THE E.W.C. OF MAA/ACM COPOLYMERS



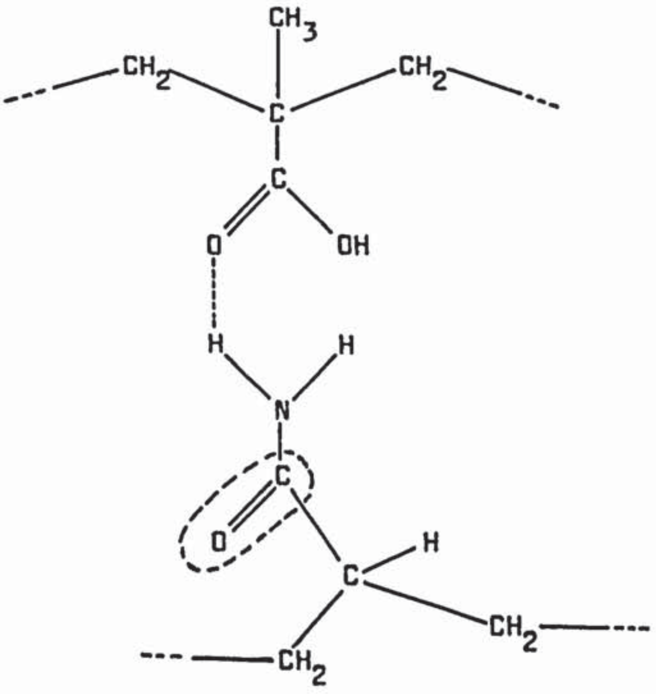
to the design of copolymers with certain properties when the bonding of water to hydrophilic groups far outweighed interchain bonding between monomer units of the polymer.

A hydrophilic/hydrophilic system of MAA/ACM was studied during this research. The E.W.C's of the homopolymers of each monomer have previously been found to be 73% for MAA and 84% for ACM.[14] However the E.W.C's of a range of copolymer compositions of these monomers shows a minimum at a ratio of about 50:50 MAA:ACM (mole%) as shown in Figure III (16).[10] The presence of such a minimum reflected the point at which the extent of hydrogel bonding between the carboxylic acid group of MAA and the amide group of

ACM reached a maximum. The nature of this interaction is shown in Figure III (17).

FIGURE III (17)

INTERCHAIN HYDROGEN BONDING BETWEEN ACM AND MAA UNITS



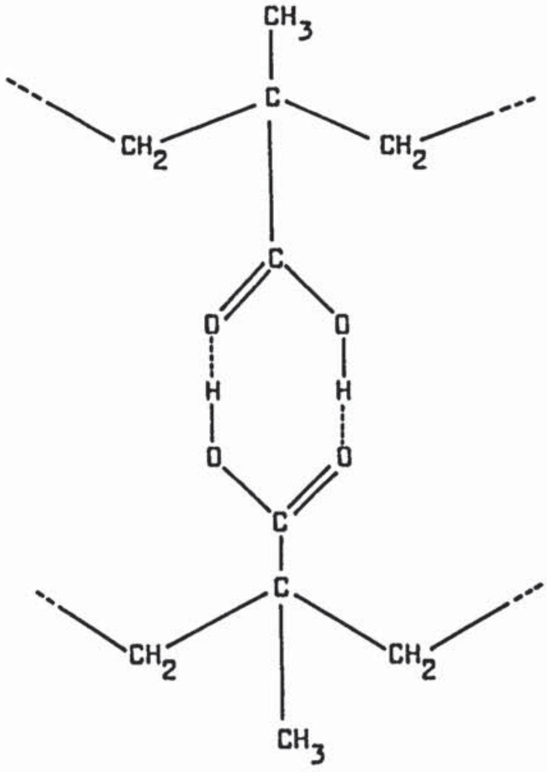
Interchain hydrogen bonding of another kind can occur between carboxylic acid groups of the polymer backbones.[15] This has been found for MAA/HEMA copolymers where the E.W.C. displayed a minimum at the 50:50 (mole%) copolymer composition as a result of interchain hydrogen bonding between carboxylic acid groups as shown in Figure III (18).

This kind of interaction forms a type of secondary cross-linking which inhibits swelling and restricts the ability of the polar hydrophilic groups to attract water molecules.

Copolymers of ACM and HEMA showed no such minimum, the E.W.C. increasing with increasing ACM component. The absence of any appreciable interchain hydrogen bonding in this system can be explained by the shielding of the carbonyl group of ACM as shown ringed in Figure III (17).

FIGURE III (18)

INTERCHAIN HYDROGEN BONDING BETWEEN MAA UNITS

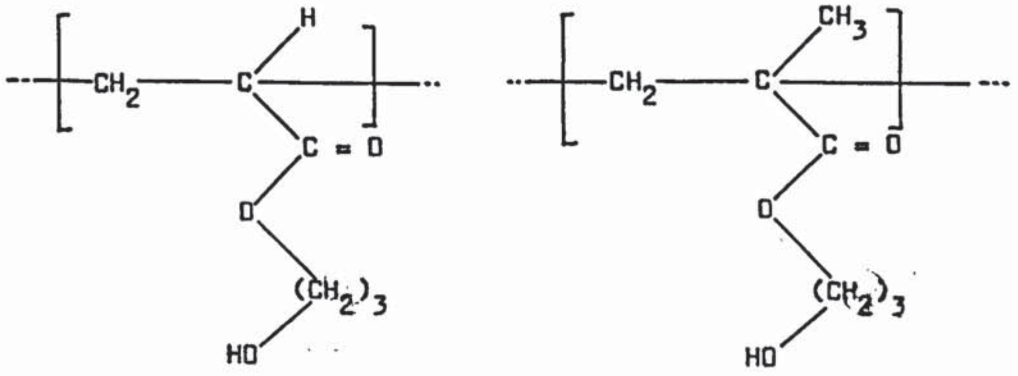


Studies have previously been reported on a number of copolymers where the effect of the presence or absence of an α-methyl group on one of the comonomers was studied. The effect of the presence of an α-methyl group on the E.W.C. of pHPMA as compared to pHPA can be seen in Figure III (19), the E.W.C. of pHPMA being 23% and that of pHPA being 50%.[11]

In the above situation it is not only the removal of the hydrophobic group which increases the E.W.C. but also the simultaneous increase in chain rotation about the polymer backbone which allows the hydrophilic groups more freedom and consequently they can be solvated more easily.

Copolymers of MAA/ACM and AA/ACM show similar differences in their E.W.C's due to the presence or absence of an α-methyl group as shown in Figure III (20).

FIGURE III (19)



pHPA
E.W.C = 50%

pHPMA
E.W.C = 23%

An investigation furthering the work of Goulding[111] on the effect of initial monomer dilution on the E.W.C. of 50:50 (mole%) ACM/MAA copolymers was carried out by Middleton.[15] The earlier work had shown dramatic increases in E.W.C. for MAA/ACM copolymers over periods between one month and seven months. Changes in E.W.C. from 45% to 75% were also reported (after one month's hydration) when the amount of water in the comonomer mixture was increased from 0 to 10% (by wt). The polymer would be formed with water molecules already associated with the hydrophilic groups thus leading to a decrease in the amount of interchain hydrogen bonding and an increase in E.W.C.

As regards the increase in the E.W.C. of these preparations with time it was suggested that interchain hydrogen bonding between hydrophilic groups was in competition with water-polymer binding. Over a period of time water present in the matrix would increasingly assert itself over interchain attractions, bonding to the hydrophilic groups itself and causing a gradual rise in E.W.C.

Studies on the change of E.W.C. with time for different types of comonomers showed no such dramatic E.W.C. increase with time. It was

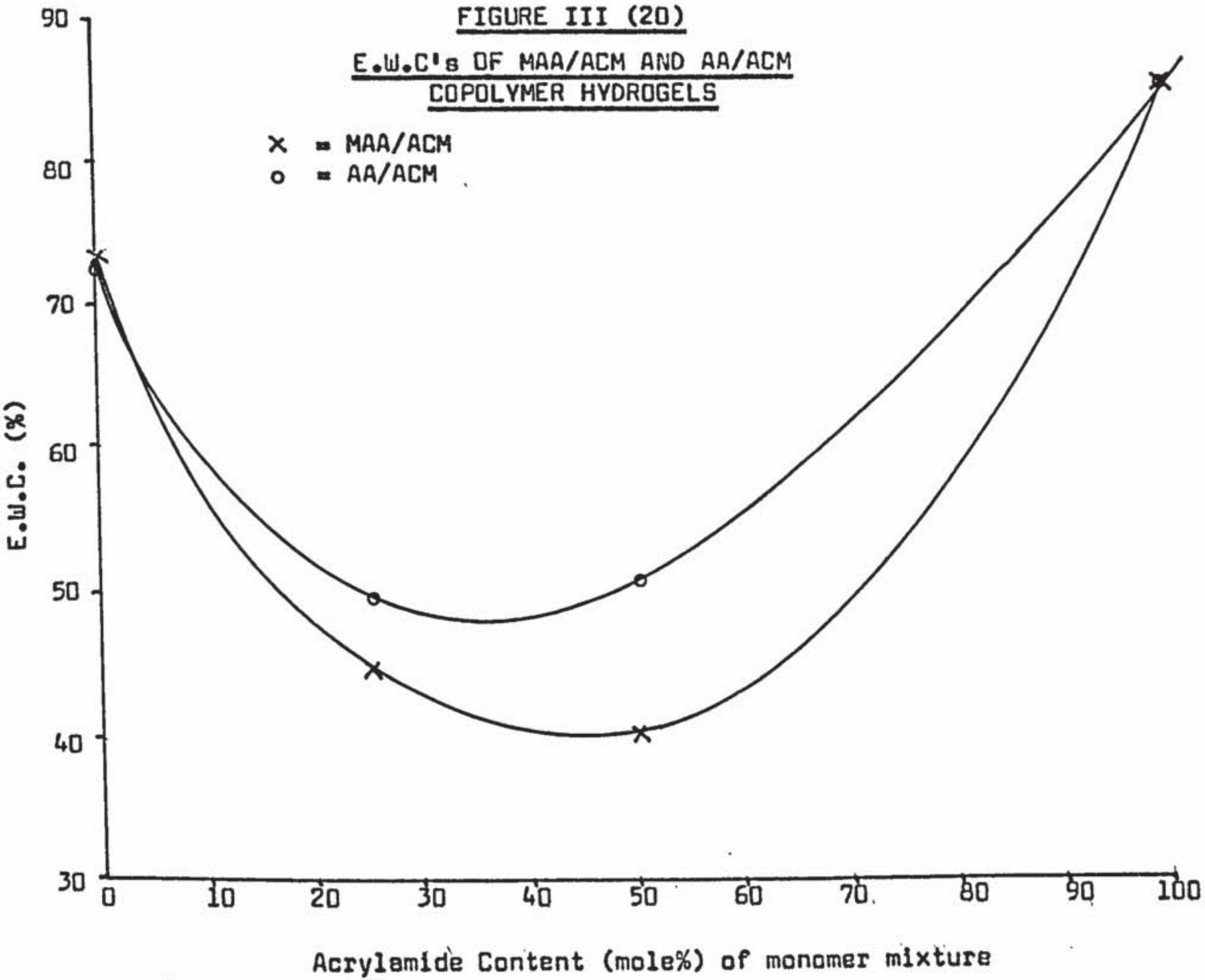


FIGURE III (20)

E.W.C's OF MAA/ACM AND AA/ACM COPOLYMER HYDROGELS

suggested that the presence of the polar carbonyl group on the amide unit of amide/carboxylate interactions, which was not directly involved in interchain interactions, attracted water molecules to the interchain hydrogen bonding site and made it more susceptible to solvation. The same process would not occur at carboxylate/carboxylate sites since both the hydroxyl and carbonyl groups participated in interchain hydrogen bonding.

Such suggestions were tentative and further studies on such systems are needed. The author considers that the effect of pH on the ionisation of MAA might provide a more likely explanation for the increasing E.W.C. of MAA/ACM copolymers with time due to rupture of interchain hydrogen bonds. Work presented later in this chapter on similar systems at pH's likely to suppress the ionisation of MAA showed no such increase in E.W.C. with time.

III.4.xi Hydrophilic/Hydrophilic/Hydrophobic Systems

A study on the effect of the incorporation of various amounts of styrene into an equimolar copolymer of MAA/ACM[10] demonstrated an unusual minimum. Figure III (21) shows this behaviour which suggests the existence of competing forces.

The initial decrease in E.W.C. was in effect the same behaviour seen earlier for HEMA/styrene copolymers where the hydrophilicity of the system was gradually reduced. The increase in E.W.C. above 5 mole% of styrene was considered to be the result of the disruption of interchain hydrogen bonding by the bulky phenyl group of styrene. The exposed hydrophilic groups would then be hydrated leading to an increase in E.W.C.

In effect, between 5 and 10 mole% styrene any increase in the hydrophobicity of the polymer was overcome by the increase in hydrophilicity due to disruption of interchain hydrogen bonds. A limiting value for this effect was reached between 10 and 15 mole% styrene, the hydrophobicity of the styrene being able to cancel out any increase in E.W.C. as fewer interchain hydrogen bonds were left to be disrupted.

Once again, the changes in the freezing and non-freezing water components of the system were of interest and this data is shown in Table III 8.[16] Some of this information is also shown in Figure III (22). It was apparent that the observed rise in water content above 5 mole% styrene was not associated with any increase in the amount of freezing water.[10] Figure III (22) shows a steady decrease in the amount of freezing water over the whole styrene concentration range so that the increase in E.W.C. above 5 mole% styrene was due to an increase in the non-freezing water

FIGURE III (21)

THE EFFECT OF STYRENE INCORPORATION ON THE E.W.C'S OF EQUIMOLAR MAA/ACM COPOLYMERS

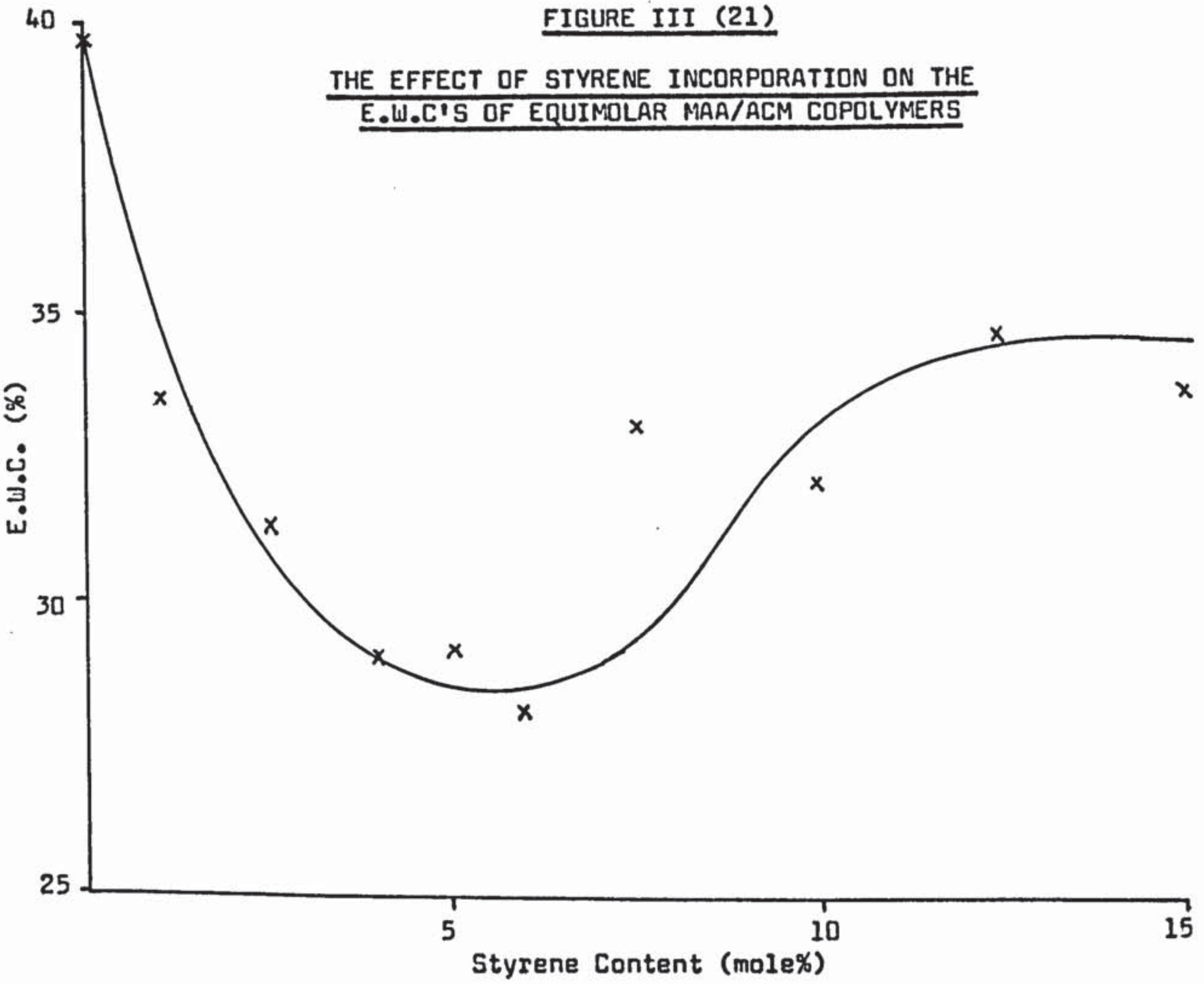


FIGURE III (22)

THE EFFECT OF STYRENE INCORPORATION ON THE FREEZING WATER CONTENTS OF EQUIMOLAR MAA/ACM COPOLYMERS

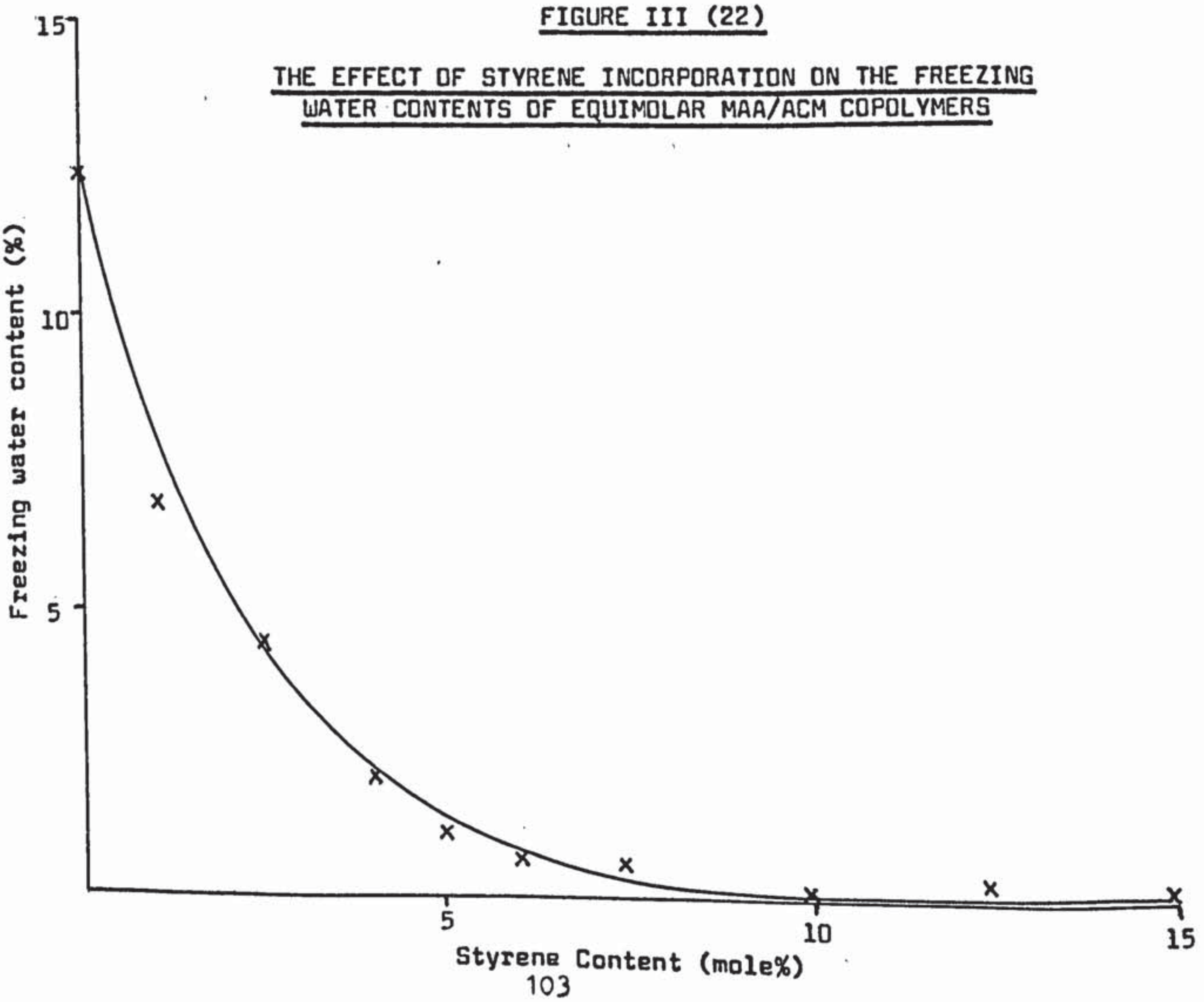


TABLE III B

WATER BINDING PROPERTIES OF METHACRYLIC ACID/ACRYLAMIDE/STYRENE HYDROGEL PREPARATIONS

Molar Composition (MAA:ACM:STY)	E.W.C. (%)	Freezing Water Content (%)	Non-Freezing Water Content (%)
49.5:49.5:1.0	33.5	6.8	26.7
48.75:48.75:2.5	31.3	4.4	26.9
48.0:48.0:4.0	29.1	2.1	27.0
47.5:47.5:5.0	29.2	1.1	28.1
47.0:47.0:6.0	28.1	0.7	27.4
46.25:46.25:7.5	33.1	0.7	32.4
45.0:45.0:10.0	32.1	0.1	32.0
43.75:43.75:12.5	34.7	0.4	34.3
42.5:42.5:15.0	33.8	0.3	33.5

component of the system. The decrease in the amount of interchain hydrogen bonding above 5 mole% styrene therefore only affected the amount of non-freezing water to any appreciable degree.

III.4.xii The Effect of Hydration Temperature on the E.W.C. of Hydrogels

The E.W.C's of many hydrogels have been shown to be dependant on the temperature at which they were hydrated.[11] The results suggest that two opposing and competing forces, which were temperature related, affected the E.W.C's of hydrogels since many preparations displayed a minimum in E.W.C. at intermediate hydration temperatures. An increase in temperature led to an increase in hydrophilic hydration and also expanded the polymer network thus allowing increased water absorption. There may also be an associated breakdown of interchain hydrogen bonding contributing to the

E.W.C. increase depending on the type of composition studied.

A competing force was due to the formation of hydrophobic bonds. The formation of such bonds, Van der Waal's interactions between non-polar groups, is an endothermic process and has been shown to exist in polymer-water systems.[43] The occurrence of hydrophobic hydration is entropically unfavoured, water being structured in the vicinity of a non-polar compound.[112] It has been suggested that these hydrophobic bondings act as "cross-links" and reduce the E.W.C. of the preparation. The existence of hydrophobic bonding in pHEMA hydrogels was postulated by Refojo,[79] who studied the effects of microsolutes in the hydrating solutions of pHEMA preparations on their E.W.C's.

The extent of the competition between these processes determined the shape of the E.W.C. - hydration temperature curve, thus explaining the minima observed for many hydrogel preparations.

Although the work of Ng[11] did not provide evidence of a minimum in the E.W.C. - hydration temperature curves of pHEMA or HEMA/STY 90:10 (mole%) the E.W.C. of pHEMA has been previously reported as displaying a minimum E.W.C. of 36% at about 60°C.[78]

III.4.xiii The Effect of Solutes in the Hydrating Medium on the E.W.C's of Hydrogels

The biomedical and physiological applications of certain hydrogels have meant that studies on the effects of solutes such as sodium chloride and urea in the equilibrating solutions of hydrogels have been carried out.

The reported deswelling effects of increasing concentrations of sodium chloride in the equilibrating solutions of pHEMA preparations[107] has also been observed by Ng[11] for a number of hydrogel preparations. The decrease in E.W.C. was found to be greatest for high E.W.C. preparations.

It was suggested that the presence of sodium chloride in solution enhanced the water structure, resulting in an increase in hydrophobic bonding and a subsequent decrease in E.W.C.

An extensive study by Refojo[79] led to the postulation of a secondary structure in pHEMA additional to its covalently cross-linked structure. This would be stabilised by hydrophobic interactions between α-methyl groups resulting in so highly a cross-linked arrangement that the covalent cross-links were overshadowed. It was suggested that the secondary structure was responsible for the preparation's swelling behaviour. The increase in the swelling of pHEMA when in dilute urea solutions was considered to be due to the disruption of hydrophobic cross-links in a manner not dissimilar to the way urea denatures proteins by the disruption of hydrophobic stabilisation. (Similar studies have since been conducted by other workers that claim to either support[113] or at least partially reject[114] the earlier claims.)

III.4.xiv Permeability Characteristics of Hydrogels

Studies on the permeation of various compounds through hydrogels are cited in the literature. Such studies involved the fields of contact lens design, reverse osmosis, drug release and medication. The results of such studies have been previously summarised[11,13,14] and only certain aspects of relevance to the research undertaken here will be reproduced.

An extensive investigation by Refojo[115,116,117] into the water permeability of many materials, including pHEMA, enabled the calculation of the water diffusion coefficients of the various hydrogels and their "pore radius". The average pore radius was meant to indicate the size of molecules that could penetrate the network studied. The pore radius for pHEMA was found to be 4 Å but this value is likely to be low since fluorescein (molecular radius 5.5 Å) has been shown to be absorbed into pHEMA hydrogels.[118]

Refojo's results indicated that for the same types of hydrogels, increasing E.W.C. led to an increase in water permeability. He also concluded that in general water flow for hydrogels of E.W.C. <60% was diffusive whereas for hydrogels of E.W.C. >60% it was predominantly viscous flow.

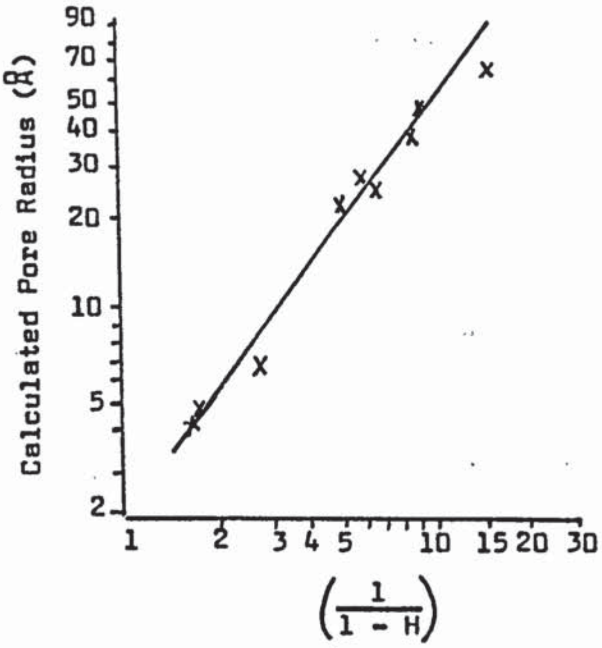
Yasuda et al[119,120,121] investigated the permeability of sodium chloride through hydrogels of various water contents and demonstrated a linear dependance of the logarithm of the permeability coefficients on the reciprocal of the volume fraction of water in hydrogels of water content >25%. Figure III (23) shows a plot of the calculated pore radius of each of a series of methacrylate hydrogels against their swelling ratio (where H is the E.W.C. divided by 100).[122] Use of this graph enables the average pore radius of a hydrogel of a different water content to be calculated. For example pHEMA (E.W.C. = 40%) would have a theoretical pore radius of 3 Å and so would be expected to only be permeable to molecules whose molecular diameters are approximately equal to or less than 6 Å.

Such a calculation was borne out by Ratner and Miller[123] who plotted the ratio of the diffusion coefficient of the molecular species through hydrogels to that found in free solution against the molecular radius of various solute molecules. Molecules such as glucose and some amino-acids were reported to penetrate pHEMA hydrogels but larger molecules such as polysaccharides did not.[11]

Sodium chloride permeability has been shown to be affected by chemical structure as well as E.W.C.[124] The modification of pHEMA hydrogels by

FIGURE III (23)

DEPENDANCE OF CALCULATED PORE RADIUS
ON THE SWELLING RATIO OF METHACRYLATE HYDROGELS



the introduction of ionogenic groups brought about by copolymerisation with for example methacrylic acid (MAA) or 2-(diethylamino) ethyl methacrylate (DEAEMA) was carried out. Lower sodium chloride permeation was observed for membranes containing acidic or basic groups than was recorded for neutral membranes. This was as would be expected according to the Donnan's excluding effect where the reduction in transport of a co-ion will slow down the transport of the salt as a whole because of the need to conserve electroneutrality.

Ampholytic membranes showed the highest sodium chloride permeability and this was attributed to the accelerated diffusion of both ions due to the electric charge.

The pH in such systems can be critical since this determines the degree of ionisation of functional groups in the membrane. The presence of such ionic groups can also profoundly influence E.W.C.[125] The conversion of MAA to its sodium carboxylate salt in HEMA/MAA copolymers dramatically increased the E.W.C. of the preparations. However, the conversion of the dimethyl amino group of dimethyl amino ethyl methacrylate (DMAEMA)/HEMA copolymers to its hydrochloride salt did not show any such dramatic effect on E.W.C.

III.5 Hydration Characteristics of Hydrogels Prepared During this Research

III.5.1 E.W.C's and Freezing/Non-Freezing Water Compositions

The polymerisation conditions for all preparations have been presented in Table III A. The data relating to the water contents of the series of acrylamide compositions prepared during this research are summarised in Table III C. As can be seen from this Table water swollen pACM cross-linked with 1% (by wt) MBACM was found to have an E.W.C. of 89.3% of which 80.1% was freezing water and 9.2% was non-freezing water. It was apparent that the hydrophilicity of pACM was such as to imbibe large amounts of freezing water but was incapable of restricting much water into a non-freezing water fraction.

The water contents of pACM preparations of varying thickness (t) can also be compared. There was a slight but nevertheless steady increase in the E.W.C. of pACM preparations as the membrane thickness increased. Membrane thicknesses of 1 × t to 4 × t were studied (ie a range of swollen membrane thicknesses of about 0.05 to 0.25 cm). This would seem to suggest that there was a small error involved in the process of cutting sample discs for E.W.C. determination. As discs were cut a small amount of water may have been "squeezed out" of the sample, the amount squeezed out accounting for a smaller proportion of the water in the sample as membrane thickness increased. The "true" value of the E.W.C. of this hydrogel composition was thus considered to be nearest to the E.W.C.

TABLE III C

NATURE OF WATER IN poly(ACRYLAMIDE) PREPARATIONS

Composition*	E.W.C. (%)	Freezing Water Content (%)	Non-Freezing Water Content (%)
pACM swollen in water	89.3	80.1	9.2
pACM 2 x t (water swollen)	89.9	-	-
pACM 3 x t (water swollen)	90.0	-	-
pACM 4 x t (water swollen)	91.5	-	-
pACM 5% (by wt) MBACM (water swollen)	82.0	-	-
pACM 10% (by wt) MBACM (water swollen)	74.8	-	-
pACM swollen in 0.1M L/M	86.2	76.1	10.1
pACM swollen in 0.1M C/M	86.4	71.6	14.8
pACM swollen in 0.1M NaCl	89.8	79.7	10.1
pACM swollen in 0.79M NaCl	86.1	-	-
pACM swollen in 1.0M NaCl	85.4	59.1	26.3
pACM swollen in 1.5M L/M	57.2	40.7	16.5
pACM swollen in 1.5M C/M	56.3	40.5	15.8
pACM swollen in 1.5M NaCl	82.6	55.2	27.4
pACM inc 0.1M L/M	82.2	62.7	19.5
pACM inc 0.1M C/M	82.6	65.6	17.0
pACM inc 0.1M NaCl	88.6	-	-
pACM inc 0.79M NaCl	82.1	50.7	31.4
pACM swollen in 0.2M NaCl	87.8	-	-
pACM swollen in 0.4M NaCl	87.3	-	-
pACM swollen in 0.6M NaCl	86.7	-	-
pACM swollen in 0.8M NaCl	85.8	-	-
pACM swollen in 1.0M NaCl	85.4	-	-
pACM swollen in 1.5M NaCl	82.6	-	-

* All preparations cross-linked by 1% (by wt) MBACM except where stated.

L/M = Lincomycin.HCl
C/M = Clindamycin.HCl
x t = number of gaskets used in mould
inc = antibiotic solution incorporated during polymerisation and polymer swollen in same solution.

found for the 4 x t sample. The difference between the range of values found here for E.W.C. (89.3 to 91.5%) and that found previously (85.0%)[14] may also be accounted for in terms of technique as well as the polymerisation parameters involved.

The data obtained for pACM preparations cross-linked with between 1.0 and 10.0% (by wt) MBACM (Table III C) showed a steady decrease in E.W.C. with increasing levels of MBACM. The data, together with that reported previously,[14] can be seen in Figure III (24). The previously reported data was for acrylamide preparations cross-linked with EDMA and photopolymerised using uranyl nitrate as a photoinitiator. A monomer:solvent ratio of 60:40 was also used. Because of the large differences in the polymerisation parameters in each case the reasons for the more dramatic decrease in E.W.C. with increasing levels of cross-linking agent in the previous study are not clear. However, the lower E.W.C's of preparations cross-linked with EDMA than was found for the use of MBACM has already been discussed and would seem a reasonable explanation in this case also. The E.W.C. reduction being the result of increased hydrophobic bonding and lower cross-linking agent polarity.

The effect of introducing antibiotics into pACM preparations on their E.W.C's and freezing/non-freezing water compositions can also be seen in Table III C.

pACM preparations swollen in 0.1M aqueous solutions of either Lincomycin or Clindamycin showed no significant difference in E.W.C. depending on which antibiotic solution was used. However, the E.W.C's of both these preparations had dropped slightly from the E.W.C. value of water swollen pACM. This effect may be due to either the osmotic influence of the antibiotic solution effectively deswelling the hydrogel or competition

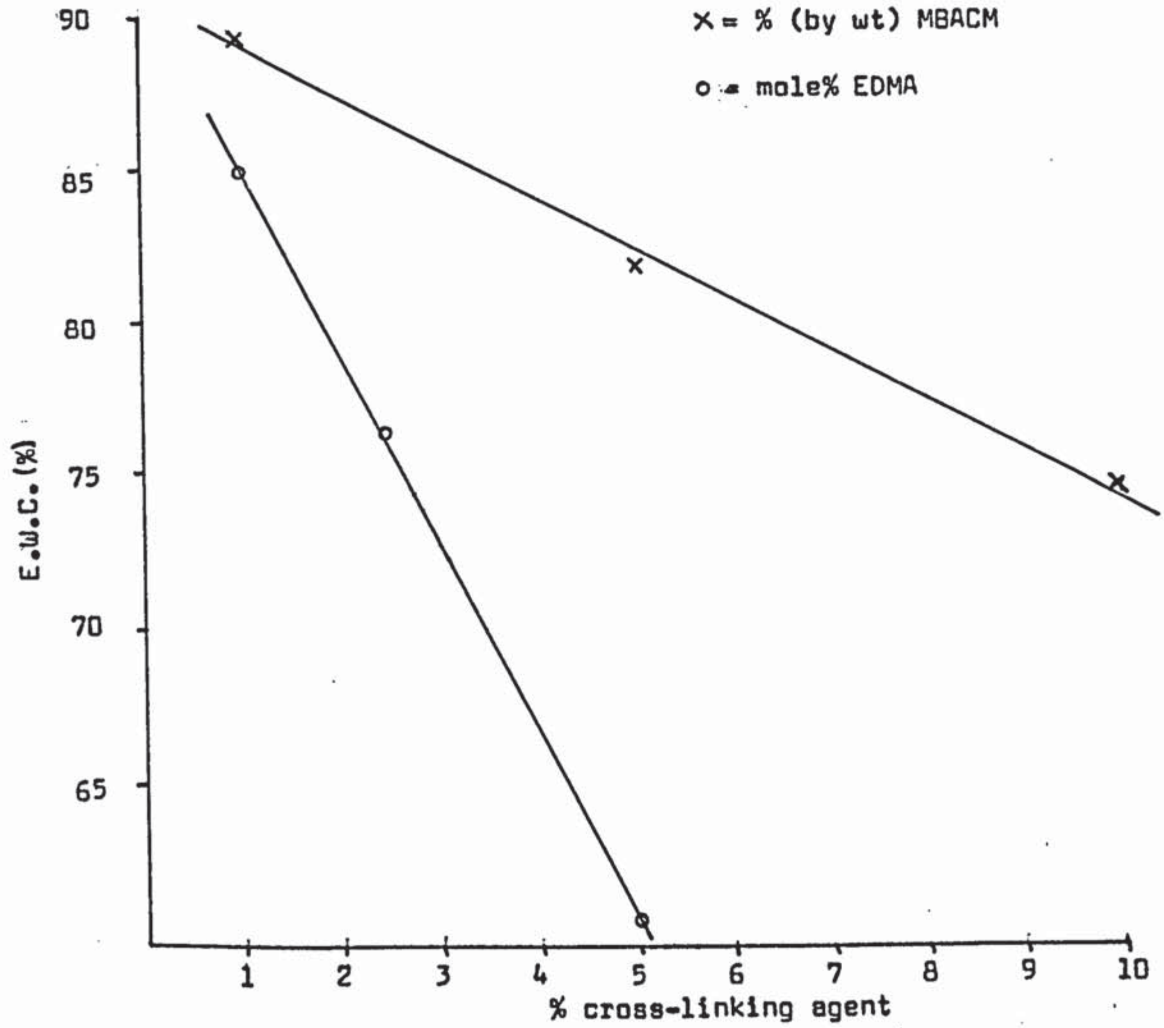


FIGURE III (24)

THE EFFECT OF THE AMOUNT AND TYPE OF CROSS-LINKING AGENT ON THE E.W.C. OF pACM HYDROGELS

for the hydrogen bonding sites of the matrix between the antibiotic and water. It was also found that the E.W.C's of pACM preparations swollen in 0.1M Clindamycin solution had higher non-freezing water components than the corresponding Lincomycin preparations. This effect was small and was contrary to the observations made on pACM preparations swollen in 1.5M aqueous solutions of either Lincomycin or Clindamycin. Since any effect of this kind would be expected to be greater the higher the concentration of antibiotic solution used, the results of the dilute antibiotic studies must be considered questionable until repeated. However, there is evidence to suggest that Clindamycin is able to structure significantly more "bound" water about it than can Lincomycin. The effect was largest at high solution concentrations of the antibiotics and the results of these studies have been presented in Chapter IV.

As has already been mentioned, pACM preparations swollen in 1.5M aqueous solutions of either Lincomycin or Clindamycin did not show any significant variation in E.W.C. or freezing/non-freezing water components. There was however a large reduction in the E.W.C's of these preparations when compared to water swollen pACM. The 1.5M antibiotic solutions were the equivalent of about 40% (by wt) aqueous antibiotic solutions and the osmotic influence of these solutions was considered responsible for the large deswelling effect. The reduction of E.W.C. can be easily accounted for by the decrease in the freezing water content alone. The slight increase in the non-freezing water components was considered to be the result of drug absorption to the matrix since any solute absorbed will add to the weight of a sample of the preparation and hence influence the E.W.C. determined. However this additional weight would not be detected as part of the freezing water fraction of the E.W.C. and thus would be erroneously assigned to the non-freezing water fraction.

An added complication to the above interpretations would be due to absorbed antibiotic displacing water from the matrix as well as affecting the E.W.C. by nature of its weight, however the antibiotics used were in general found to increase both the E.W.C. and the freezing water components of a range of hydrogels throughout this work (see later) and comparisons of the freezing water components of E.W.C's of different preparations were still considered valid.

Table III C also shows the observed reduction in E.W.C. of pACM preparations into which 1.0M aqueous solutions of Lincomycin or Clindamycin had been incorporated during polymerisation, when compared to water swollen pACM. (This technique has been described in Chapter II.) In this case the incorporated preparations were produced by substituting the 75% (by wt) water used in the starting mixture with 75% (by wt) 0.1M aqueous antibiotic solutions. The increase in the extent of deswelling of pACM found for antibiotic incorporated preparations as compared with preparations swollen in antibiotic solutions and the higher non-freezing water contents of the antibiotic incorporated preparations were considered indicative of the large amount of antibiotic introduced into the matrices through incorporation. The amount of freezing water determined in Clindamycin incorporated pACM was slightly greater than that found in Lincomycin incorporated pACM. This was the first of many similar observations on a variety of antibiotic incorporated hydrogels in which Clindamycin incorporated preparations had consistently higher freezing water contents than the corresponding Lincomycin preparations. In the case of the pACM systems the increase in freezing water content for Clindamycin incorporation as compared with Lincomycin incorporation was also accompanied by a slightly lower non-freezing water component whilst the E.W.C's of both preparations were virtually identical.

In general, the observations on the effects of the antibiotics on the nature of water in acrylamide hydrogels were overshadowed by the large "free" water contents of the systems. Before considering the results of studies of the effects of the antibiotics on the nature of water in lower E.W.C. hydrogel compositions, it is worth considering the remaining data in Table III C on the effect of the introduction of sodium chloride into pACM hydrogels.

pACM preparations swollen in 0.1M sodium chloride solutions showed less deswelling than was seen with 0.1M antibiotic solutions. The deswelling effect was related to the mass of solute present in solution hence when pACM was swollen in a solution of equivalent mass of sodium chloride as that found in a 0.1M antibiotic solution (ie 0.79M) the extent of deswelling was found to be the same.

The same effect was observed for 1.5M sodium chloride solutions since a 1.5M sodium chloride solution was 8% (by wt) sodium chloride whereas a 1.5M antibiotic solution was 40% (by wt) antibiotic, hence the extent of deswelling was less for the 1.5M sodium chloride solution than for the 1.5M antibiotic solutions. This effect was again repeated for pACM preparations into which either sodium chloride or antibiotic had been incorporated during polymerisation.

The results in Table III C also showed that the non-freezing water component of pACM/sodium chloride preparations was greater than for the corresponding pACM/antibiotic preparations. This change may be the result of the solute weight effect on EWC described previously, sodium chloride possibly being accumulated in the matrix at a higher level than that found for antibiotic. Alternatively the observations may well be genuine changes in non-freezing water components due to the additional stabilisation of water structure by sodium chloride. The explanation for these observations can only be resolved with a knowledge of the partition coefficients of both sodium chloride and the antibiotics in such systems.

The determination of these partition coefficients was attempted, however the attempt was not successful for the antibiotic systems since the assay was not sufficiently sensitive and so no comparisons between sodium chloride and antibiotic systems could be drawn. The results that were obtained are presented in Chapter VI.

The final observations that can be made from the data presented in Table III C concern the extent of deswelling of pACM preparations according to the concentration of sodium chloride solution used for swelling. The results are also shown in Figure III (25) which illustrates the linear dependance of the extent of deswelling on the swelling solution sodium chloride concentration. (Error limits of ± 0.5% allow for complete linearity).

The results obtained concerning the nature of water in various pHEMA hydrogels are summarised in Table III D.

TABLE III D

NATURE OF WATER IN pHEMA PREPARATIONS

Composition*	E.W.C. (%)	Freezing Water Content (%)	Non-Freezing Water Content (%)
pHEMA swollen in water	35.5	12.8	22.7
pHEMA swollen in 1.5M L/M	36.0	-	-
pHEMA swollen in 1.5M C/M	41.8	-	-
pHEMA swollen in 1.5M NaCl	25.7	-	-
pHEMA inc 10% 1.0M L/M	39.4 ± 0.4‡	10.1 ± 4.0‡	29.3 ± 4.0‡
pHEMA inc 10% 1.0M C/M	40.8 ± 0.9‡	20.2 ± 6.1‡	20.6 ± 6.1‡

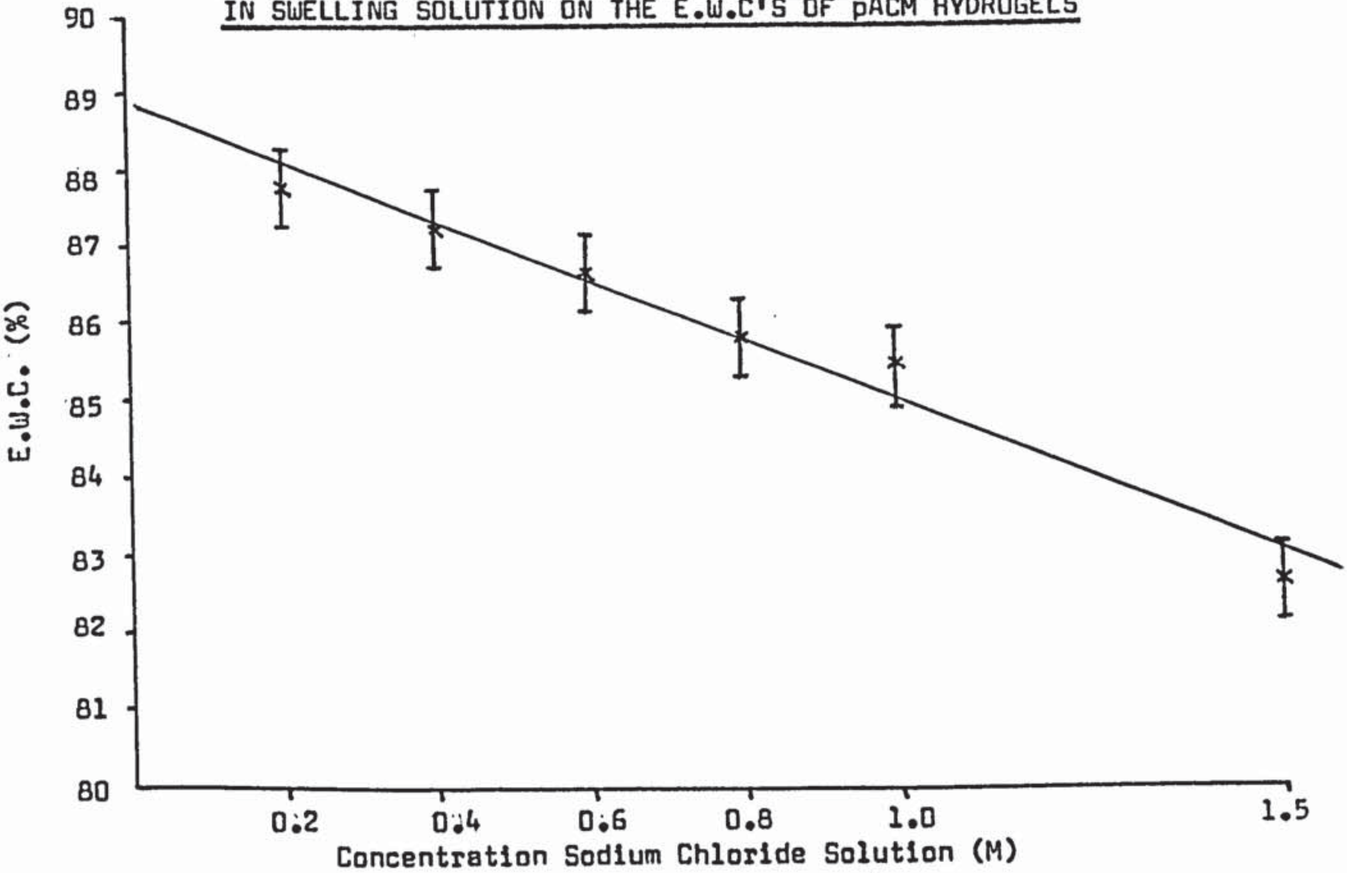
‡ The errors shown are included to show the problems encountered with this particular composition. Whilst the error limits for the E.W.C's were acceptable, the freezing water contents were found to vary between samples and on thermal cycling. These observations were indicative of the type of water structures postulated in Chapter IV.

* All preparations were cross-linked by 1% (by wt) EDMA.

It was apparent from Table III D that pHEMA preparations swollen in 1.5M aqueous Clindamycin solutions had higher E.W.C's than the corresponding Lincomycin preparations and water swollen pHEMA. The E.W.C's of pHEMA preparations swollen in 1.5M sodium chloride solution

FIGURE III (25)

EFFECT OF SODIUM CHLORIDE CONCENTRATION IN SWELLING SOLUTION ON THE E.W.C'S OF pACM HYDROGELS



were surprisingly low considering the fact that the sodium chloride solution was only 8% (by wt) as compared to the 40% (by wt) antibiotic solutions.

Once again, whether or not the deswelling was mainly due to the osmotic influence of the swelling solution or increased absorption of sodium chloride by the matrix cannot yet be ascertained but the value of E.W.C. obtained for pHEMA swollen in 1.5M NaCl (25.7%) was in agreement with that found by Refojo[79] (27.0%).

The E.W.C's found for pHEMA preparations with either Lincomycin or Clindamycin incorporated during polymerisation, were not significantly different from each other. The determination of precise values for the freezing and non-freezing water contents of these preparations proved difficult and a larger number of samples were analysed than was usual in order to gain a better indication of their true values. The values shown in Table III D were considered a fair representation of those values commonly found, the large error limits demonstrating the occasional variation in results that could occur from sample to sample or between sample runs. These difficulties were considered to be indicative of the kind of structural changes and modifications undergone by water in these preparations as shown in differential scanning calorimetry studies presented in Chapter IV.

The considerable change in the freezing/non-freezing water compositions of these preparations was considered significant and was a continuation of the trend first seen for pACM preparations. Whilst the E.W.C's of pHEMA preparations incorporating either Lincomycin or Clindamycin were about equal, the amount of freezing water found in pHEMA preparations incorporating Clindamycin was approximately double that found for the corresponding Lincomycin preparations (a simultaneous reduction in non-freezing water content also being observed).

Table III E shows the results obtained for various HEMA/STY hydrogel preparations. The HEMA/STY preparations studied were of similar type to those already discussed for pACM and pHEMA. Completing this series with HEMA/STY allowed the effects of the antibiotics and sodium chloride on the nature of water in hydrogels to be studied over a wide range of E.W.C's and freezing/non-freezing water ratios.

The results for HEMA/STY preparations swollen in either 0.1M Lincomycin, Clindamycin or sodium chloride showed no significant variations in E.W.C. or freezing/non-freezing water content, the strong water structuring forces of the hydrogel apparently remaining prevalent.

TABLE III E

NATURE OF WATER IN HEMA/STY PREPARATIONS

Composition*	E.W.C. (%)	Freezing Water Content (%)	Non-Freezing Water Content (%)
HEMA/STY swollen in water	23.1	1.0	22.1
HEMA/STY swollen in 0.1M L/M	22.3	0.9	21.4
HEMA/STY swollen in 0.1M C/M	22.7	1.2	21.5
HEMA/STY swollen in 0.1M NaCl	22.4	0.9	21.5
HEMA/STY swollen in 1.5M L/M	29.3	-	-
HEMA/STY swollen in 1.5M C/M	42.3	-	-
HEMA/STY swollen in 1.5M NaCl	16.7	-	-
HEMA/STY inc 10% 1.0M L/M	28.8	1.2	27.6
HEMA/STY inc 10% 1.0M C/M	34.6	5.4	29.2

* All preparationsHEMA/STY (90:10) (mole%) and cross-linked by 1% (by wt) EDMA

When higher solution concentrations (1.5M) of these solutes were used to swell HEMA/STY copolymers the E.W.C. of the preparations swollen in 1.5M sodium chloride solution were lowered as expected. However, HEMA/STY preparations swollen in 1.5M solutions of either Lincomycin or Clindamycin had elevated E.W.C's when compared to water swollen HEMA/STY, the elevation being markedly greater using Clindamycin than for the use of Lincomycin.

The data presented in Table III E for HEMA/STY preparations with antibiotics incorporated during polymerisation also showed higher E.W.C's for antibiotic incorporated preparations than for water swollen preparations. Once again, the Clindamycin incorporated preparations had higher E.W.C's than the corresponding Lincomycin preparations. The freezing/non-freezing water content data for these preparations reinforced the trend already seen in that the freezing water content of the Clindamycin incorporated HEMA/STY preparations was greater than that found for the corresponding Lincomycin preparations. The non-freezing water component of the Clindamycin incorporated preparation was also found to be slightly greater than that found for the Lincomycin incorporated preparation, both of which were higher than water swollen HEMA/STY. This was not surprising since the increased E.W.C's found for these preparations could not reasonably be expected to be confined to an increase in the freezing water fraction in hydrogels where non-freezing water structuring forces predominated.

Whereas HEMA/STY preparations both incorporating antibiotics and those with antibiotics swollen into them showed E.W.C. increases due to Clindamycin over the E.W.C. found with Lincomycin (the HEMA preparations swollen in antibiotics also showing this trend) the effect was not found for HEMA preparations with antibiotics incorporated during polymerisation. However, it would seem that the presence of both antibiotics in hydrogel preparations can stimulate increases in both the E.W.C. and the freezing water content of the hydrogel relative to the water swollen preparation. This was contrary to the deswelling effect seen for solutes such as sodium chloride and suggests that both antibiotics are extremely hydrophilic and possess strong water structuring capabilities.

The results of determinations of E.W.C. and freezing/non-freezing water composition of various MAA/ACM and MAA/ACM/STY preparations are shown in Table III F. These results were possibly the most difficult to interpret but were certainly the most interesting as regards modifications to the nature of water in hydrogels brought about by the antibiotics.

The particular swelling properties of MAA/ACM copolymers cross-linked with EDMA as reported by Middleton[15] and Goulding[111] have been discussed

TABLE III F

NATURE OF WATER IN MAA/ACM AND MAA/ACM/STY PREPARATIONS

Composition*	E.W.C. (%)	Freezing Water Content (%)	Non-Freezing Water Content (%)
MAA/ACM swollen in water	‡37.6 at 2.5 weeks ‡68.9 at 16 weeks	‡36.0 at 10 weeks ‡48.5 at 16 weeks	- 20.4 at 16 weeks
MAA/ACM inc 10% 1.0M L/M	37.5 at 4.5 weeks 37.7 at 16 weeks	1.8 at 7 weeks 2.3 at 16 weeks	35.7 35.4 at 16 weeks
MAA/ACM inc 10% 1.0M C/M	38.8 at 4.5 weeks 39.2 at 16 weeks	6.7 at 7 weeks 7.3 at 16 weeks	32.1 31.9 at 16 weeks
MAA/ACM/STY swollen in water	27.5 at 2.5 weeks 28.0 at 16 weeks	0.1 at 10 weeks	27.4
MAA/ACM/STY inc 10% 1.0M L/M	19.4 at 4.5 weeks 19.6 at 16 weeks	0.0 at 7 weeks	19.4
MAA/ACM/STY inc 10% 1.0M C/M	21.1 at 4.5 weeks 21.2 at 16.0 weeks	o.0 at 7 weeks	21.1

* All preparations cross-linked by 1.0% (by wt) MBACM, and stored at 23°C.

‡ Preparations swollen at 37°C for 2 weeks and at 23°C for the rest of the time.

earlier in this chapter. It had been reported that the E.W.C's of these preparations increased with time up to at least 30 weeks hydration time. This process had been explained as the gradual solvation of interchain hydrogen bonds. However, the pH of the system used by Goulding may well have been of a value at which ionisation of the methacrylic acid units occurred which might also be expected to influence the hydration process. Work carried out during the course of this research suggested no such elevation of E.W.C. with time at room temperature for similar systems cross-linked with MBACM rather than EDMA.

Goulding[111] also reported that the E.W.C's of MAA/ACM preparations increased with increasing initial dilution of the starting mixture with water. This was explained in terms of increased solvation of hydrophilic groups before polymerisation took place and interchain hydrogen bonds could form. These results are shown in Table III G.

TABLE III G

AFFECT OF INITIAL DILUTION ON THE E.W.C. OF MAA/ACM COPOLYMERS[111]

% (by wt) Water in Starting Mixture	E.W.C. (%) at 4 weeks	E.W.C. (%) at 30 weeks
0.0	45.2	64.2
2.0	49.2	69.0
5.0	56.8	72.4
7.5	68.5	84.3
10.0	75.3	86.7

It would seem reasonable to assume that the same process would occur in MAA/ACM/STY preparations but with correspondingly lower values of E.W.C. due to the presence of styrene.

Table III F also showed that the E.W.C's of MAA/ACM/STY preparations both swollen in water and incorporating either Lincomycin or Clindamycin did not vary with hydration time at room temperature over a period of 16 weeks. This was as was found for the MAA/ACM preparations incorporating Lincomycin and Clindamycin. However, the same E.W.C. stability with time was not actually demonstrated for water swollen MAA/ACM copolymers. It was unfortunate that such E.W.C. stability for MAA/ACM could not be confirmed, all stocks of membranes from this batch having been used in the study of the especially interesting permeability properties of this composition (see Chapter VI). The only E.W.C. determinations that could be made after a long hydration time were therefore on membranes that had been soaking at 37^{0}C for about two weeks, the rest of the hydration time being at room temperature (23^{0}C).

The results of the effect of increased hydration temperature on E.W.C. with regard to all the water swollen hydrogels studied will be considered later but the results for the water-swollen MAA/ACM and MAA/ACM/STY preparations cross-linked with MBACM, as shown in Table III J, are also of relevance here.

It was apparent that the MAA/ACM/STY preparations whose E.W.C's were stable with time at 23^{0}C, were not stable at hydration temperatures of 37^{0}C and 45^{0}C where marked E.W.C. increases were found. Since antibiotic incorporated preparations of MAA/ACM and MAA/ACM/STY also did not vary in E.W.C. with time at 23^{0}C it was not unreasonable to assume that the E.W.C. of MAA/ACM swollen in water was also stable with hydration time. This being the case then the E.W.C. of this preparation was also found to substantially increase at elevated hydration temperatures.

All the MBACM cross-linked copolymers used in this study did not seem to vary in E.W.C. with time at 23^{0}C. However, it was suggested in earlier studies that MAA/ACM copolymers cross-linked with EDMA did vary in E.W.C. with time at room temperature. If the earlier studies can be confirmed then this would suggest that the shorter cross-links present in MBACM cross-linked preparations are able to prevent solvation of interchain hydrogen bonds by water which readily occurred in EDMA cross-linked preparations. The "tighter" MBACM cross-linked preparations would also be expected to have lower E.W.C's than their EDMA cross-linked counterparts. This was certainly observed for MAA/ACM/STY preparations when compared to previously determined values on preparations cross-linked with EDMA. The lower E.W.C. of MBACM cross-linked MAA/ACM copolymers compared with their EDMA cross-linked counterparts was not very large but was complicated by the lack of knowledge of the length of hydration time used in the previous work. Previously obtained values are shown in Table III H.

TABLE III H

PREVIOUSLY REPORTED WATER CONTENT VALUES OF MAA/ACM AND MAA/ACM/STY COPOLYMERS

(Hydrated at Room Temperature)

Composition	E.W.C. (%)	Freezing Water Content (%)	Non-Freezing Water Content (%)
pACM, 1% EDMA	85.0[14]	-	-
pHEMA, 0.9% EDMA	36.0[11]	-	-
HEMA/STYRENE (90/10)(mole%)	22.8[11]	-	-
MAA/ACM (50:50)(mole%), 1% EDMA	40.0[13, 14]	12.4[13]	27.2[13]
MAA/ACM/STY (43.75:43.75:12.5)(mole%), 1% EDMA	34.7[16]	0.4[16]	34.3[16]

The increased E.W.C's of these preparations as interchain hydrogen bonding was disrupted at elevated hydration temperatures would be expected to be irreversible. This was shown by the limited data on MAA/ACM swollen in water which spent 2 weeks of its total hydration time at 37°C, the remaining hydration time was at 23°C. The final E.W.C. value was intermediate to that found for samples hydrated for two weeks at 37°C and 45°C. This is further evidence showing the gradual disruption of interchain hydrogen bonding with time and suggests that the E.W.C. values shown for MAA/ACM and MAA/ACM/STY water swollen preparations at 37°C and 45°C in Table III J are fairly arbitary values and not stable or true equilibrium values of water content.

The freezing/non-freezing water composition of MAA/ACM/STY as compared with the previously obtained values (Table III H) showed the E.W.C.'s of the MBACM cross-linked preparations to be lower than the E.W.C.'s of EDMA cross-linked preparations, contrary to previous reports of the effects of cross-linking agents on E.W.C.[15] This might suggest some involvement of MBACM units in interchain hydrogen bonding. However, the lack of data on the freezing/non-freezing water composition of water swollen MAA/ACM preparations used in this study, hydrated only at 23°C does not allow any comparison to previous values to be made. A similar ratio of freezing/non-freezing water in water swollen MAA/ACM cross-linked with MBACM to that found in the EDMA cross-linked preparations[13] would be expected (ie about 30:60, freezing:non-freezing water).

Bearing these observations in mind the results for MAA/ACM and MAA/ACM/STY preparations incorporating the antibiotics are particularly interesting, (Table III F). In both cases the incorporation of either Lincomycin or Clindamycin produced a hydrogel of exceptionally high non-freezing water content. The MAA/ACM preparations did not show any variation in E.W.C. between hydrogels incorporating antibiotic and those swollen in water whereas for MAA/ACM/STY preparations the E.W.C's of antibiotic incorporated preparations were lower than for water swollen preparations. The latter observation was contrary to the trend seen for other hydrogels where antibiotic incorporation increased the E.W.C. relative to water swollen preparations.

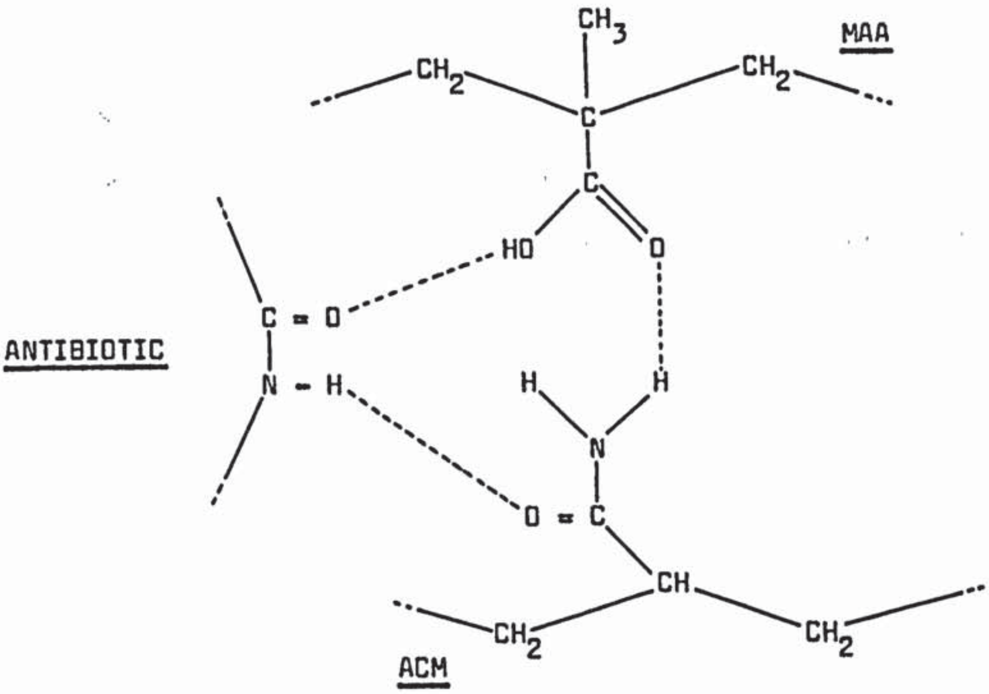
However, the previously observed trend of Clindamycin incorporated preparations having higher freezing water components than the corresponding Lincomycin preparations was maintained for MAA/ACM copolymers but not MAA/ACM/STY preparations. The MAA/ACM/STY preparations were found to have no detectable freezing water when antibiotics were incorporated.

The observation that the E.W.C's of water swollen and antibiotic incorporated MAA/ACM preparations were similar was surprising bearing in mind the work of Goulding[11] on the effect of initial dilution of MAA/ACM preparations on their E.W.C's as shown earlier in Table III G. Since the antibiotic solutions used for incorporation were approximately equivalent to the incorporation of 7% (by wt) water into the starting mixture then this much water alone (no interference from the antibiotic) would be expected to result in an immediate increase in E.W.C. of about 20%. No such increase in E.W.C. was observed. The lack of any increase in E.W.C. due to solvation of hydrophilic groups before interchain hydrogen bonds could form was thus due to the presence of the antibiotic. In effect, the addition of about 3% (by wt) antibiotic with about 7% (by wt) water to the monomer mixture resulted in a hydrogel in which the vast majority of the water was non-freezing as compared to an estimated 60% non-freezing water in a water swollen preparation.

This effect was considered to be the result of hydrogen bonding interactions between antibiotics and the hydrogen bonds utilised in interchain bonding. There are many possible types of arrangement. Figure III (26) shows a "trimer" arrangement of hydrogen bonded species. The hydrogen bond between MAA and ACM would be of the type already postulated (Figure III (17)), but would now be supplemented by an additional stabilising bridge via the antibiotic. This allows the previously shielded carbonyl group of ACM to participate in interchain hydrogen bonding via the antibiotic. The strength of the hydrogen bonding capability of the N-H and C=O groups of the antibiotics would make this type of interaction quite feasible.

FIGURE III (26)

HYDROGEN BONDED "TRIMER" IN ANTIBIOTIC INCORPORATED MAA/ACM COPOLYMERS



The drawback with the arrangement shown in Figure III (26) was that the preparations contained mainly non-freezing water but in the "trimer" a lot of hydrophilicity has been "tied up" in intermolecular bonding and so presumably the amount of "bound" water would not be particularly large. This problem was overcome by envisaging the antibiotic's position as in the centre of the matrix "pore", interacting with the monomeric units via water bridges. This arrangement would allow stabilisation of the matrix and a high level of non-freezing water. It could also be envisaged that this arrangement would lead to a propagated water structure throughout the matrix, in a manner described by Pauling[40] resulting in the high level of non-freezing water found. Alternatively, the "trimer" arrangement might be incomplete leaving hydrogen bonding groups free to structure water.

The "seeding" of a propagated water structure was favoured as a model since such a small amount of antibiotic on its own would be incapable of "binding" the amount of water found to be non-freezing in these preparations.

Both antibiotics would participate in the "trimer" in the same manner but the orientation of the remainder of the antibiotic molecule could be different for each antibiotic due to the influence of the Cl/OH group changes. This would then allow Clindamycin incorporated preparations to demonstrate the slightly higher freezing water components than were found for Lincomycin preparations throughout these studies.

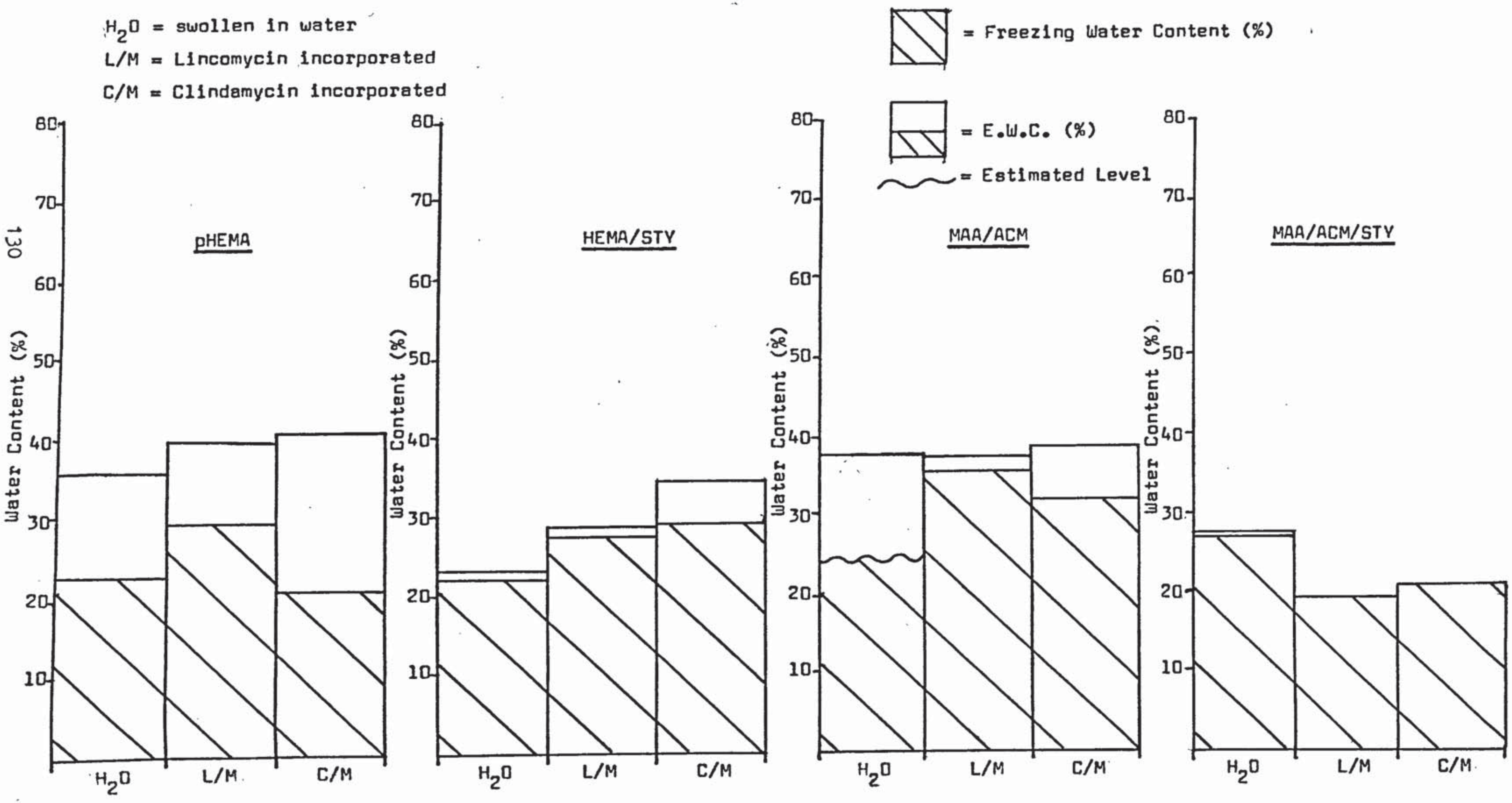
The effect of antibiotic incorporation into MAA/ACM/STY preparations was a reduction in E.W.C. of approximately the same extent for each antibiotic. As was previously mentioned, this was contrary to the previous trend. It was evident that the presence of styrene modifies the interaction model for MAA/ACM/ANTIBIOTIC outlined above. If the effect of styrene was to disrupt the "trimer" or similar arrangement then assuming that the MAA/ACM interaction remained, the effect of the presence of antibiotic in the matrix would surely be to increase the E.W.C. in the manner seen earlier for preparations swollen in antibiotic solutions. Disruption of the "trimer" due to styrene was thus considered unlikely. It was possible that the styrene units would be orientated in a manner so that there was no disruption of the antibiotic/matrix interactions but that the E.W.C. was lowered by hydrophobicity of the styrene groups. The majority of the forces in the matrix would still result in an almost totally non-freezing water network but the hydrophobicity of styrene prevented the accumulation of any freezing water. The lowering of E.W.C. due to the presence of antibiotic as compared to the water swollen preparation would in part be because of the restriction of the matrix water content to "bound" water only and also due to displacement of water from the matrix due to the physical presence of the antibiotics. Another possible cause might be the reduction in hydrophilic hydration due to hydrophobic bonding between styrene and the relatively hydrophobic parts of the antibiotics.

In conclusion, despite the problems in assessing the water content of MAA/ACM and MAA/ACM/STY preparations, these compositions with antibiotic incorporated during polymerisation stand out as preparations in which a drastic change in the nature of water present has been brought about by the presence of fairly low levels of antibiotic.

Throughout all the preparations studied, the antibiotics were seen to affect both the E.W.C. and freezing water content in a trend consistent with which antibiotic was present. The data is summarised in Figure III (27). Although it can be seen that the greatest influences of the antibiotics were seen for the lower E.W.C. compositions, there was no clearly discernible trend. This was mainly the result of the interactions postulated to be occurring in the MAA/ACM and MAA/ACM/STY preparations. The overall conclusion being that the extent of water structuring influences of Lincomycin and Clindamycin were affected by both the chemical nature of their environment and also its freezing/non-freezing water composition.

FIGURE III (27)

VARTIATION IN E.W.C. AND FREEZING WATER CONTENT OF A RANGE OF HYDROGEL PREPARATIONS SWOLLEN IN WATER OR WITH LINCOMYCIN OR CLINDAMYCIN INCORPORATED AND HYDRATED AT 23°C



III.5.ii Density Determinations of Various Hydrogel Preparations

The densities of samples were determined using the method already described in Chapter II. Before discussing the results obtained some of the problems encountered during the determination of the density of samples will be discussed.

In a density gradient column the density of the liquid mixture increases from top to bottom. It was usually found that the calibration of columns showed that their density was not linear with height over the whole range of densities used.

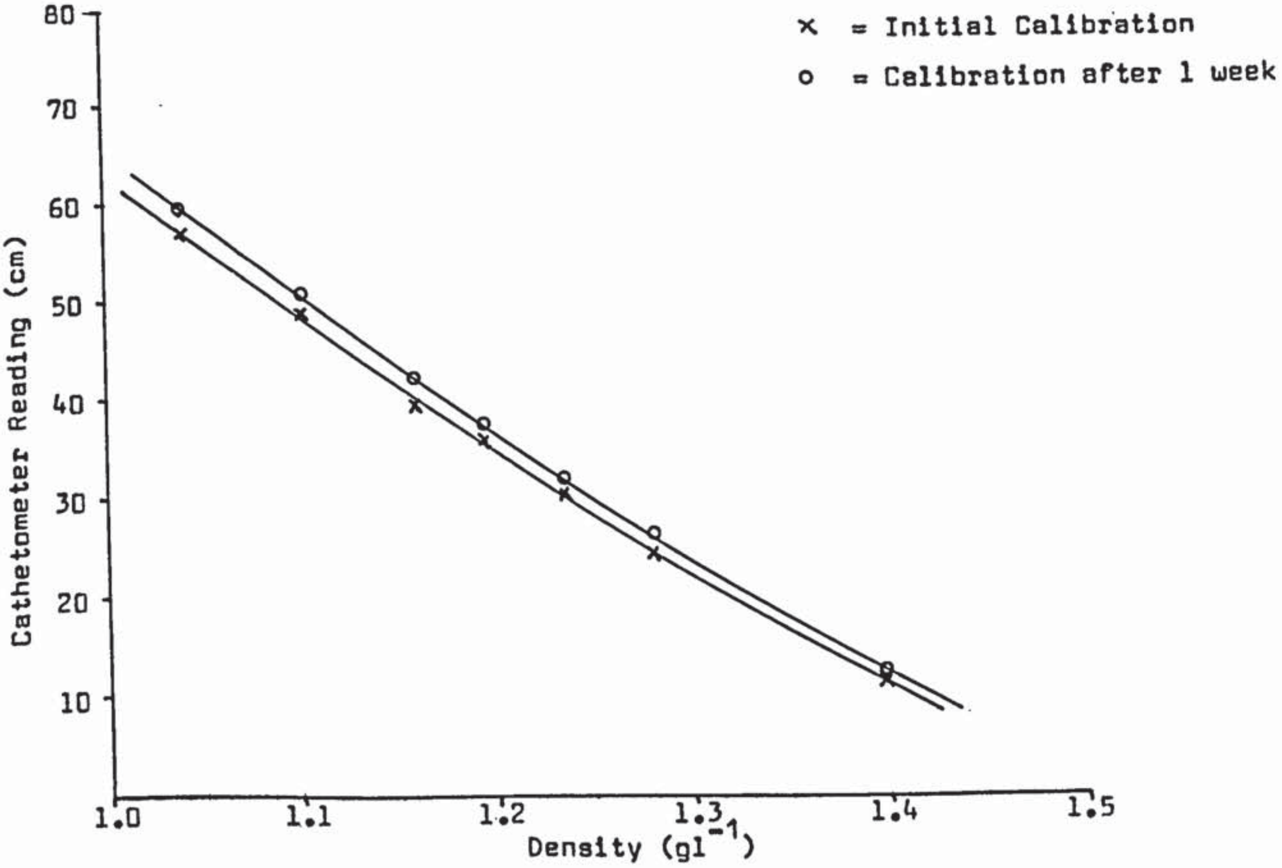
An example of this non-linearity is shown in Figure III (28) where column density was plotted against marker float height.

The non-linearity was mainly due to difficulties in controlling the rate of mixing of the liquids and the subsequent flow rate into the column. The repeated insertion and removal of the sweep basket into and out of the column was also found to alter the calibration as well as general ageing of the column as one part of the liquid column diffused into another and as impurities in test samples leached into the column liquid. Figure III (28) also shows the calibration of the column after one week's use. The column was always calibrated before any density measurements were made and if a significant change in the calibration was detected a new column was prepared.

The density of a preparation was determined by taking the cathetometer height reading of the sample in the column at equilibrium and reading off the corresponding density from the calibration curve. The average density of a number of samples being taken as the density of that preparation. In some cases a final equilibrium was not observed, the samples reaching a level at which they did not visibly appear to be moving after which they gradually changed position. The sample readings were taken at the point of visible equilibrium since samples could then be observed to either slowly increase or decrease in height with time after they had initially "settled". The changes in height were due to the uptake of either the "lighter" or "heavier" liquid from the column. Taking sample readings at the initial settling point removed most of the swelling effects from the measurements but small errors would have been unavoidable.

FIGURE III (28)

EXAMPLE OF DENSITY GRADIENT COLUMN CALIBRATION CURVE



Other errors in the density measurements could arise from the presence of small air bubbles attached to the sample surface during measurement which would lead to a lower apparent density than the true value. The

TABLE III I

DENSITIES IN HYDRATED AND DEHYDRATED STATES, EXPERIMENTAL E.W.C'S AND CALCULATED E.W.C'S FOR SAMPLES HYDRATED AT 23°C

Composition	Density (Hydrated) (gcm^{-3})	Density (Dehydrated) (gcm^{-3})	E.W.C. (Calculated) (%)	E.W.C. (Experimental) (%)
Samples Swollen in Water				
pACM	1.006	1.332	96.8	89.3
pHEMA	1.189	1.284	27.9	35.5
HEMA/STY	1.215	1.260	14.1	23.1
MAA/ACM	1.209 at 11 weeks	1.315	27.6	37.6 at 2.5 weeks
MAA/ACM/STY	1.237 at 11 weeks	1.260	7.1	27.5 at 2.5 weeks
Samples Incorporating 10% 1.0M Lincomycin				
pHEMA	1.176	1.270	29.3	39.4
HEMA/STY	1.205	1.270	19.8	28.8
MAA/ACM	1.212 at 8 weeks	1.299	23.8	37.5 at 4.5 weeks
MAA/ACM/STY	1.256 at 8 weeks	1.273	4.9	19.4 at 4.5 weeks
Samples Incorporating 10% 1.0M Clindamycin				
pHEMA	1.180	1.270	28.0	40.8
HEMA/STY	1.184	1.274	27.5	34.6
MAA/ACM	1.201 at 8 weeks	1.297	26.7	38.8 at 4.5 weeks
MAA/ACM/STY	1.250 at 8 weeks	1.273	6.7	21.1 at 4.5 weeks

presence of any residual moisture in the sample due to incomplete dehydration would also result in a lower apparent density. It was considered that these errors were minimal since the values of sample densities obtained were in good agreement with previously reported values (see later).

The results of the density determinations on a number of preparations hydrated at 23°C are shown in Table III I and values for samples hydrated at various temperatures for two weeks are shown in Table III K. Tables III I and III K also summarise the calculated or theoretical E.W.C's determined from the density values in the dehydrated and hydrated states according to the method described in Chapter II.

The results shown in these tables for MAA/ACM and MAA/ACM/STY may be subject to the limitations discussed previously regarding their hydration properties. This apart, many interesting observations could be made from the data. Data in Table III I can be compared with that previously reported. Ng[11] found dehydrated and hydrated density values for pHEMA of 1.279 and 1.169 respectively. Other workers determined the dehydrated value as 1.286[126] and hydrated value as 1.170[127]. The corresponding values of density for dehydrated and hydrated pHEMA obtained during this study were 1.284 and 1.189. As can be seen the agreement with previously reported dehydrated values was good but there was a slight difference in the hydrated density values. The experimentally determined E.W.C. of pHEMA was also slightly lower than that previously reported. This was adequately explained by the fact that whereas 1% (by wt) of cross-linking agent had been used in these studies, 1 mole% of cross-linking agent had been used in the previous study. This would lead to differences in the extent of cross-linking of the preparations.

The previously reported density values of HEMA/STY (90:10) (mole%) in dehydrated and hydrated states[11] of 1.259 and 1.204 respectively compared favourably with the corresponding values of 1.260 and 1.215 determined during this study.

The results presented in Table III I showed that the densities of the various antibiotic incorporated hydrogel preparations in both dehydrated and hydrated states showed little difference depending on whether Lincomycin or Clindamycin was used. The only exception was for HEMA/STY preparations where preparations incorporating Lincomycin were less dense than the corresponding Clindamycin preparations. This factor accounted for the difference between the theoretical (calculated) E.W.C's of Clindamycin and Lincomycin incorporated HEMA/STY preparations.

Since the theoretical E.W.C's were all calculated in the same manner, a comparison of these values provides a convenient method of appraisal of the density data.

The theoretical E.W.C. values for pHEMA preparations swollen in water or with Lincomycin or Clindamycin incorporated were approximately the same. The actual E.W.C. values of these preparations were on average about 10% greater than the theoretical values, although throughout this study the general trend in hydration was similar to that seen previously, in that antibiotic incorporated preparations generally had higher water contents than the water swollen preparations.

The data for HEMA/STY preparations either swollen in water or with Lincomycin or Clindamycin incorporated showed that the extent of hydration increased in the manner water swollen < Lincomycin incorporated < Clindamycin incorporated, a trend also seen with the experimental E.W.C. values. Once more there was approximately a 7% difference between experimental and calculated E.W.C. values (calculated values being lowest). Such differences between experimental and calculated E.W.C. values were also reported by Ng[11] who attributed them to small errors in density measurements which markedly affected the theoretical (calculated) E.W.C. values.

The previously described experimental E.W.C. trends for MAA/ACM/STY were also found for calculated E.W.C. values, where the increasing hydration was of the order Lincomycin incorporated $<$ Clindamycin incorporated $\ll$ water swollen. Although the experimental E.W.C. values found for MAA/ACM preparations were broadly similar the calculated values for Lincomycin incorporated MAA/ACM were slightly lower than the values for the corresponding Clindamycin incorporated and water swollen preparations. The general trend of Clindamycin incorporated preparations having higher hydration levels than the corresponding Lincomycin incorporated preparations was consistent with the previously reported trends in freezing water contents of these preparations.

III.5.iii <u>Variation of E.W.C. with Hydration Temperature</u>

The effect of sample hydration at a number of temperatures on E.W.C. was studied. Samples of membranes that had previously been hydrated at 23°C were placed in freshly distilled water and incubated at either 37°C or 45°C for a further two weeks, after which time the water contents of the samples were determined. The results of these studies are shown in Table III J and plotted in Figure III (29).

It was apparent from the results shown in Table III J that the hydration temperatures studied had very little effect on the E.W.C. of the HEMA/STY preparations used. pACM preparations showed a slight increase in E.W.C. as the hydration temperature increased. This was probably due to

TABLE III J

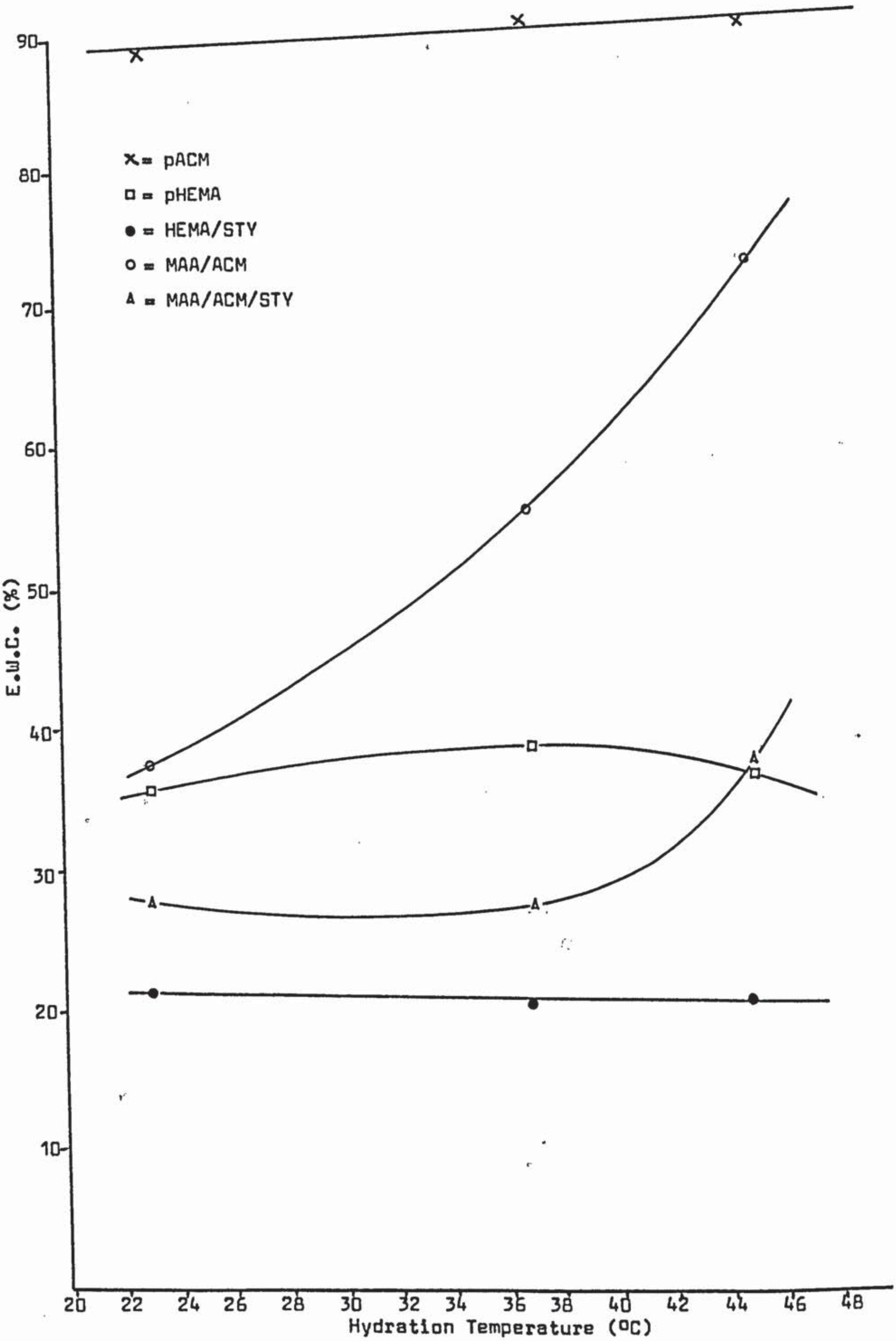
EFFECT OF HYDRATION TEMPERATURE ON THE E.W.C'S OF WATER SWOLLEN HYDROGEL PREPARATIONS

Composition	E.W.C. (%)
$23^{o}C$ Hydration	
pACM	89.3
pHEMA	35.5
HEMA/STY	23.1
MAA/ACM at 2.5 weeks	37.6
MAA/ACM/STY at 2.5 weeks	27.5
$37^{o}C$ Hydration	
pACM	91.5
pHEMA	38.4
HEMA/STY	20.2
MAA/ACM at 4 weeks	55.5
MAA/ACM/STY at 4 weeks	27.2
$45^{o}C$ Hydration	
pACM	91.3
pHEMA	37.0
HEMA/STY	20.7
MAA/ACM at 4 weeks	73.5
MAA/ACM/STY at 4 weeks	37.5

Where times of hydration are indicated this includes prior hydration at $23^{o}C$ plus two week's hydration at the relevant temperature.

FIGURE III (29)

VARIATION OF E.W.C. WITH HYDRATION TEMPERATURE FOR VARIOUS WATER SWOLLEN HYDROGELS



increased hydrophilic hydration and polymer network expansion as discussed earlier.

The results of the study on pHEMA hydration seemed to suggest a slight maximum in E.W.C. at about 37°C for the hydration temperature range studied. This was considered indicative of the previously reported minimum in the E.W.C. of this preparation at a hydration temperature of 60°C.[78] The nature of the competing forces which govern such changes in E.W.C. with hydration temperature have also been discussed earlier.

The results of the hydration of MAA/ACM and MAA/ACM/STY preparations at elevated temperature have already been discussed. It appeared that increasing hydration temperature provided enough energy to allow the disruption of interchain hydrogen bonds and the solvation of the hydrophilic groups. This led to the observed dramatic increase in hydration of these preparations as temperature increased. If future experiments confirm the stability of the E.W.C. at 23°C of water swollen MAA/ACM copolymers with time as has been found for water swollen MAA/ACM/STY, then it would appear that using MBACM to cross-link these preparations confers stability of E.W.C. at room temperature not found for preparations cross-linked with EDMA. It would also appear that increased hydration temperature supplies sufficient energy to overcome such stabilisation and so an increase in E.W.C. results.

It can be postulated that the E.W.C's of MAA/ACM and MAA/ACM/STY will eventually reach a maximum value when all interchain hydrogen bonds have been solvated. Only this point can be considered as the true equilibrium water content. The E.W.C. data for these preparations shown in Table III J at 37°C and 45°C hydration temperatures must therefore be considered as transient water content values of networks in which only a proportion of interchain hydrogen bonds were solvated.

III.5.iv Variation of Sample Densities and Theoretical (Calculated) E.W.C's with Hydration Temperature

Samples of preparations hydrated at elevated temperatures used in the above study were also used for the determination of their densities in both the dehydrated and hydrated states. The theoretical (calculated) E.W.C's of the preparations were also determined according to the method already described. The data are presented in Table III K and are also plotted in Figure III (30).

The calculated values of E.W.C. were found to be lower than the actual values of E.W.C. as had been noted earlier. However, the general trends for the variation of the extents of hydration with temperature were very similar to those seen earlier for the variation of experimentally determined (actual) E.W.C's with hydration temperature.

The only exception was the higher theoretical E.W.C. of pACM at 23°C than was experimentally determined, the theoretical values at hydration temperatures of 37°C and 45°C behaved in the manner seen previously for their actual E.W.C's at these hydration temperatures.

Table III K also shows that the dehydrated densities of pACM, pHEMA and HEMA/STYRENE also decreased as hydration temperature increased from 23°C to 37°C but no further change was observed between 37°C and 45°C. The MAA/ACM and MAA/ACM/STY preparations showed a continuous but very slight increase in density as hydration temperature increased from 23°C to 45°C although this was not considered significant. The changes in hydrated density of all these preparations were consistent with the changes in theoretical E.W.C. shown in Figure III (30). These observations would suggest that for pACM, pHEMA and HEMA/STY preparations the expansion of the network increases up to 37°C and then levels off since

TABLE III K

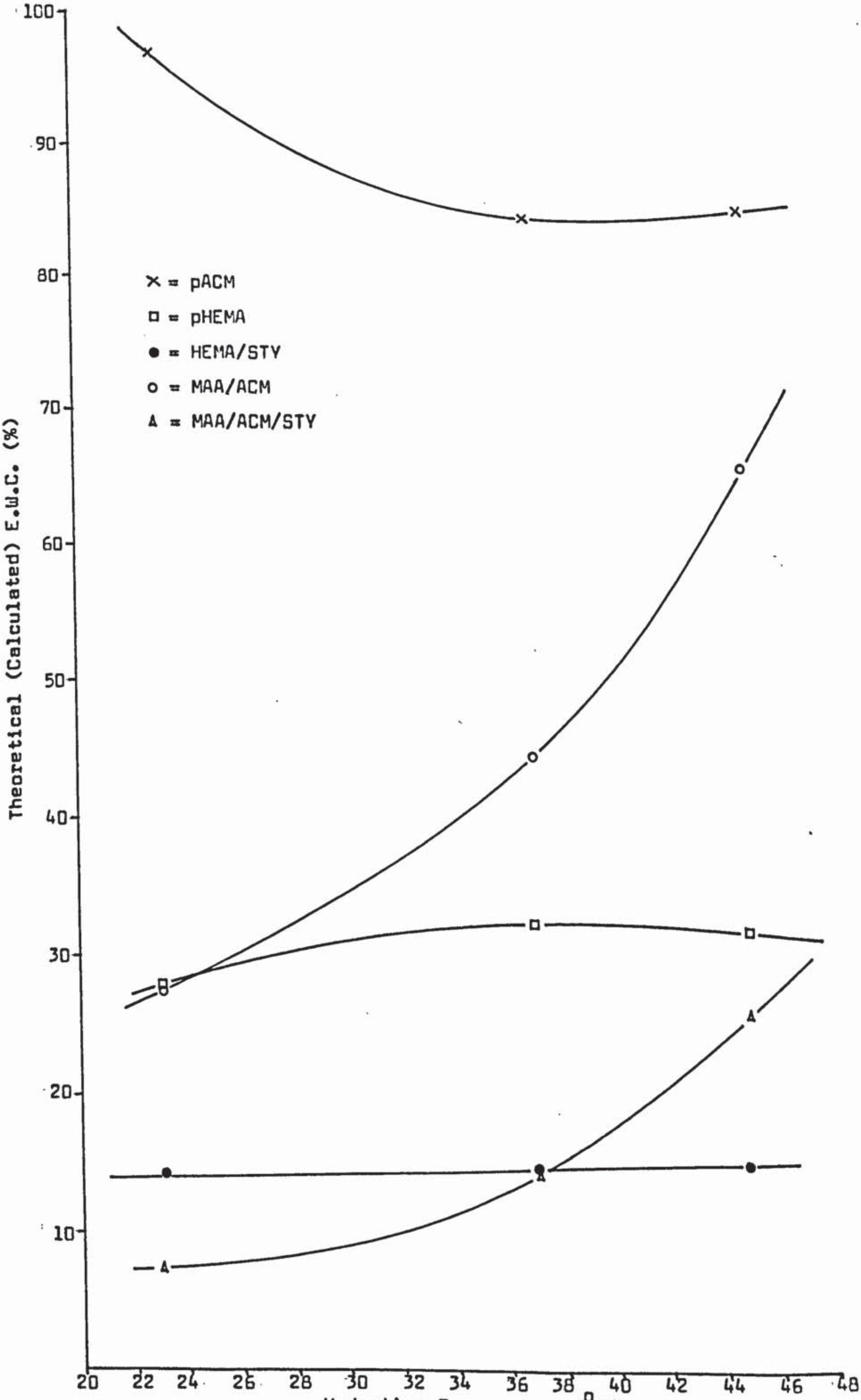
DENSITIES IN HYDRATED AND DEHYDRATED STATES, EXPERIMENTAL E.W.C'S AND CALCULATED E.W.C'S FOR SAMPLES HYDRATED AT 23°C, 37°C and 45°C (TWO WEEKS HYDRATION AT 37°C AND 45°C) FOR WATER SWOLLEN HYDROGELS

Composition	Density (Hydrated) (gcm^{-3})	Density (Dehydrated) (gcm^{-3})	E.W.C. (Calculated) (%)	E.W.C. (Experimental) (%)
23°C Hydration				
pACM	1.006	1.332	96.8	89.3
pHEMA	1.189	1.284	27.9	35.5
HEMA/STY	1.215	1.260	14.1	23.1
MAA/ACM	1.209 at 11 weeks	1.315	27.6	37.6 at 2.5 weeks
MAA/ACM/STY	1.237 at 11 weeks	1.260	7.1	27.5 at 2.5 weeks
37°C Hydration				
pACM	1.038	1.320	84.2	91.5
pHEMA	1.170	1.275	32.3	38.4
HEMA/STY	1.208	1.251	14.0	20.2
MAA/ACM	1.155 at 4 weeks	1.321	44.4	55.5 at 4 weeks
MAA/ACM/STY	1.218 at 4 weeks	1.263	13.9	27.2 at 4 weeks
45°C Hydration				
pACM	1.037	1.321	84.6	91.3
pHEMA	1.173	1.276	31.5	37.0
HEMA/STY	1.206	1.251	14.7	20.7
MAA/ACM	1.091 at 4 weeks	1.323	65.3	73.5 at 4 weeks
MAA/ACM/STY	1.184 at 4 weeks	1.265	25.6	37.5 at 4 weeks

Where time of hydration is indicated this includes prior hydration at 23°C plus two weeks hydration at the relevant temperature.

FIGURE III (30)

VARIATION OF THEORETICAL (CALCULATED) E.W.C. WITH HYDRATION TEMPERATURE FOR VARIOUS WATER SWOLLEN HYDROGELS



the lower densities found reflected a more open matrix. This apparent tailing off of network expansion might be considered as the breakdown of supplementary hydrophobic cross-links of the type postulated by Refojo[79] although the behaviour of the pACM preparations could not be accounted for in such a way.

It is appropriate to mention here that in all the preparations synthesised so as to incorporate antibiotics during polymerisation crystallisation of the antibiotics "locked" into the matrix could be observed when the preparations were dehydrated. This was taken as evidence of high levels of the antibiotics being trapped within the matrices of these hydrogels.

The studies in this chapter have demonstrated the existence of larger and possibly more stable hydration shells associated with Clindamycin when incorporated into hydrogels than with Lincomycin in similar preparations. The two antibiotics were able to alter the freezing/non-freezing water ratio of hydrogel preparations. It also appeared that the extent and nature of the effects of the drugs on the water structure in the hydrogels was dependant on the type of water environment to be found in the water swollen hydrogels.

The next logical step was to study more of the nature of the differences in these structuring effects, and such work has been presented in the following chapter. However, bearing in mind the objectives of the research undertaken and the reported differences in the irritancy of the antibiotics, it was also important to study the ways in which the water structuring effects of the antibiotics influenced certain membrane transport processes. The results of permeability studies on hydrogel membrane systems have been presented in Chapters V and VI.

CHAPTER IV

DIFFERENTIAL SCANNING CALORIMETRY (D.S.C) STUDIES

IV.1 Introduction

Differential Scanning Calorimetry (D.S.C.) was used to quantitatively determine the amounts of "freezing" and "non-freezing" water in a variety of sample types and compositions during the course of this research. A full account of sample preparation and analysis by D.S.C. has already been presented in Chapter II.

Differential scanning calorimetry also provides information concerning the kind of thermal transitions taking place in samples as seen by the structure of the peak or peaks observed on the D.S.C. trace.

The results of freezing and non-freezing water content determinations of samples using D.S.C. have already been presented in Chapter III, where equilibrium water content data and the results of other characterisation studies were also discussed.

This chapter will largely be confined to the discussion of the nature of the D.S.C. traces themselves in relation to sample type and composition, thermal transitions observed and thermal history of the sample.

IV.2 Applications of Differential Scanning Calorimetry

Thermal analysis techniques, and differential scanning calorimetry in particular, have been used to study a variety of systems in recent years.

Early calorimetric investigations on the nature of water in wool can be found in the literature[128,129] and studies of water in cellulosic materials[130,131] Collagen[132], Bovine Serum Albumin[133] and Deoxyribonucleic acid (D.N.A.),[134] are also well documented.

The use of D.S.C. in various modes has enabled studies of the freezing/melting behaviour of salt solutions[135] in porous materials, hydration forces in phospholipid multilamellar systems,[136,137] aqueous polymer solutions and gels[138,139,140] and the state of water in microbial cells[141] to be carried out.

Accounts of D.S.C. studies of phase behaviour of aqueous poly(alkylene oxide) solutions,[142] calorimetric investigations of the non-equilibrium freezing behaviour of aqueous systems[37] and studies of the movement of water in hydrogels using D.S.C,[85] are also to be found.

More diverse studies, although not necessarily concerned with water structure, which employed D.S.C. include the study of the interaction of synthetic polymers with cell membranes and model membrane systems,[143] studies of the thermal transitions of the water-splitting complex of Photosystem II of chloroplast membranes,[144] low density lipoproteins and lipids[145] and human erythrocyte ghost proteins.[146] These examples are mentioned not only because they demonstrate the wide-reaching versatility of D.S.C. but also because certain aspects of the thermograms obtained were comparable with observations reported in this chapter.

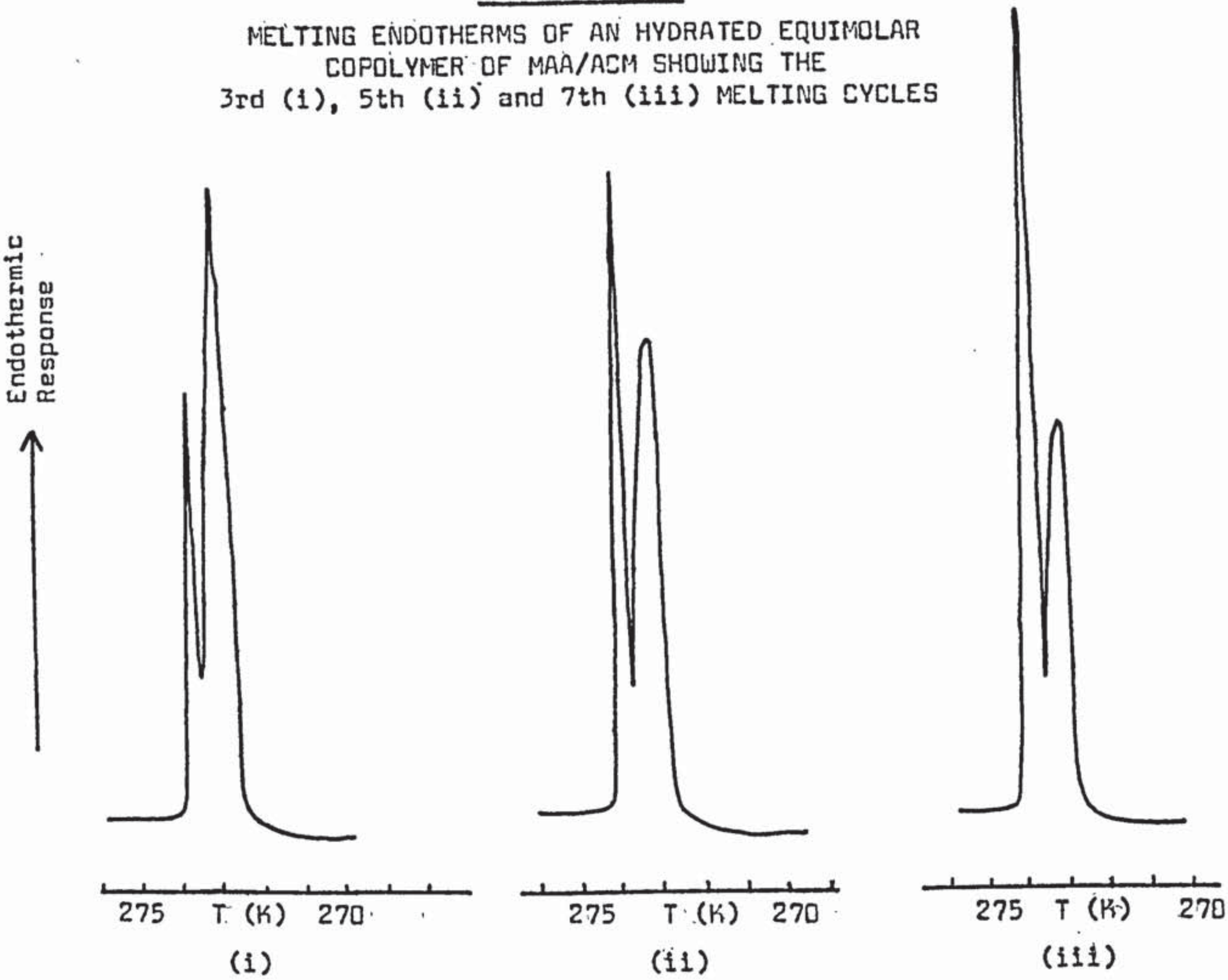
IV.3 Recent D.S.C. Studies on Hydrogel Systems

IV.3.1 The Melting of Water in Hydrogels

In recent years, during extensive studies on the water binding properties of hydrogels, workers within this research group have recorded observations of a "fine structure" to D.S.C. traces obtained.[10,16] In such instances the thermograms did not show the simple melting behaviour exhibited by pure water but were more complicated. Figures IV (1) (i), (ii) and (iii) illustrate this behaviour as seen in the melting endotherm of an

FIGURE IV (1)

MELTING ENDOTHERMS OF AN HYDRATED EQUIMOLAR COPOLYMER OF MAA/ACM SHOWING THE 3rd (i), 5th (ii) and 7th (iii) MELTING CYCLES



equimolar copolymer of acrylamide and methacrylic acid. Figure IV (1) shows how the two peaks found at 273K and 274K changed in size on temperature cycling between 293K and 233K. The 274K peak was seen to increase in size on temperature cycling with a simultaneous decrease in the size of the 273K peak. Figures IV (1) (i), (ii) and (iii) show these changes at the 3rd, 5th and 7th cycles respectively. Given the presence of water pores in hydrogels[121,115,147] the effects of temperature cycling were visualised in terms of "increased perfection of crystallisation"[10] of the water in the network. The area under the 274K peak was assigned as the melting of "pure" water in the system (bulk-like water) since it recrystallised and melted in a manner indistinguishable from that of pure water. The mechanical stressing of the network due to expansion and the incomplete randomisation of the crystalline structures on raising the temperature to 293K were implicated in the process.

IV.3.ii The Crystallisation of Water in Hydrogels

The freezing exotherm obtained for the same equimolar copolymer of acrylamide and methacrylic acid discussed above, was also of considerable interest. This can be seen in Figure IV (2). The freezing exotherm consisted of a sharp peak and a broader more complex peak well separated from each other. The area under the sharp peak of a sample not previously temperature cycled and the area under the 274K peak of the melting endotherm (Figure IV (1)) showed good correlation, the sharp peak of the freezing exotherm then being assigned as the freezing of water of the same type (ie bulk-like) as found under the 274K melting endotherm peak. The broader more complex peak was considered indicative of a multiple nucleation process.

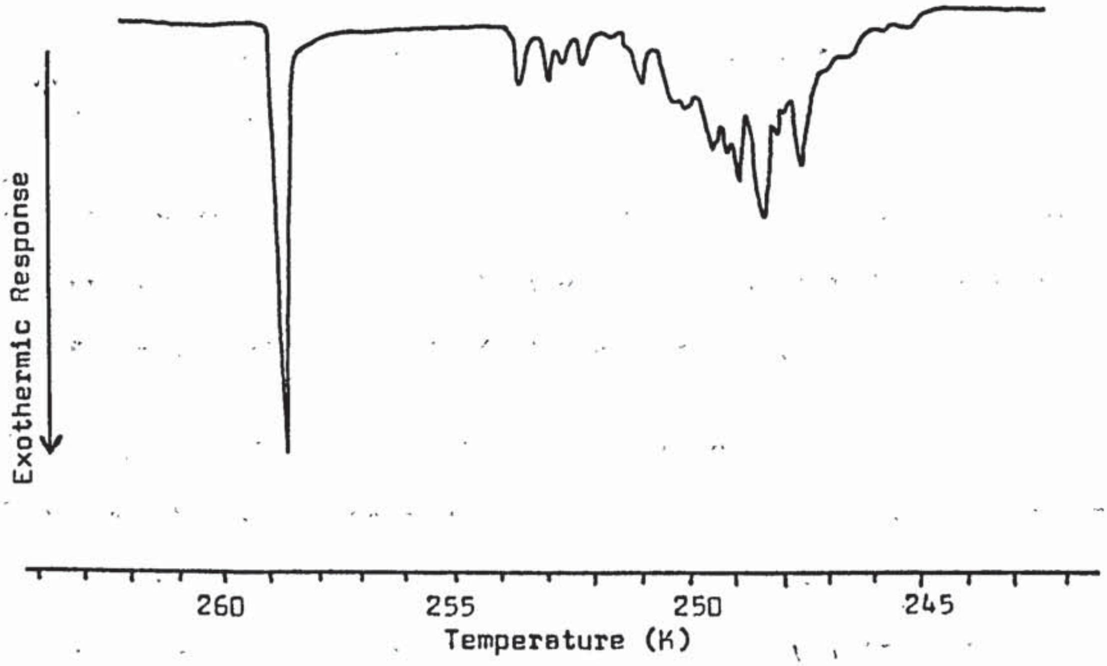
IV.3.iii The Development of a Continuum Model for Water in Hydrogels

From the observations cited so far in this chapter it was apparent that there was a difference between the various descriptions of water states (see Chapter I) and the experimental results of members of this research group. The theoretical concepts of the water states described in Chapter I did not necessarily correspond with the results of any particular experimental technique and so any distinction made between "free" and "bound" water using different techniques would be affected by the presence of intermediate states of water.

These considerations led to the development of the concept of a continuum of water states between water hydrogen bonded to functional groups of the

FIGURE IV (2)

FREEZING EXOTHERM OF AN HYDRATED EQUIMOLAR COPOLYMER OF METHACRYLIC ACID AND ACRYLAMIDE



polymer and water relatively unaffected by its polymeric environment. The crystallisation of such a continuum of water states would be affected by the polymeric environment and would therefore take place after the crystallisation of "pure" water in the system. Such a concept agreed well with the experimental observations of this research group.

IV.4 Preliminary D.S.C. Studies

IV.4.1 Introduction

Differential scanning calorimetry has been used to study not only the behaviour of water in hydrogels but also the effects of the antibiotics under investigation on water structure in solution and in hydrogels. The results of these studies will now be presented.

IV.4.ii D.S.C. Studies of the Melting Behaviour of Low Concentration Aqueous Antibiotic Solutions

Samples of the aqueous antibiotic solutions were introduced into aluminium sample pans using a Hamilton Microlitre Syringe. The sample pans were then hermetically sealed and treated in the manner previously described in Chapter II.

The melting endotherms obtained for 0.1M aqueous solutions of clindamycin hydrochloride, lincomycin hydrochloride and clindamycin phosphate can be seen in Figures IV (3) (i), (ii) (iii) respectively.

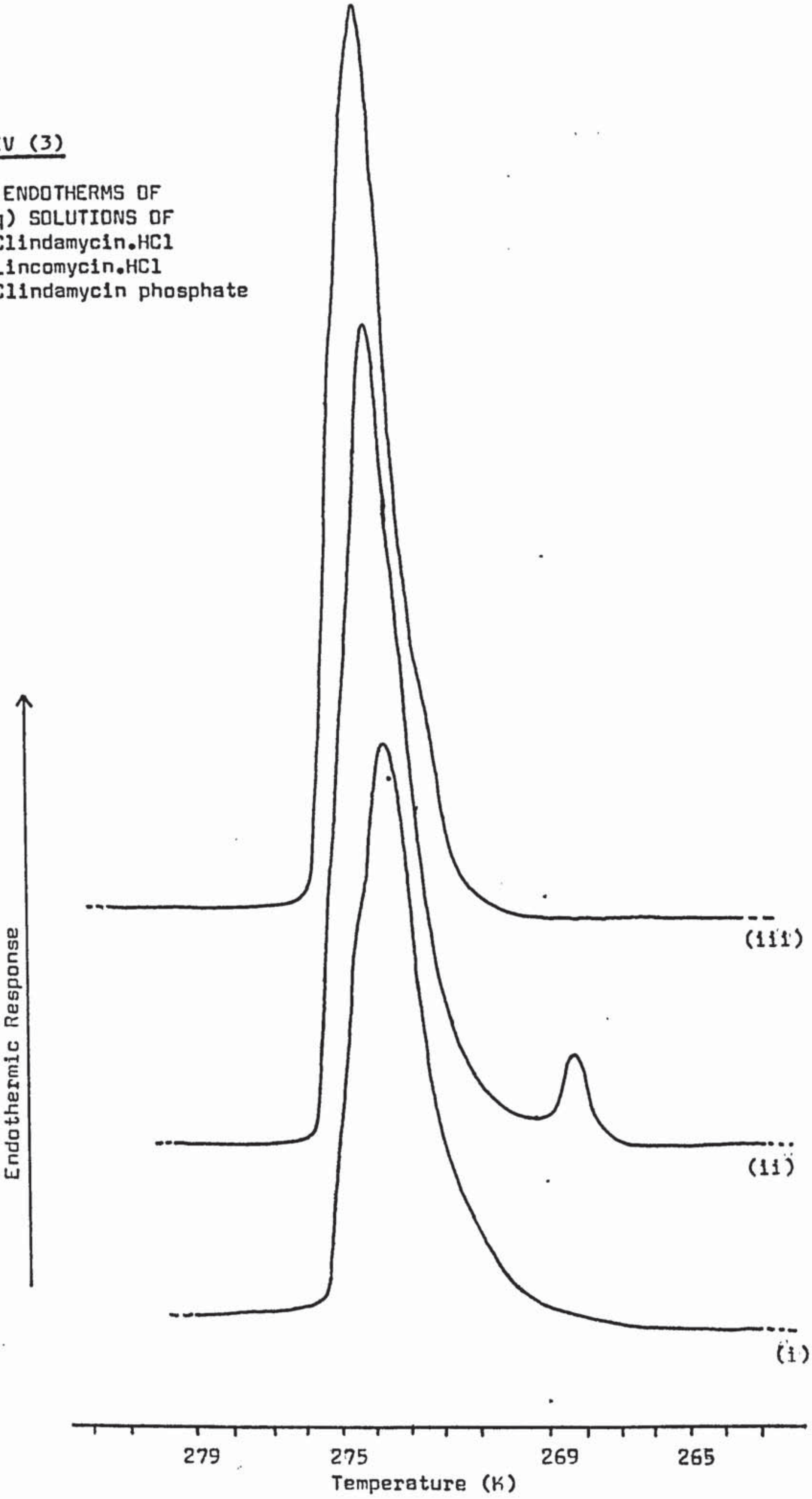
Aqueous solutions of lincomycin hydrochloride underwent an additional small thermal transition prior to the main endothermic response that was not observed for the other antibiotic solutions. The main peak in all these thermograms occurred at about 274K and was correlated with the peak position for the melting of a pure water sample.

The "preliminary peak" found for Lincomycin solutions at 269K was considered as water in some way influenced or "restricted" in its movement by the presence of the antibiotic but not bound to the molecule. Water influenced in such a way would freeze "reluctantly" and at a lower temperature than pure water. The crystalline structure of frozen restricted water would not adopt as perfect an order of crystallisation as the pure water component and as a direct consequence of this the subsequent heating of the sample would show the melting of the restricted water component before the pure water component as the less stable poorly ordered crystalline regions of the water melted first.

Once frozen and subsequently remelted the restricted water component could be considered to behave in two different ways. At a point just after its melting transition, but before the onset of the melting of the

FIGURE IV (3)

MELTING ENDOTHERMS OF
0.1M (aq) SOLUTIONS OF
(i) Clindamycin.HCl
(ii) Lincomycin.HCl
(iii) Clindamycin phosphate



pure water peak, it was possible that the restricted water would immediately re-freeze since it was below 273K, only to remelt with the pure water component at a higher temperature.

Alternatively, once "destabilised" by melting, the restricted water component could be considered as remaining in this more fluid form but in close proximity to the Lincomycin molecule which re-exerted its structuring influence on this water fraction and prevented it from immediately re-freezing.

There are two pieces of evidence which support the latter hypothesis. First, if the restricted water component was to melt and then immediately recrystallise then there would be an associated exothermic response associated with the re-freezing process of the same magnitude as the endothermic response due to melting of the restricted water fraction. This would be of the general nature reported for the cold-crystallisation behaviour at initial stages in the recrystallisation of amorphous cellulose with water,[148] pACM/water and Guar/water systems.[139] No such behaviour was demonstrated at this stage in the studies.

The second piece of evidence was based on quantitative data of the type presented in Chapter III. Assuming that the total area under the thermogram (both transitions) accounted for 100% of the water present (ie no bound water) then the melting of the restricted water fraction, its immediate refreezing and subsequent re-melting with the "pure" water fraction would result in this water fraction making twice the contribution to the area of the thermogram than would be expected. This would mean that the calculated amount of freezing (and hence melting) water in the sample would exceed 100%. The situation was complicated by the existence of a small bound water fraction for Lincomycin in solution

(Clindamycin showed a larger bound water fraction - see later). The results did not provide any evidence to support the occurrence of the re-incorporation of melted restricted water into a frozen pure water component.

It can be seen from Figure IV (4) that the restricted water fraction of lincomycin hydrochloride solutions (aq) could be detected in solutions of concentration as low as 0.01M.

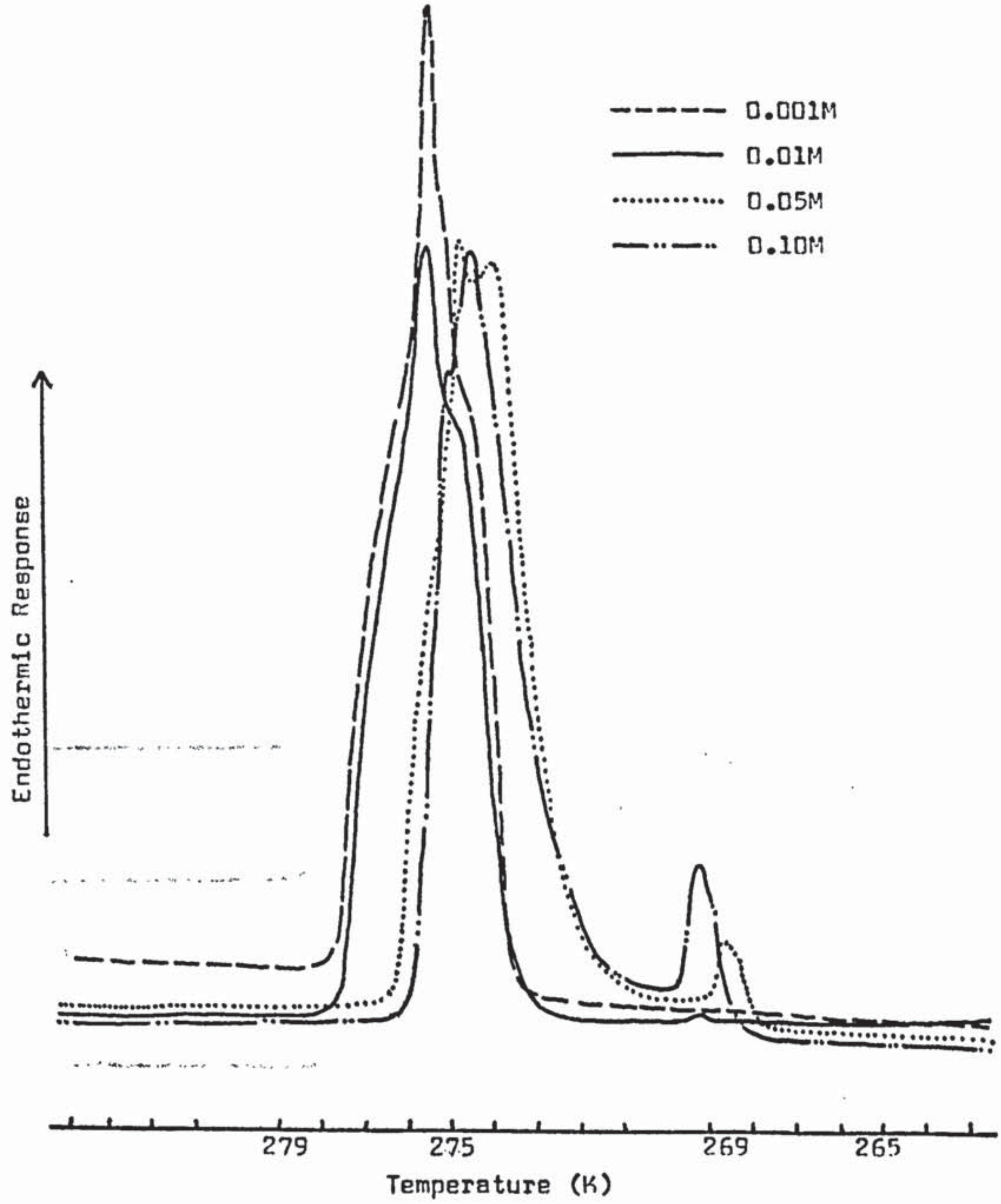
These early D.S.C. studies on dilute aqueous antibiotic solutions suggested that although Lincomycin and Clindamycin are almost identical chemically, the "non-irritant" Lincomycin appeared to have an associated water-structuring effect that was not seen with the "irritant" Clindamycin. Whilst this difference was encouraging a theory directly relating a water-structuring ability with irritancy was clearly inappropriate. This was further complicated by the finding that other "non irritant" solutes displayed the same simple melting behaviour in solution as the "irritant" Clindamycin.

IV.4.iii D.S.C. Studies on the Melting of Low Concentration Aqueous Solutions of Various Other Solutes

Figures IV (5) (i), (ii), (iii) and (iv) show the D.S.C. traces obtained for aqueous solutions of Erythromycin Lactobionate (E/M/L) ((i) and (ii)) Erythromycin Glutamate (E/M/G) ((iii)) and Sodium Chloride ((iv)). The 0.01M solutions of E/M/L and E/M/G displayed no unusual melting behaviour, however, a split peak was observed for a 0.1M solution of E/M/L (E/M/G was insoluble in water at this concentration) the second peak increasing at the expense of the first peak on temperature cycling, a trend observed for the melting peaks of aqueous Lincomycin solutions. 0.1M sodium chloride solution did not show any peculiarities, the broader peak

FIGURE IV (4)

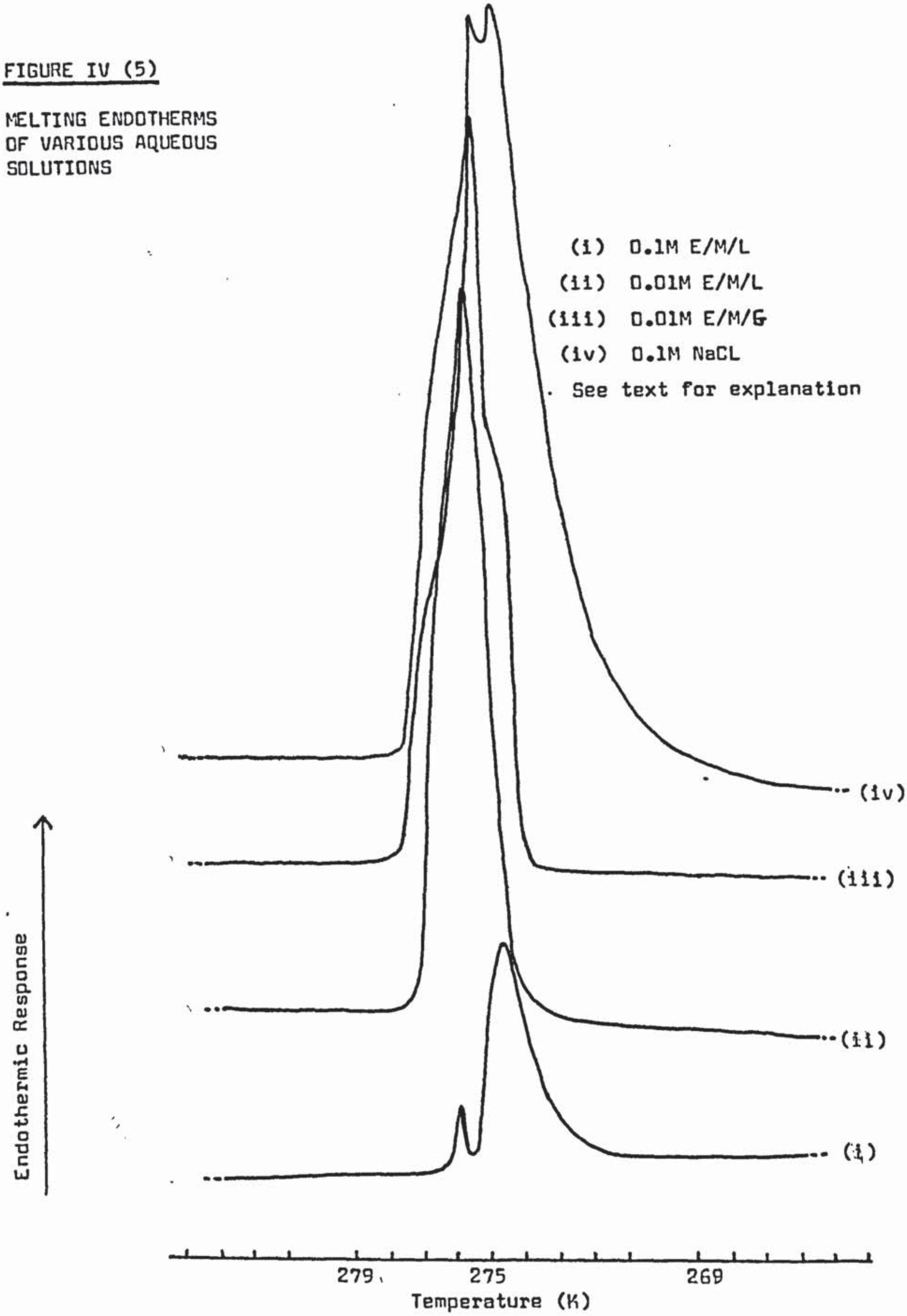
MELTING ENDOTHERMS OF AQUEOUS LINCOMYCIN HYDROCHLORIDE SOLUTIONS



observed being attributed to a melting/freezing point depression effect. Higher concentration sodium chloride solutions demonstrated a eutectic composition peak (see later) and the similarity of the E/M/L thermograms to these later results, especially since the E/M/L solutions were close to their solubility limits, led to the suggestion that the melting peaks of 0.1M E/M/L solutions were due to eutectic composition and pure water

FIGURE IV (5)

MELTING ENDOTHERMS OF VARIOUS AQUEOUS SOLUTIONS



components. The further investigation of solutions of the erythromycins was not pursued in order to prevent further diversification of this research.

IV.4.iv D.S.C. Studies of the Melting Behaviour of High Concentration Aqueous Antibiotic Solutions

A range of aqueous solutions of Lincomycin and Clindamycin of 1% (by wt) to 40% (by wt) were prepared. The higher end of this concentration range approached the solubility limits of the compounds.

Thermograms obtained are shown in Figure IV (6) for Lincomycin solutions and Figure IV (7) for Clindamycin solutions (10% $^w/_w$ solutions of the antibiotics corresponded to about 0.25M solutions). It can be seen from these figures that the increase in the antibiotic concentration up to approximately 10% by wt improved the resolution of thermal transitions occurring in the solutions so that three peaks were now seen for Lincomycin solutions and two peaks were seen for Clindamycin solutions. Further antibiotic concentration increases showed Clindamycin to develop an additional low temperature transition at a lower temperature than the "restricted" water transition of Lincomycin previously seen but at the same temperature as Lincomycin displayed a small exothermic transition. No exothermic transition was observed for Clindamycin solutions. Throughout this work peak temperatures will be compared since the multiple peak structures often obscured peak onset temperatures.

Dealing with the Lincomycin solution behaviour first, the preliminary peak of "restricted" water found in dilute solution at about 269K was found at the same temperature for all the solution concentrations studied (although peak temperatures were generally ± 1.0°). The size of this peak was observed to increase with increasing antibiotic concentration but was found to decrease on temperature cycling as the 274K transition increased. The melting behaviour of the 274K peak was indistinguishable from that of bulk water and so it was assigned as a "pure" water peak.

FIGURE IV (6)

MELTING ENDOTHERMS OF LINCOMYCIN HYDROCHLORIDE AQUEOUS SOLUTIONS (% $^{w}/_{w}$)

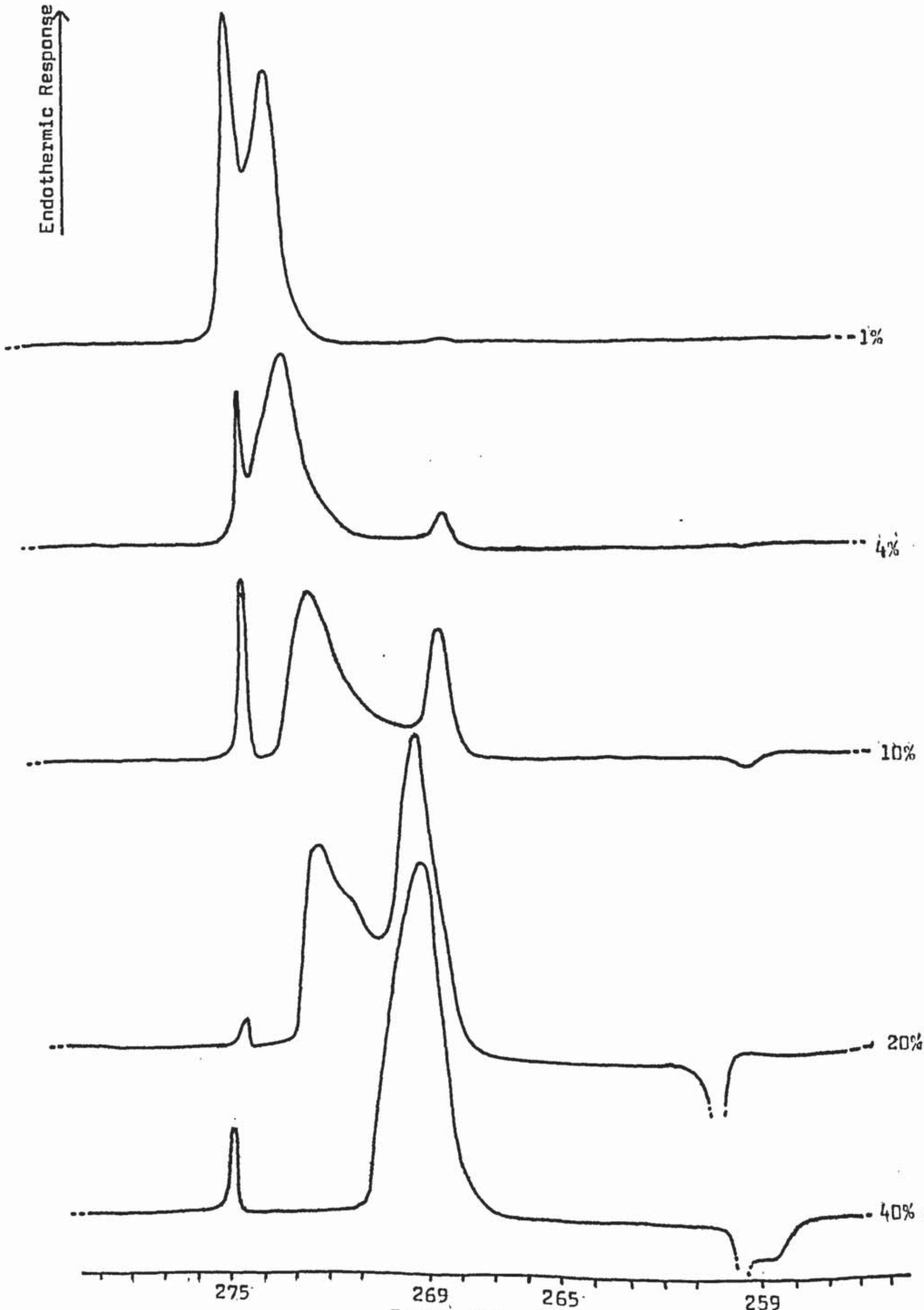


FIGURE IV (7)

MELTING ENDOTHERMS OF CLINDAMYCIN HYDROCHLORIDE AQUEOUS SOLUTIONS (% w/w)

The transition between the 269K and 274K peaks accounted for the majority of the freezing/melting water in the samples. This peak was broad and was seen to move to progressively lower temperatures as the Lincomycin concentration increased. This behaviour was consistent with the depression of the freezing/melting point of water by Lincomycin even though the observed depression of freezing point was slightly greater than could be accounted for by the amount of Lincomycin present. However, this was adequately accounted for by the increased cryoscopic effect due to the dissociation of Lincomycin free base and hydrochloride.

The results obtained during these studies on Lincomycin solutions seemed to challenge conventional attitudes to cryoscopic behaviour since a depressed water transition and a pure water transition were observed in the same sample of Lincomycin solution. Normally the depression of freezing point effect would be considered to extend throughout the whole solution with the exception in this case of the restricted water fraction which was under a stronger influence of the antibiotic.

The exothermic transition found at low temperatures for Lincomycin solutions will be included within the discussion on the melting behaviour of Clindamycin solutions. However, at this stage it was possible to envisage a number of water shells forming a continuum throughout which Lincomycin exerted a progressively weaker influence. The innermost and smallest layer would be the fraction of water "bound" to the antibiotic (see later). This would be surrounded by the "restricted" water component which in turn would be surrounded by the bulk of the water in the solution in a "depressed" water state. The final fairly small water component would lie outside any appreciable sphere of antibiotic influence and would display "bulk-like" freezing/melting properties.

Figure IV (6) shows some other interesting features. The 20% (by wt) Lincomycin solution scan clearly shows a shoulder on the transition of the depressed water component. Such observations of more complex peak definitions were erratic but certainly not uncommon. In most instances the appearance of small additional peaks or shoulders was transient, often disappearing after only one temperature cycle. Similar observations were made in later studies and were taken as further evidence of the existence of a continuum of water states in which spontaneous but transient stabilisation of additional water fractions could from time to time occur.

Turning now to the D.S.C. observations on Clindamycin solutions, as was mentioned earlier, the intermediate concentration antibiotic solutions (approximately 0.1M to 0.25M) only provided evidence for three peaks for Lincomycin solutions and two peaks for Clindamycin solutions. However, when solution concentrations of about 0.25M to 1.5M (ie approximately 10% ($^w/w$) to 40% ($^w/w$)) were studied a third low temperature peak was found for Clindamycin solutions that had previously been overlooked (Figure IV (7)). This peak was found at about 259K, ten degrees lower than the "restricted" water transition seen for Lincomycin solutions. This peak showed the same characteristics as the Lincomycin 269K peak in that its position remained constant whilst its size increased with increasing Clindamycin solution concentration. The 274K peak for Clindamycin was found to increase at the expense of the 259K peak and was assigned as "pure" water. Clindamycin solutions also showed the same "depressed" water transition behaviour with increasing solution concentration as had been seen for Lincomycin solutions. The extent of the depression of the Clindamycin "depressed" water peak was slightly lower than that of the Lincomycin "depressed" water peak at high solution concentrations. This small effect was nevertheless consistent with the observed association of Clindamycin in high concentration aqueous solutions.

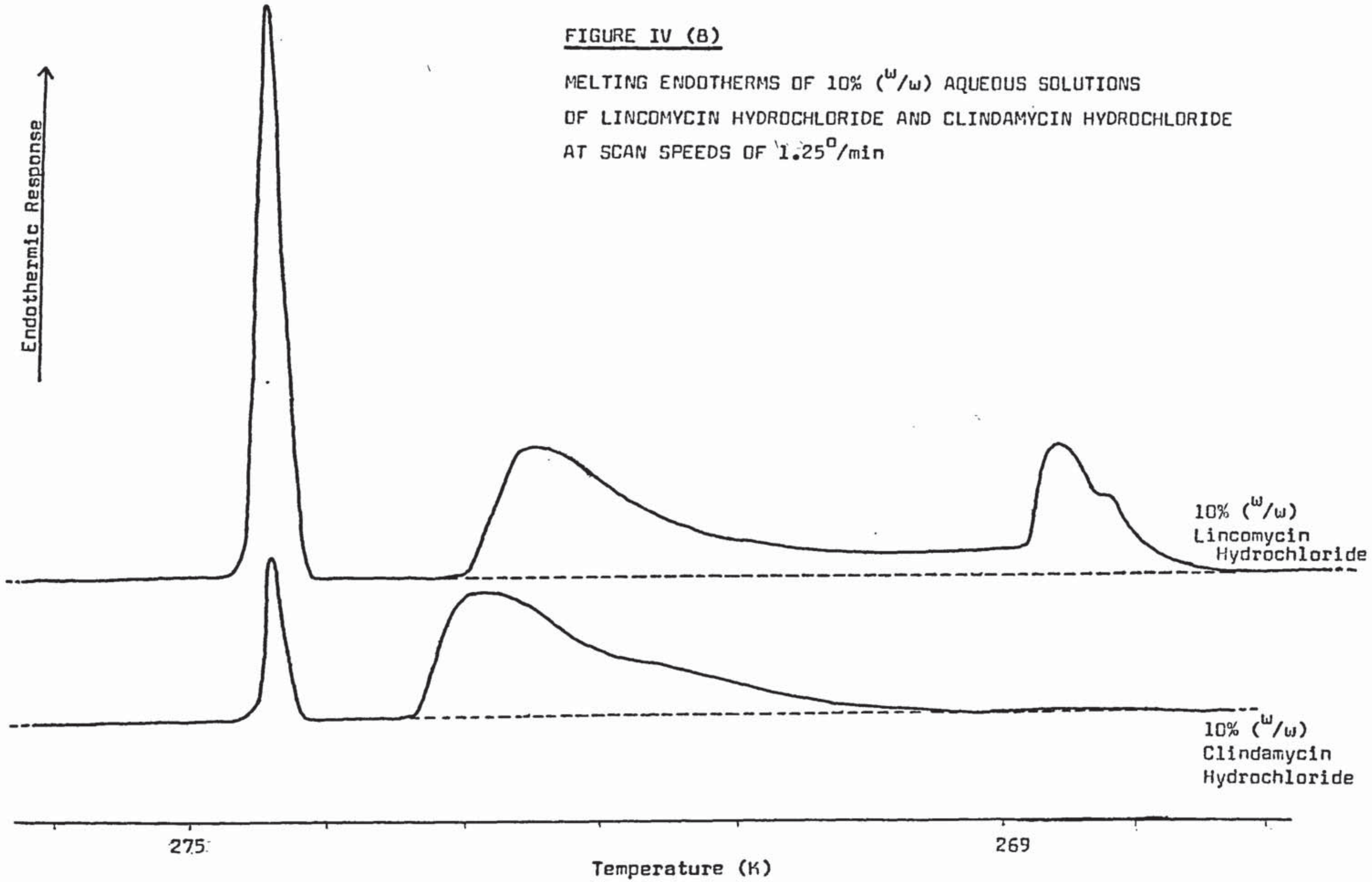
As has already been mentioned Lincomycin solution did not demonstrate an endothermic transition at 259K but at high concentration levels an exothermic response at precisely this temperature was found. The size of the response was proportional to the concentration of Lincomycin in solution, the temperature of the transition remaining unaffected by concentration. The size of the exothermic response was generally off scale and therefore unquantifiable.

The transitions observed for both antibiotics in aqueous solution were reproducible without exception and Figure IV (8) illustrates the clear separation of the transitions of both antibiotics when in 10% ($^w/w$) aqueous solutions as seen by D.S.C. at low scan speed (1.25^o/min). The observation that the traces did not return to the baseline between the "restricted" water transition and "depressed" water transition of Lincomycin in solution was taken as further evidence of the continuum of water states or "merging in" of the water shells described earlier for Lincomycin in aqueous solution. Such water shells could also be envisaged for Clindamycin in aqueous solution with absence of the 269K "restricted" water shell but its possible replacement by a 259K "restricted" water fraction. Figure IV (8) also shows another transient shoulder on the "restricted" water transition of a Lincomycin solution.

The appearance of an exothermic transition in Lincomycin solutions at 259K and an endothermic transition at the same temperature in Clindamycin solutions has interesting implications if the transitions are true water structuring effects. However, mention must first be made of an alternative explanation for the low temperature transitions of both antibiotic solutions.

FIGURE IV (B)

MELTING ENDOTHERMS OF 10% ($^{w}/_{w}$) AQUEOUS SOLUTIONS OF LINCOMYCIN HYDROCHLORIDE AND CLINDAMYCIN HYDROCHLORIDE AT SCAN SPEEDS OF 1.25°/min



A detailed discussion and a further investigation into this alternative explanation has been presented later in this chapter. The explanation was based on work of Hager and Macrury[142] on the phase behaviour and water binding of poly(alkylene oxide) solutions. D.S.C. traces of not dissimilar appearance to those presented here were obtained and the lowest temperature endothermic transition was explained in terms of the melting of a crystalline eutectic. The characteristics of eutectics include a constant melting point, as seen for the 269K and 259K transitions of Lincomycin and Clindamycin solutions respectively. However, a number of other observations might suggest a water structuring explanation to be the more reasonable. It was possible to suggest from phase diagrams (see later) that the 269K and 259K transitions were the eutectic points of aqueous Lincomycin and Clindamycin solutions respectively. However, it seemed unreasonable that two almost identical compounds could show a difference of 10 degrees in their eutectic temperatures. Also, the 269K peak was not the lowest temperature transition found for Lincomycin solutions which displayed an exothermic transition at 259K, any explanation must satisfactorily explain this behaviour. A compromise was possible in that 259K was indeed the eutectic temperature of Clindamycin in solution and was also the eutectic temperature of Lincomycin in solution which showed unusual exothermic behaviour as the melting eutectic water fraction was influenced by the water structuring forces of Lincomycin as manifest in its 269K endothermic transition.

It was not possible to positively demonstrate either explanation during the course of this research but the immediate implications of the D.S.C. results remained unaffected by such indecision in that it had been shown that the antibiotic/water interaction processes occurring in aqueous Lincomycin solutions were different from those found in aqueous Clindamycin solutions.

Throughout the rest of this chapter the D.S.C. observations have been interpreted in terms of water structuring effects. Should the 259K Clindamycin solution transition be shown to be a eutectic temperature then these explanations can be easily modified. It was considered unlikely that the 269K transition in Lincomycin solutions could be shown to be a eutectic temperature since studies presented later in this chapter found endothermic transitions for Lincomycin in hydrogel preparations lower than 269K (and above 259K) alongside the 269K transition, (unless a series of eutectic compositions at different temperatures are envisaged).

Adopting the water structuring hypothesis (albeit cautiously), and returning to the 259K transitions of both antibiotic solutions, the exothermic response of Lincomycin solutions (recorded previously for many water swollen hydrogels at various temperatures) could be considered to be associated with a certain amount of re-ordering of the crystalline structure of that water fraction. Unstable frozen water regions could undergo breakdown and regrowth of crystalline structure as part of a more stable crystalline form. Within this context the 259K endothermic transition of Clindamycin solutions could be considered as the partial melting of a fraction of water previously structured by Clindamycin that immediately became under the influence of Clindamycin again and so was prevented from refreezing immediately. In the case of the 259K exothermic transition demonstrated by Lincomycin solutions the transition was additional to the 269K transition of the restricted water fraction and this would suggest that the transitions for both antibiotic solutions at 259K were due to the same type of structural influence of the antibiotics on water. It would seem reasonable to suggest that Lincomycin uses the same forces that Clindamycin used to structure its 259K restricted water fraction to influence a similar water fraction itself. However, unlike Clindamycin, Lincomycin does not succeed in structuring a definite water fraction as seen at 259K, it only produces an unstable structure over which its forces are unable to maintain their effect. The result of this would be the collapse of the weak structure on heating and its immediate refreezing, possibly into the frozen pure water fraction, rather than remaining under the restricting influence of the antibiotic, hence the exothermic transition.

A thorough investigation of the relationships between the amount of antibiotic present and the size of each water fraction could not be carried on the limited data available. Further studies are required before any significant conclusions can be drawn.

IV.4.v D.S.C. Studies of Water in Hydrogel Matrices

Before the effects of changing water environment on the water structuring properties of antibiotics in hydrogel preparations could be investigated, a study of the "untreated" water swollen hydrogels was necessary.

The equilibrium water contents and freezing water and non-freezing water compositions of the hydrogels referred to in the remainder of this chapter have already been presented in Chapter III.

Figure IV (9) shows the melting behaviour of water swollen pACM and the effect of the subsequent temperature cycling of the sample. The E.W.C. of such a sample was about 90%, of which 80% was freezing water. The broad endothermic transition displayed no additional structuring prior to the main transition which was split in a manner similar to that illustrated in Figure IV (1). The effect of temperature cycling was to increase the 275K "pure water" component at the expense of the 274K component.

FIGURE IV (9)

MELTING ENDOTHERMS OF WATER SWOLLEN pACM HYDROGEL AFTER SUCCESSIVE TEMPERATURE CYCLES

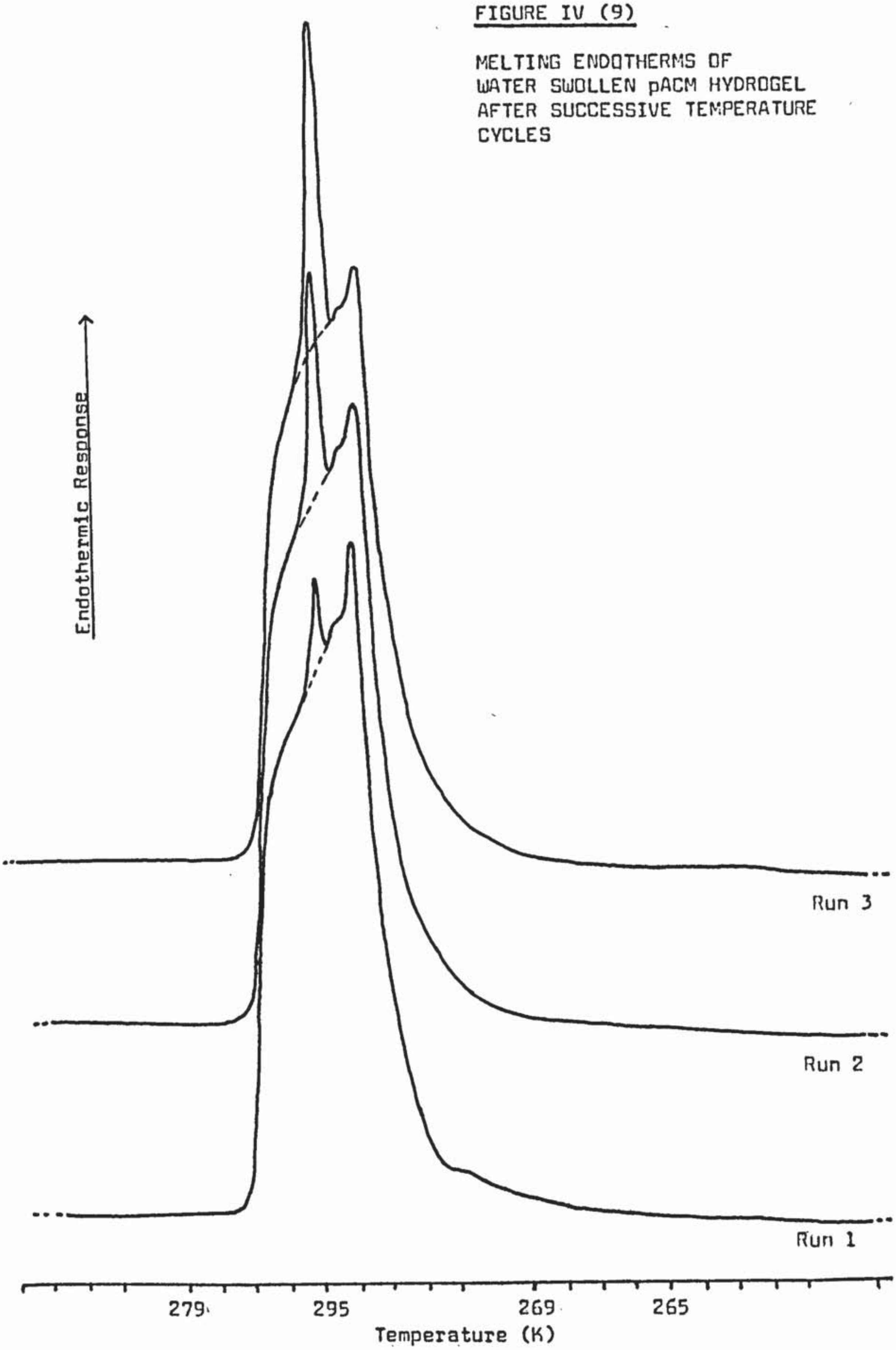


Figure IV (9) also shows, by means of a dotted line, that the 275K "pure" water transition was effectively superimposed on a broader 274K transition. This broad transition was imperfectly crystallised water due to interaction with the polymer matrix.

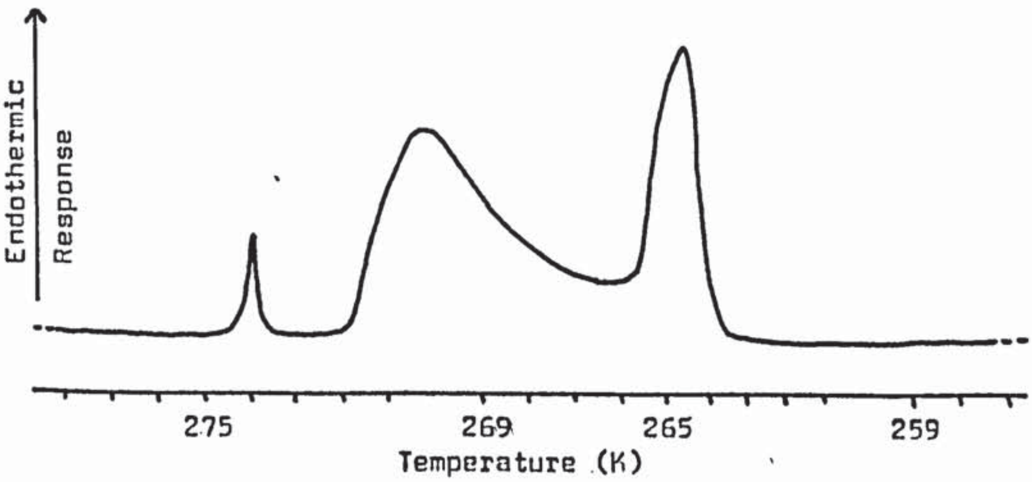
The D.S.C. study of a solution of acrylamide monomer in water (10:90, ACM:H_2O) would be expected to show a depressed water peak at -2.9^0 from the melting temperature of "pure" water. The results obtained can be seen in Figure IV (10) which shows the unexpected fine structure found. The peak at 274K could easily be identified as the melting of pure water in the system. The 271K peak was assigned as the depressed water peak with its characteristically broad profile, the extent of depression agreeing well with the theoretical value of -2.9^0. In actual fact the depression could have been expected to be greater than -2.9^0 since after subtraction of the 274K pure water component the amount of water in the depressed water fraction would be smaller and hence the effective acrylamide concentration and resultant depression of freezing/melting point would be greater.

It was tempting to assign the 265K transition to a "restricted" water fraction and such an assignment might well be valid but since no corresponding structure was found for the same components in a polymerised system (Figure IV (9)) it was possible that the 265K transition represented the melting of a crystalline eutectic of the type described by Hager and Macrury.[142] Whilst such differences between the thermal behaviour of polymerised and unpolymerised acrylamide were interesting a complete understanding of the processes occurring in aqueous solutions of monomers was not essential to this research and such studies were not pursued to any depth.

The trace obtained by D.S.C. analysis of a sample of pHEMA swollen in water can be seen in Figure IV (11). The figure shows the traces

FIGURE IV (10)

MELTING ENDOTHERM OF ACRYLAMIDE MONOMER IN AQUEOUS SOLUTION (ACM/H_2O, 10:90 by wt)

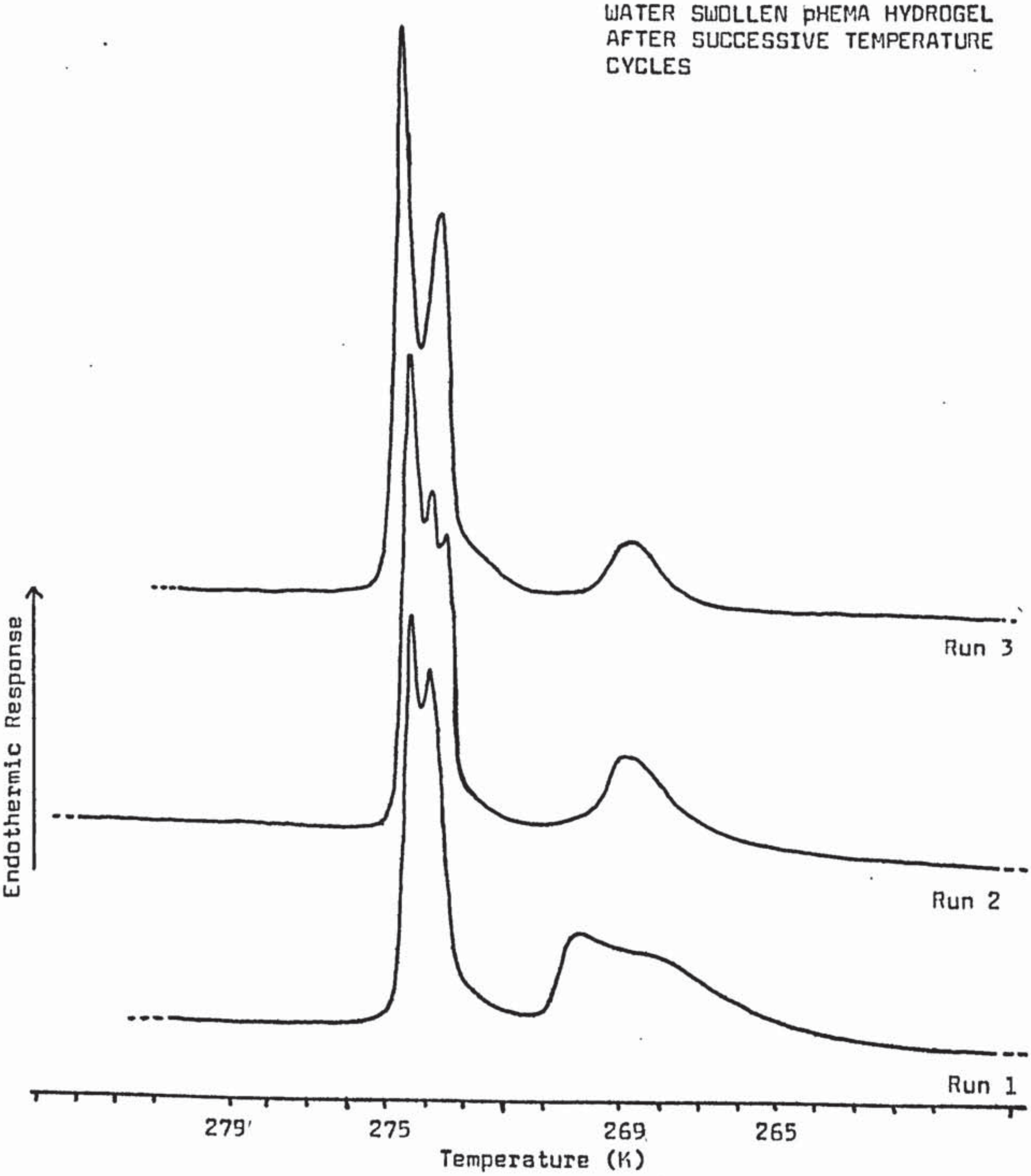


obtained from successive temperature cycles of the same sample. The E.W.C. of such a sample was about 36% of which 13% was freezing water and 23% was non-freezing water.

As was seen for water swollen pACM in Figure IV (9) the peak at 273.5K may be assigned to the melting of a slightly imperfect crystalline water

FIGURE IV (11)

MELTING ENDOTHERMS OF WATER SWOLLEN pHEMA HYDROGEL AFTER SUCCESSIVE TEMPERATURE CYCLES



fraction and the 274.5K peak to the "pure" water fraction which was seen to increase at the expense of the 273.5K transition on temperature cycling. The 273.5K peaks also became more clearly defined and well separated during this process as the broader characteristics of the 273.5K water peak became more evident. Assuming these assignments to be correct, the transition occurring at 269K could then be considered as a "restricted" water fraction. This assignment would be supported in so much as the theoretical depression of water due to unpolymerised HEMA monomer in an aqueous solution of the same HEMA/water composition as was present in the hydrogel would be about -26.5^{0} from the melting transition of pure water. (The 269K transition was at the same temperature as the "restricted" water transition associated with aqueous solutions of Lincomycin.) The 269K transition for water swollen pHEMA on the first run of the sample displayed a marked shoulder, this was considered further evidence of occasional transient stabilisation of water fractions within the continuum of water states as described earlier. A similar transient structure was observed between the 273.5K and 274.5K transitions on the second run of the same sample.

The D.S.C. trace of an aqueous HEMA monomer solution of the same composition of HEMA and water as was found in a water swollen pHEMA preparation (35% water, 65% HEMA monomer, by wt) can be seen in Figure IV (12).

The 273K pure water transition and 268K restricted water transition seen for water swollen pHEMA were both again found in aqueous HEMA monomer solution. Should further studies show the position of the 268K peak to be independant of solution composition then this transition will have been isolated as due to either eutectic formation or water structuring, although the latter was favoured by the author.

FIGURE IV (12)

MELTING ENDOTHERM OF HEMA MONOMER IN AQUEOUS SOLUTION (HEMA/H_2O, 65:35 by wt)

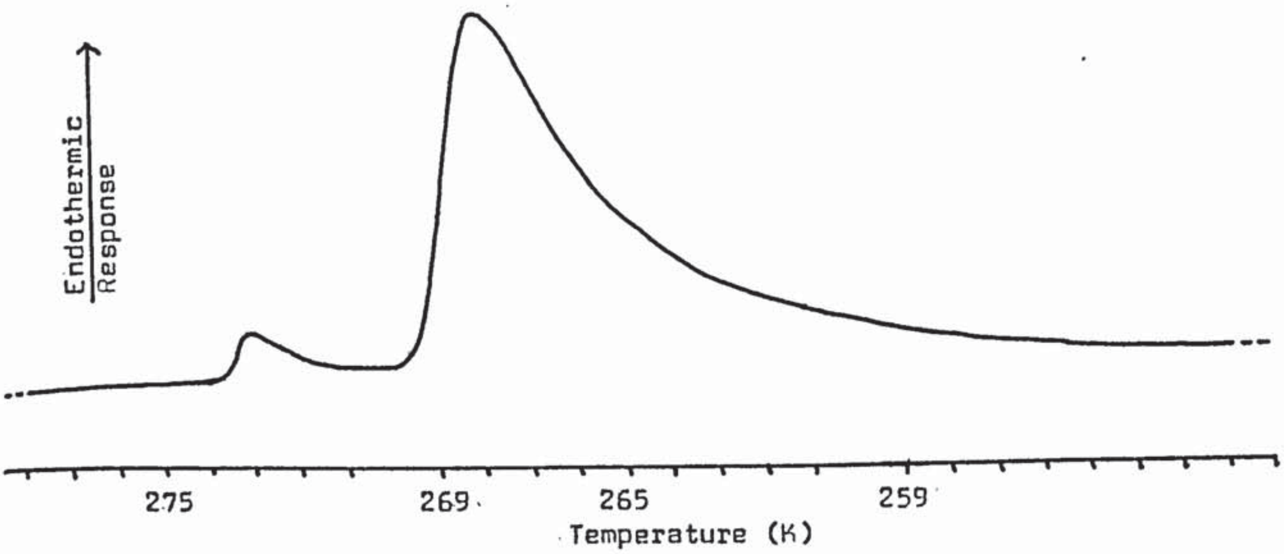


Figure IV (13) shows the D.S.C. trace of water swollen HEMA/STY (90:10) (mole%) copolymer (E.W.C. = 23%, of which only 1% was freezing water). The 275K "pure"water transition was found to increase at the expense of the 274K transition on temperature cycling. The D.S.C. trace of an aqueous solution of a monomer mixture of HEMA/Styrene (90:10) (mole%) of the composition monomer mixture:H_2O, 80:20 (by wt) can be seen in Figure IV (14). This solution had a similar monomer/water composition as that of water swollen HEMA/STY. The 274.5K transition of pure water was found as well as a 268.5K transition not seen in the corresponding water swollen HEMA/STY polymer. The depression of the freezing/melting point of water due to the HEMA monomer content of this solution alone would theoretically be over 50 degrees. The interpretation of the 268.5K transition would therefore have to be either one of eutectic formation or water structuring. However, the various solution compositions studied in which monomer solutions showed a transition at about 269K would not be expected to have the same eutectic temperature and so it was considered

FIGURE IV (13)

MELTING ENDOTHERM OF
WATER SWOLLEN
HEMA/STY (90:10)(mole%)
COPOLYMER

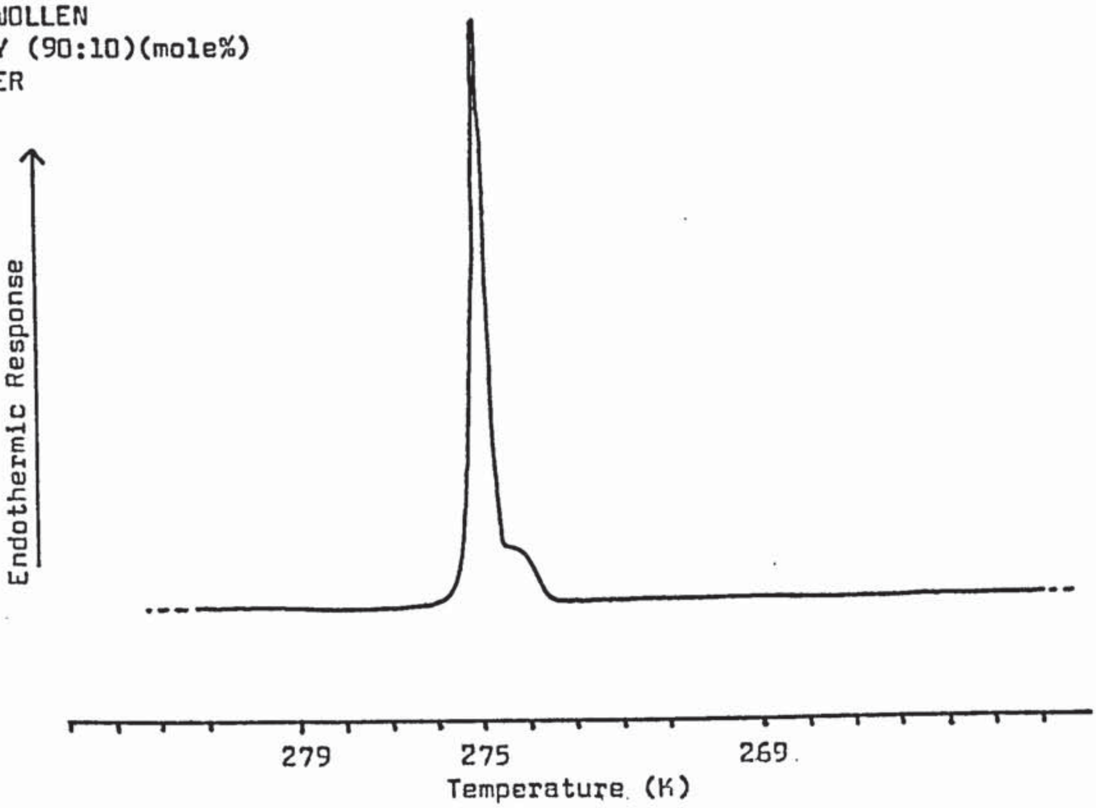
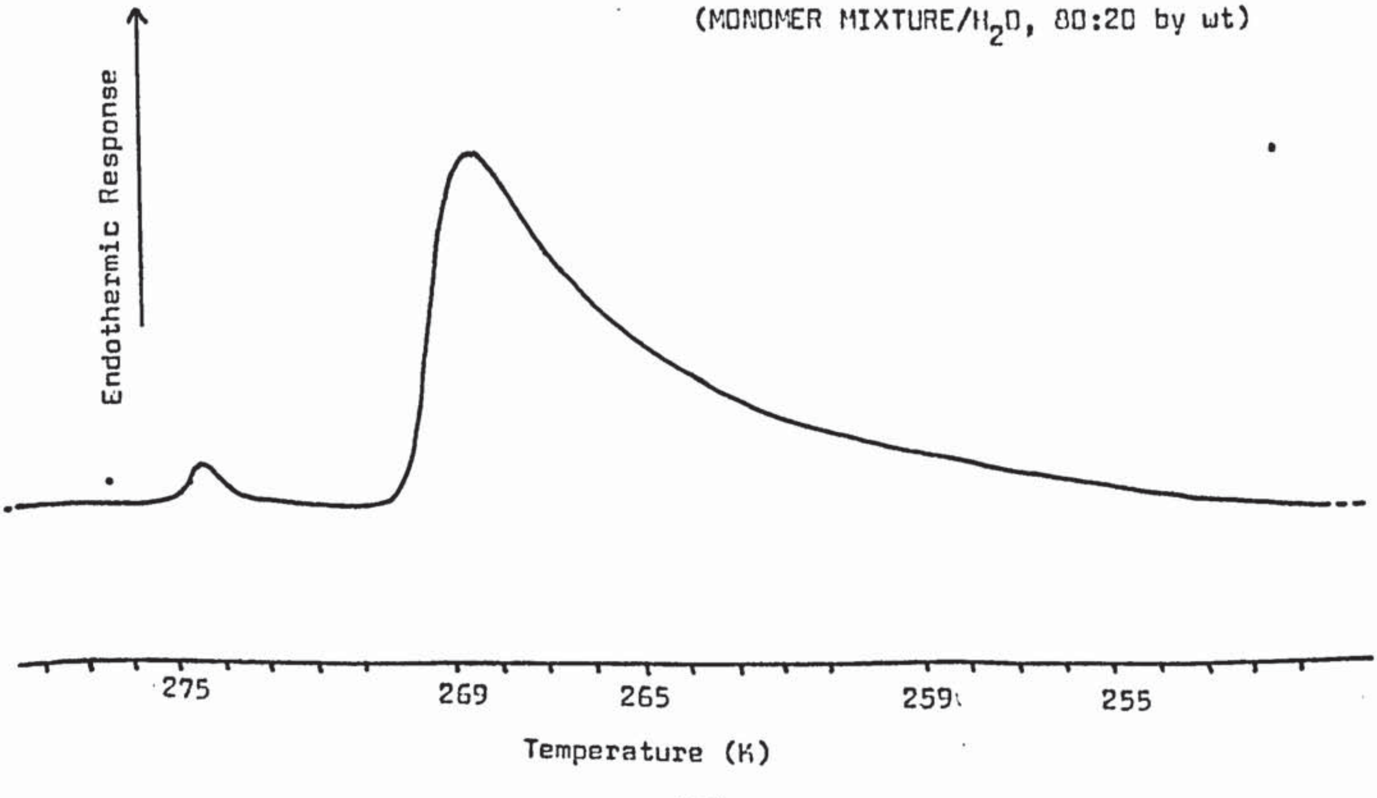


FIGURE IV (14)

MELTING ENDOTHERM OF A HEMA/STY
MONOMER MIXTURE (90:10) (mole%)
IN AQUEOUS SOLUTION

(MONOMER MIXTURE/H_2O, 80:20 by wt)



likely that this temperature corresponded to the melting of a particular type of water structure common to all water-structuring species. Transitions at this temperature were also found for aqueous solutions of Lincomycin and HEMA/water systems in both polymerised and unpolymerised states. In the aqueous monomer solutions the 269K transition was greater the smaller was the water component of the system, but HEMA/water systems were the only preparations to demonstrate this transition in both the polymerised and unpolymerised state. This may well reflect something of the nature of the way in which water structuring forces must be arranged in order to maximise their effects.

IV.4.vi D.S.C. Studies on Antibiotic/Water/Hydrogel Interactions

Using the assignments of the thermal transition temperatures of the various water fractions described so far, the effects of the antibiotics on the type and temperature of these transitions could now be studied.

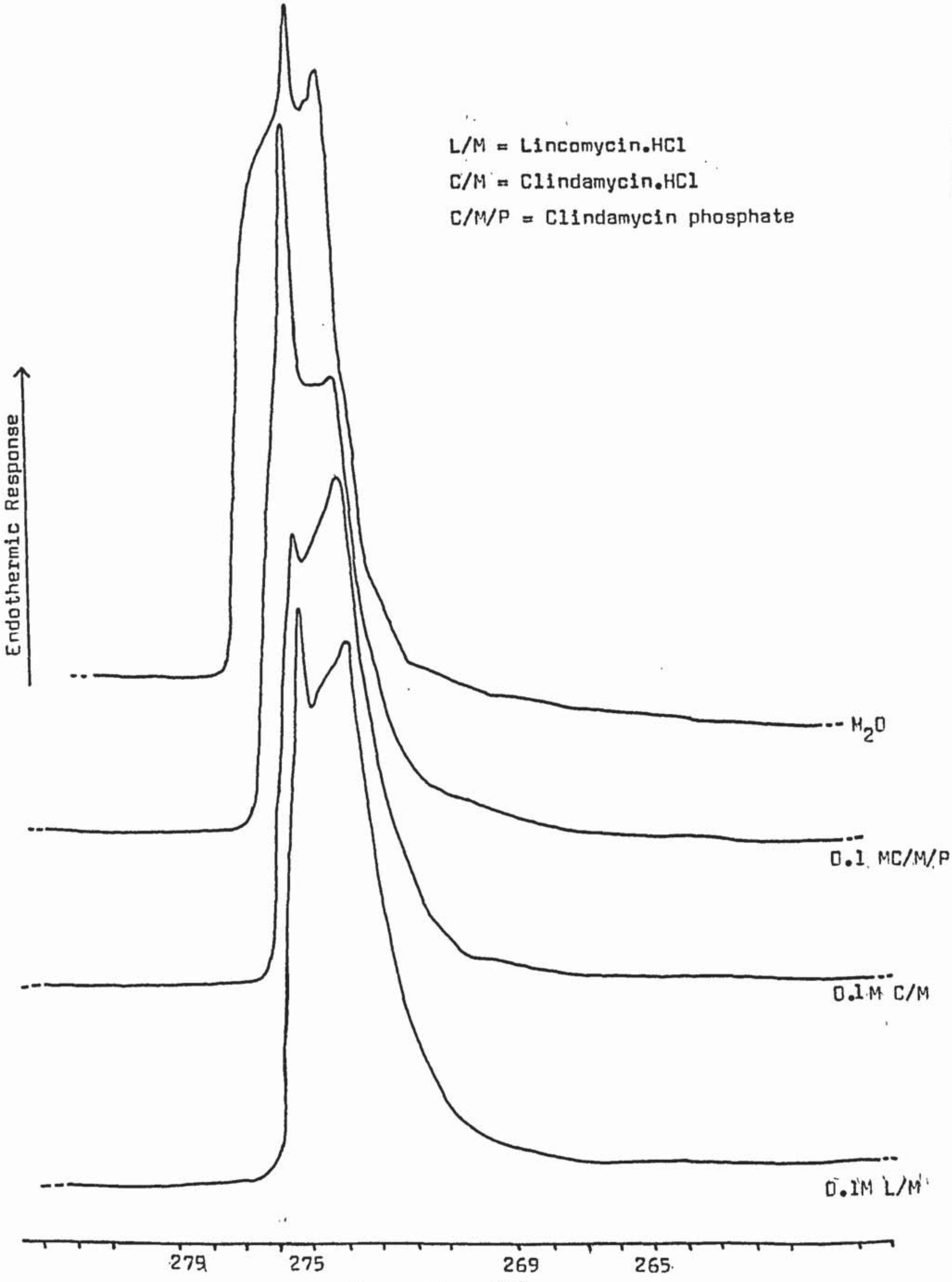
Figure IV (15) shows the melting endotherms of pACM preparations swollen in various solutions and all the preparations shown had similar thermal profiles. The lack of any additional low temperature transitions was the result of the high dilution of the antibiotics in the water of the preparation (the pACM preparations were already pre-swollen with water (75% H_2O:25% ACM) (by wt) before completion of swelling in the antibiotic solutions).

The split peak structures of all the transitions seen in Figure IV (15) were considered the result of imperfect water crystallisation processes due to physical interaction with the polymer matrix as already described.

The pACM preparations swollen in 0.1M Lincomycin and 0.1M Clindamycin solutions had E.W.C's of 86.2% and 86.4% respectively of which more than

FIGURE IV (15)

MELTING ENDOTHERMS OF pACM HYDROGELS SWOLLEN IN VARIOUS SOLUTIONS (aq)



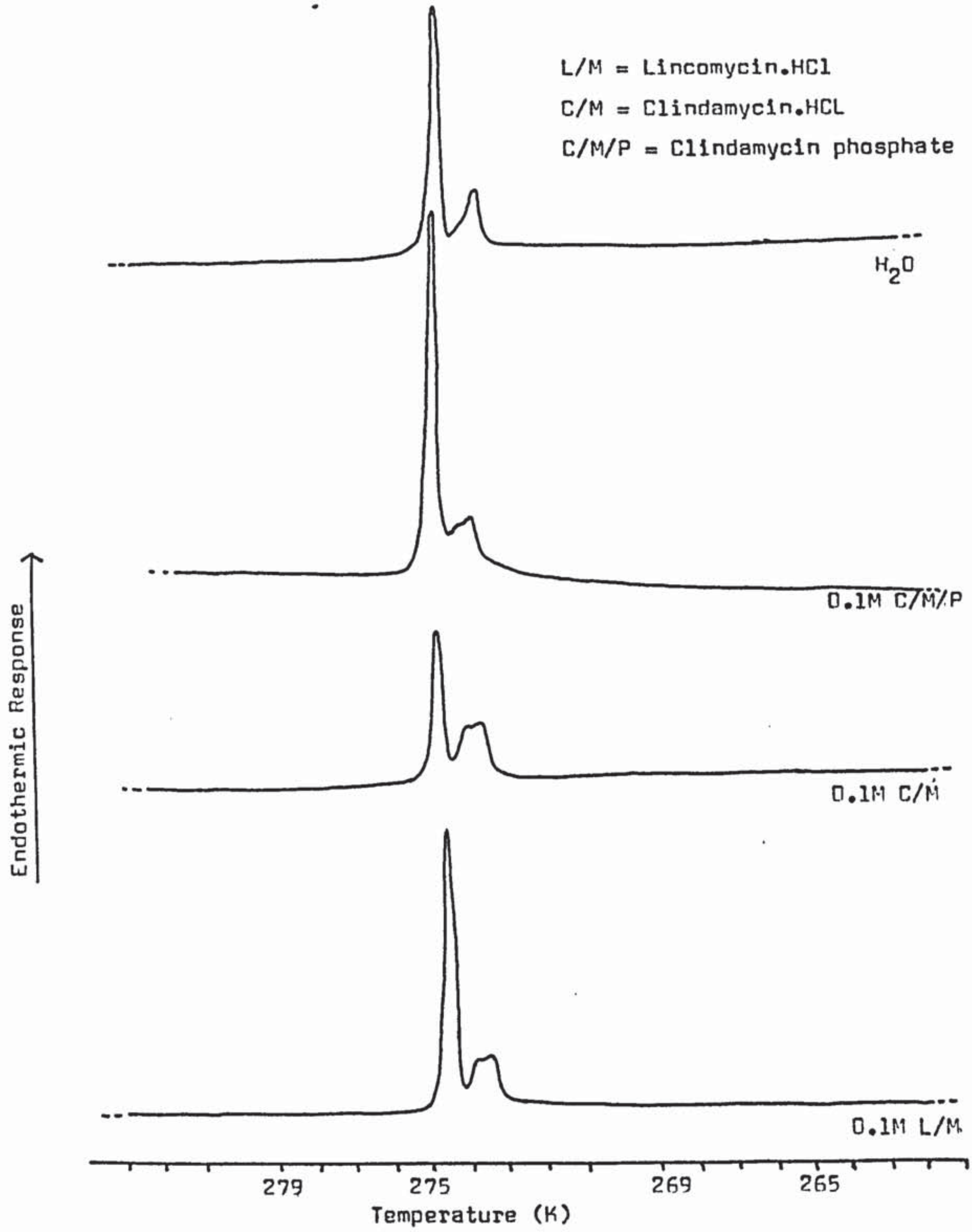
70% was freezing water in each case, but corresponding HEMA/STY (90:10) (mole%) preparations had E.W.C's of 22.3% and 22.7% (of which only about 1% was freezing). The melting endotherms of these and other HEMA/STY preparations can be seen in Figure IV (16). It was apparent that even at opposite extremes of E.W.C. and freezing water contents to those found in pACM preparations, the antibiotic concentrations in the systems were too low to demonstrate any low temperature thermal transitions. All the preparations seen in Figure IV (16) showed the split peak behaviour associated with the melting of an impure crystalline water fraction (273.5K) followed by the melting of a pure water fraction (274.5K).

In order to increase the contribution of any water structuring effects to the thermal behaviour of hydrogel systems containing antibiotics, a series of hydrogels swollen in about 1.5M antibiotic solutions (aq) (40% by wt antibiotic) were prepared. The D.S.C. traces obtained from studies on pACM hydrogels swollen in 1.5M aqueous solutions of Lincomycin or Clindamycin can be seen in Figure IV (17).

The Lincomycin and Clindamycin preparations displayed pure water peaks at about 276K and 275K respectively and these water fractions were found to increase with temperature cycling. The next transition common to both preparations was the broad endothermic response at about 270K to 271K. Because of the high levels of antibiotic present in the swelling solutions used and the appearance of the peaks, these transitions were assigned to the melting of depressed water fractions. The Lincomycin preparation also displayed a shoulder (not transient) on the low temperature side of the depressed water transition and this was considered to be the same restricted water peak seen in previous D.S.C. studies of Lincomycin solutions. Both preparations displayed small endothermic

FIGURE IV (16)

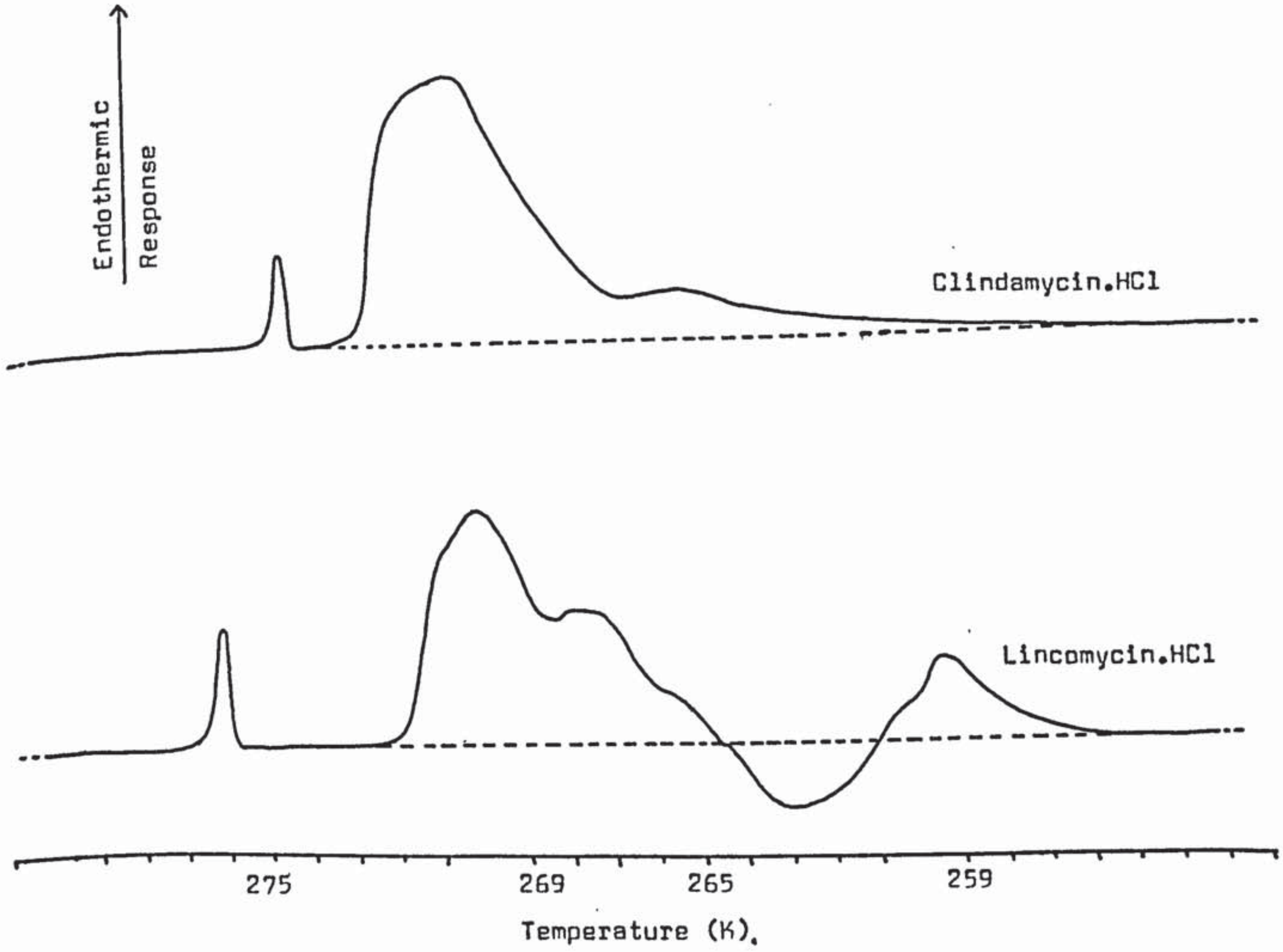
MELTING ENDOTHERMS OF HEMA/STYRENE (90:10)(mole%) COPOLYMERS SWOLLEN IN VARIOUS SOLUTIONS (aq)



transitions at about 265K, this appearing as an extra shoulder on the 268K shoulder of the 270K transition in the case of the Lincomycin preparation. Although the contributions of these transitions to the total endothermic areas were small they were commonly found and were

FIGURE IV (17)

MELTING ENDOTHERMS OF pACM HYDROGELS SWOLLEN IN 1.5M SOLUTIONS (aq) OF LINCOMYCIN.HCl AND CLINDAMYCIN.HCl



considered to be slightly more stable than the spontaneous and transient type of water structures already described. Once present, these transitions only disappeared slowly after many temperature cycles. The fact that both antibiotics displayed the same 265K transition was considered further evidence of both antibiotics having the same inherent water structuring abilities which could only be displayed in suitable environments.

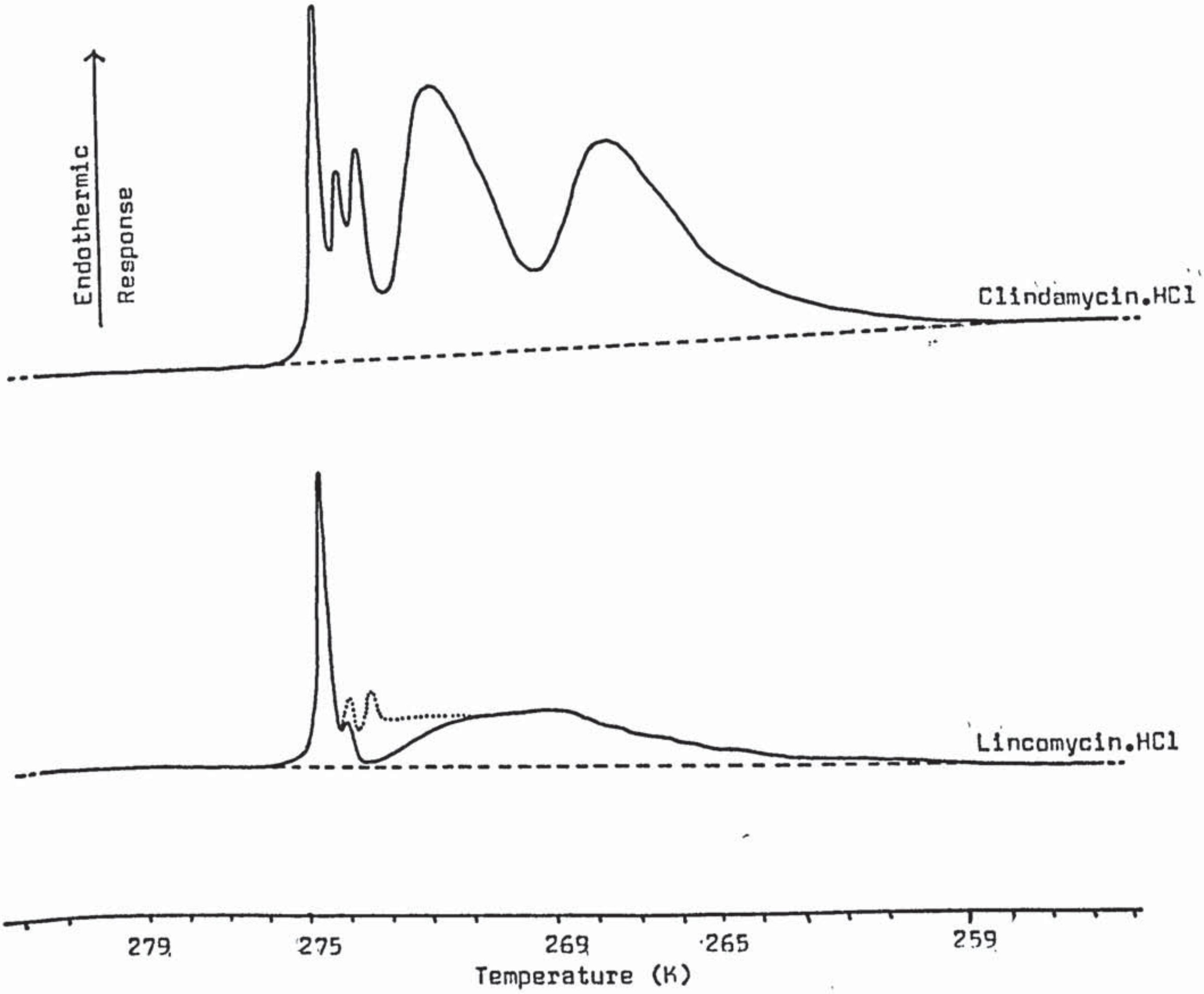
Another endothermic transition was seen at about 259K for the Lincomycin preparations (but not Clindamycin preparations). This temperature was the same temperature as that at which high concentration aqueous Clindamycin solutions displayed small endothermic transitions and high concentration Lincomycin solutions displayed small exothermic transitions. The exotherm seen for high concentration Lincomycin solutions at 259K had been described as a small fraction of water sufficiently influenced so as to allow a change in its melting point, but it did not melt as such (since no endothermic response was detected). Instead it was postulated that the water molecules in this water fraction became only free enough to immediately refreeze, hence only an exotherm was apparent. The process was thus only a reorganisation of crystalline structure.

However, the transition seen at 259K in Figure IV (17) was clearly a melting transition and was followed by an exothermic transition of similar area. This suggested that the environment of the antibiotic in this case led to a more strongly influenced water fraction at this temperature which showed definite (complete) melting behaviour and subsequent refreezing (exothermic transition). The slight high temperature shoulder on the 259K transition was a spontaneous and transient stabilisation of a small water fraction within the continuum of water states to be found in these preparations.

The D.S.C. traces obtained for the melting of HEMA/STY (90:10) (mole%) copolymers swollen in 1.5M aqueous solutions of either Lincomycin or Clindamycin can be seen in Figure IV (18) where previously identified transitions can be discerned. It was immediately apparent that the change from the high E.W.C., high freezing water content pACM systems studied before, where Lincomycin (but not Clindamycin) displayed well-defined multiple structured water fractions, to the low E.W.C., low

FIGURE IV (18)

MELTING ENDOTHERMS OF HEMA/STYRENE (90:10)(mole%) COPOLYMERS SWOLLEN IN 1.5M SOLUTIONS (aq) OF LINCOMYCIN.HCl AND CLINDAMYCIN.HCl



freezing water content systems considered here, resulted in Clindamycin (but not Lincomycin) showing well defined multiple structured water fractions. Also, whereas the E.W.C's and freezing water contents of the antibiotic solution swollen pACM preparations were similar, their E.W.C's being substantially lower than for water swollen pACM, the corresponding HEMA/STY antibiotic solution swollen preparations were both of higher E.W.C. than the water swollen preparations, and the Clindamycin preparations had higher E.W.C's than the Lincomycin preparations, thus resulting in the clearly defined peaks of the Clindamycin preparations. The 274.5K transitions were in each case correlated with the melting of pure water fractions, these fractions gradually increasing with temperature cycling of the samples. A number of smaller transitions just prior to the pure water transitions were found in both preparations. Although these were often observed for the Clindamycin preparations they were of a more spontaneous and transient character for Lincomycin preparations (as indicated by the dotted line). These were considered to be small imperfectly crystallized "pure" water fractions. The 271.5K transition of the Clindamycin preparation was considered to be the depressed water fraction. This was found to be a much smaller component in the Lincomycin preparation because of its lower water content than the Clindamycin preparation. In the case of the Lincomycin preparation the depressed water peak merged into the 269K restricted water peak. It was particularly interesting to note the large 267.5K transition for the Clindamycin preparation. It was apparent that the environment of this type of preparation was suited to Clindamycin displaying the same water structuring capability as had been seen for Lincomycin at this temperature. Thus, the 267.5K transition was considered as the melting of a Clindamycin "restricted" water fraction. A close inspection of the traces obtained from studies on the Clindamycin preparations also suggested that there may have been a further small low temperature transition at about 265K of the type seen for both Lincomycin and Clindamycin swollen pACM preparations (see Figure IV (17)) although this was largely obscured by the 267.5K transition.

At this stage in the research the implications of the water structuring effects of the antibiotics in explaining their differing extents of irritancy was rapidly becoming clear. It was apparent that Lincomycin and Clindamycin had the same inherent water-structuring capabilities but displayed them on different occasions depending on the type of water environment in which they were present and both antibiotics were also able to modify these water environments.

Explanations of the differing irritant effects of the two antibiotics, based on alteration of water structure in biological systems resulting in disruption of cellular processes that are dependant on water structuring processes, were now to some extent justifiable.

IV.5 Miscellaneous Results from D.S.C. Studies

IV.5.1 Investigation of Phase Behaviour

Following the work of Hager and Macrury,[142] the possibility of at least some of the multiple peak observations already described being due to the melting of crystalline eutectics was investigated. Hager and Macrury studied aqueous poly(alkylene oxide) systems but D.S.C. had been used previously by Smith and Pennings,[149] Wittman and St John Manley[150] and Gryte, Berghmans and Smets[151] for studies of eutectic forming polymer/ solvent systems.

Observations during this study were confined to antibiotic/water systems, the aim being the elucidation of the nature of the 259K and 269K endothermic transitions observed during the heating of aqueous Clindamycin and Lincomycin solutions respectively.

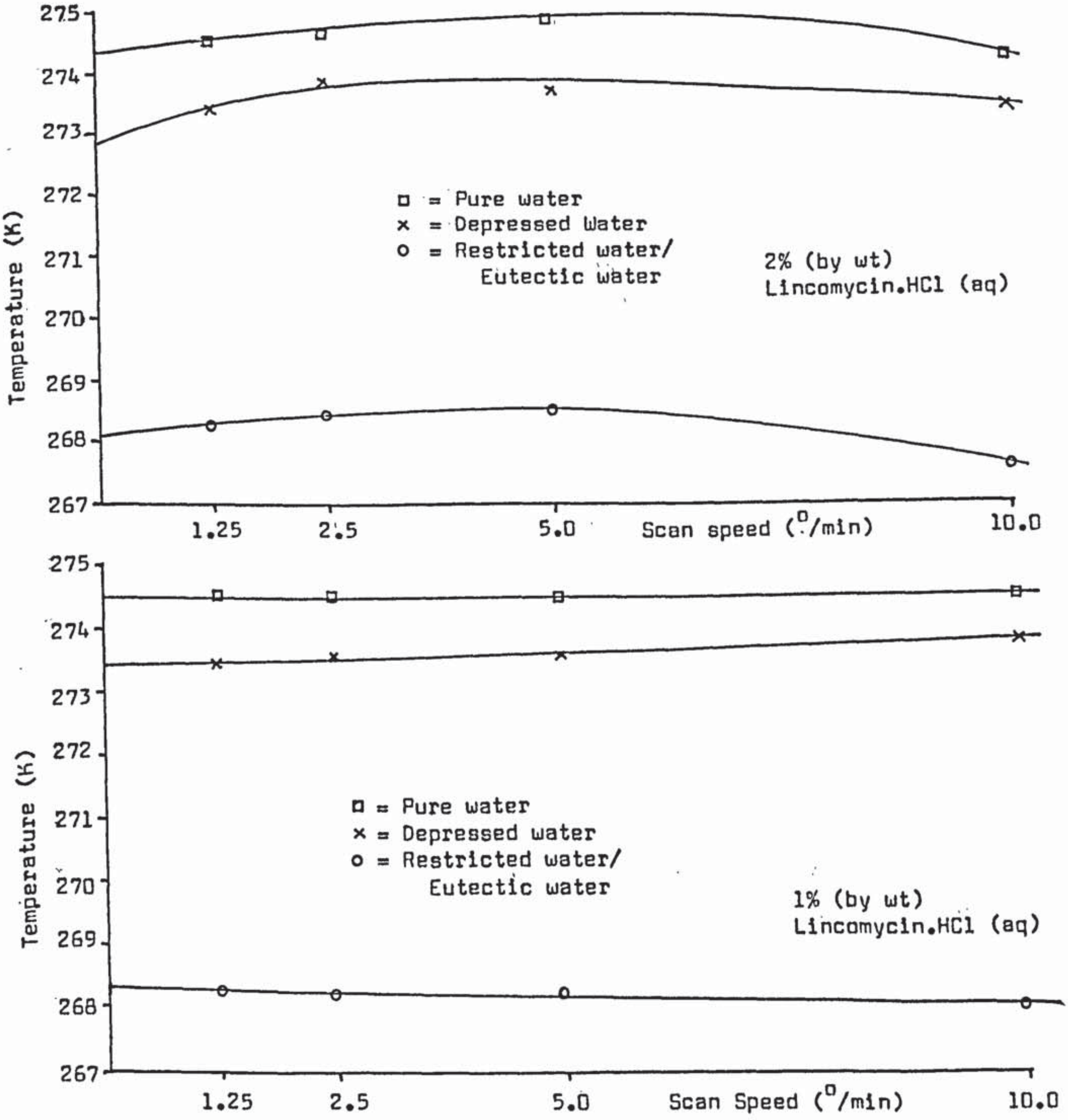
The method used to construct the phase diagrams was based mainly on the procedure described by Smith and Pennings.[149] A range of antibiotic/ water mixture compositions of both antibiotics were prepared. The range covered was from 1% (by wt) to 40% (by wt) antibiotic above which the solubility limits of the compounds were rapidly approached.

The peak temperatures of the melting transitions observed on heating samples of these solutions were plotted against the composition of the sample to produce the phase diagram. However, since the melting processes involved were to some extent dependent on the thermal lag time of the system resulting from the rate of sample heating, it was necessary to find the peak temperatures at a theoretical "zero scan rate". This in itself was a time consuming process since each sample composition needed to be scanned a number of times at each of four scan rates. The average peak temperature of each transition at each scan rate was then plotted against the relevant scan rate and extrapolated to find each peak temperature at zero scan rate. The scan rates used were 1.25°/min, 2.5°/min, 5.0°/min and 10.0°/min and examples of the extrapolated plots of average peak temperature against scan rate are shown in Figure IV (19). Since initial interest concerned only the 269K transition for Lincomycin solutions, extrapolated determinations of the zero scan rate peak temperatures were only carried out for aqueous Lincomycin solutions. However, the phase diagrams presented later also include such plots based on peak temperatures at a scan speed of 5°/min for both Lincomycin and Clindamycin solutions. The values of peak temperature determined could now be corrected for the temperature lag observed in the melting of a tin standard at various scan rates.

The phase diagrams constructed for Lincomycin/water (zero scan rate and 5°/min scan rate) and Clindamycin/water (5°/min scan rate) systems are shown in Figure IV (20) and Figure IV (21) respectively. Because of the solubility problems encountered, the phase diagram could not be extended to higher antibiotic concentration compositions. However, Figure IV (20) might seem to suggest a eutectic point at about 269K and 40% (by wt) Lincomycin/60% (by wt) water. However, it was not unreasonable to suggest

FIGURE IV (19)

EXAMPLES OF TYPICAL SCAN SPEED VERSUS
PEAK TRANSITION TEMPERATURE PLOTS FOR SOME
AQUEOUS LINCOMYCIN.HCl SOLUTIONS



that at higher concentrations of antibiotic in the samples the depressed water peak temperature could continue to fall, below the temperature of the restricted water/eutectic peak temperature, rather than gradually rising towards the melting point of the antibiotic (determined as 152-4°C for Lincomycin.HCl and 145-147°C for Clindamycin.HCl). The case for the

FIGURE IV (20)

PHASE DIAGRAM FOR LINCOMYCIN.HCl/WATER AT VARIOUS SCAN SPEEDS

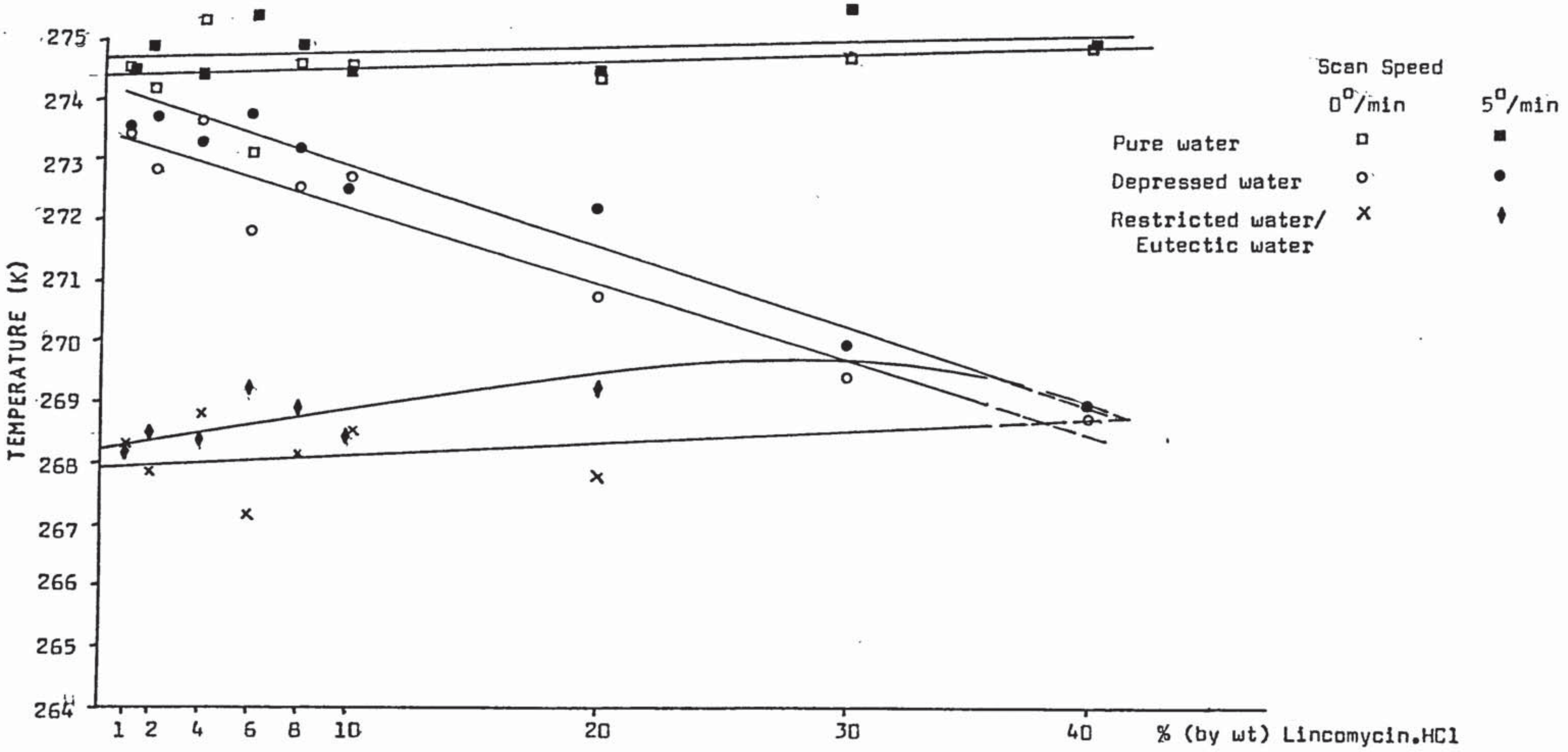
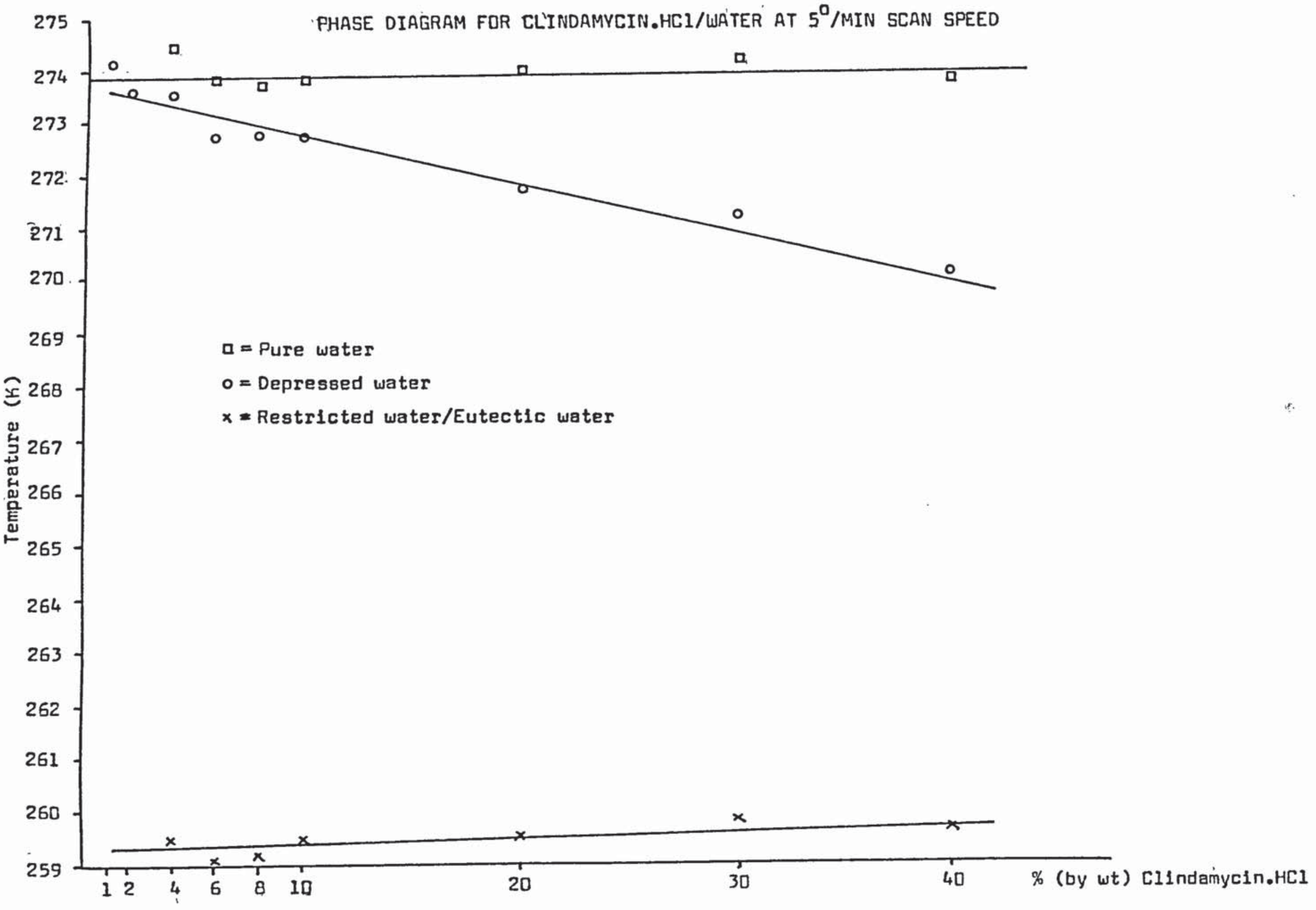


FIGURE IV (21)



PHASE DIAGRAM FOR CLINDAMYCIN.HCl/WATER AT 5°/MIN SCAN SPEED

259K transition of aqueous Clindamycin solutions being due to the melting of a eutectic would seem stronger. Figure IV (21) shows the phase diagram for the Clindamycin/water system. The depressed water peak temperature could only be envisaged as intercepting to 259K peak plot for very high concentrations of Clindamycin and even if this occurred, and was shown to be a eutectic point, it would not influence any of the other peak interpretations for the melting behaviour of water in Clindamycin/water/hydrogel systems since the 259K peak was the lowest detected transition temperature anyway. This was not the case for Lincomycin systems where many transitions have already been seen below 269K for water in Lincomycin/water/hydrogel systems. Thus unless a series of eutectic compositions and temperatures for all the transitions seen for Lincomycin/water and Lincomycin/water/hydrogel systems below and including 269K are to be proposed, an alternative explanation must be sought.

One such alternative explanation proposes that both antibiotics have similar eutectic temperatures of about 259K that being the lowest observed transition temperature for both antibiotics in any system studied so far. However, for this to be so the phase diagram for Lincomycin would have to include a large deviation in order to allow a eutectic point at such a low temperature. Sudden drops in the melting point curves of various hydrates are well known (as seen in sodium sulphate/water and ferric chloride/water systems), and since both antibiotics are known to form hydrates this complication of phase behaviour might well result in a eutectic composition melting temperature of 259K in each case.

It can also be seen from Figure IV (20) that there were significant differences between the plots of peak temperatures at 5^0/min scan rate and zero scan rate. This illustrated the importance of determining peak temperatures at zero scan rates in order to accurately follow the thermal behaviour patterns of such systems.

The problem concerning the possible formation and melting behaviour of crystalline eutectics of both antibiotics with water remains unresolved. However, it was considered unlikely that the 269K transition in Lincomycin/water systems was due to the melting of a eutectic because of the existence of lower temperature transitions. The most likely possible involvement of eutectics would be at the 259K transition for both Lincomycin/water and Clindamycin/water systems but this was far from proven. It was considered that the transitions above 259K in each case could confidently be considered as the melting of various structured water fractions for which there was only a limited number of previous reports in the literature,[139,135,140] these will be discussed later. This would then leave the 259K transitions as either structured water or eutectic melting. The overall conclusions remain unaffected by this uncertainty in that the interactions occurring in the various antibiotic/water and antibiotic/water/hydrogel systems were clearly of different character for each of the two antibiotics and apparently strongly influenced by the nature of the environment in which the antibiotic/water interactions were studied.

Throughout the remaining D.S.C. studies to be discussed in this chapter the majority of the transitions observed will be interpreted in terms of water structuring effects. However, the 259K transitions may in the course of future studies have to be re-assigned.

IV.5.ii Bound Water Levels Associated with Lincomycin and Clindamycin

Since the total area under each thermogram corresponds to the amount of freezing water in that sample and subtraction of this value from the equilibrium water content of the sample (E.W.C.) enables the amount of non-freezing water present to be determined, the number of "bound" water molecules per antibiotic molecule over a range of solution concentrations could be determined.

It was found that the least variation in the calculations of bound hydration levels occurred in the highest concentration antibiotic solutions (as might be expected) and the results are shown in Table IV A.

TABLE IV A

NUMBERS OF "BOUND" WATER MOLECULES ASSOCIATED WITH VARIOUS AQUEOUS SOLUTION CONCENTRATIONS OF LINCOMYCIN AND CLINDAMYCIN

% (by wt) Antibiotic	Number of Bound H_2O's/Antibiotic	
	Lincomycin	Clindamycin
10%	1.0 ± 1.0	6.0 ± 0.5
20%	2.9 ± 1.5	7.6 ± 0.5
30%	1.0 ± 1.0	9.2 ± 0.5
40%	2.0 ± 0.5	11.7 ± 1.5

The results indicated that there were significantly higher numbers of water molecules bound per molecule of antibiotic for Clindamycin than Lincomycin. The value for Lincomycin was typically 0.0 to 2.0 molecules of bound water and for Clindamycin 6.0 to 12.0 molecules of bound water.

A significant upward trend in the numbers of bound water molecules associated with Clindamycin as the solution concentration of the antibiotic increased was also recorded. Such an observation would suggest that a co-operative type of mechanism was occurring in aqueous Clindamycin solutions whereby with increasing Clindamycin concentration increasing numbers of water molecules per antibiotic molecule were being influenced. This process was not found with Lincomycin solutions. Co-operative type mechanisms of water structure propagation through systems have been described by Pauling[40] in explanation of anaesthetic action. The differences seen were in the amount of bound water per antibiotic molecule further illustrate the type of fundamental water structuring differences between Lincomycin and Clindamycin seen during this research.

IV.5.iii Affect of Temperature Cycling on Samples

Studies of the effects of temperature cycling of samples provided confirmation of the assignments of the pure water peaks when interpreting the multiple peak appearance of some of the thermograms already discussed.

The manner in which the pure water peak of a sample has been found to increase at the expense of less well crystallised water fractions on temperature cycling has been described previously.[10] The overall process was considered in terms of the gradual perfection of crystallisation within the system studied.

Results of D.S.C. scans of HEMA/Styrene copolymers swollen in water or aqueous 0.1M antibiotic solutions also showed a gradual incorporation of the non-freezing water fraction of the sample into the freezing water fraction. The amounts of water found to undergo such behaviour were small, typically 0.02 water molecules per functional monomer unit for samples where approximately 2.4 water molecules per functional monomer unit were found overall. These figures were comparable to similar observations of Pedley and Tighe.[10]

Unfortunately, similar processes in other preparations could not be studied over sufficient numbers of temperature cycles to enable adequate interpretation of the data. However, the affects of the presence of the antibiotic in such systems on the gradual non-freezing to freezing transition would be of considerable interest.

IV.5.iv Miscellaneous D.S.C. Studies

D.S.C. analyses were carried out on solid samples of the antibiotics used during these studies. In all cases, no deviations from the baseline were detected over the temperature ranges used during the D.S.C. studies described earlier.

D.S.C. studies were also made on dehydrated samples of certain systems and the results are presented later in this chapter.

IV.6 D.S.C. Studies on Antibiotic Incorporated Hydrogel Preparations

A series of antibiotic incorporated hydrogel preparations were synthesised which covered a range of E.W.C. and freezing/non-freezing water content systems. The preparations were used extensively during the permeability studies discussed in Chapter VI. The data pertaining to the characterisation of these preparations has already been presented in Chapter III.

Attempts have been made in this section to correlate the observations recorded here with the assignments of the various thermal transitions already seen. However, the positions of the peaks may in some cases not correlate precisely with those previously observed and whilst peak positions were found to vary within $\pm 1^{0}$ anyway it was considered that the increased antibiotic/water and antibiotic/polymer interactions in the preparations studied here may well have resulted in slightly greater fluctuations of the transitional temperatures.

IV.6.i Antibiotic Incorporated MAA/ACM/STY Preparations

D.S.C. analysis of MAA/ACM/STY preparations swollen in water or incorporating 10% (by wt) 1.0M Clindamycin.HCl or Lincomycin.HCl solutions (aq) only showed detectable transitions for water swollen samples. Figure IV (22) shows the sharp transition obtained corresponding to the melting of the pure water in the preparation at 274.5K.

IV.6.ii Antibiotic Incorporated HEMA/Styrene Preparations

The D.S.C. traces obtained from studies on water swollen and 10% 1.0M antibiotic solution incorporated HEMA/Styrene (90:10) (mole%) preparations are shown in Figure IV (23). The water swollen preparations displayed a sharp "pure" water melting transition at 275K and the melting of a slightly imperfect crystalline water component at 274K. The freezing water content of this system was 1.0%, for an E.W.C. of 23.1%.

The freezing water content of the Lincomycin incorporated preparation was comparable to that of the water swollen preparation, being 1.2% although its E.W.C. was slightly higher at 28.8%. Figure IV (23) shows that for this preparation an additional transition to that of the melting of the pure water fraction in the system at about 274K was detected at about 268K. This transition was identified as the same "restricted" water melting transition seen earlier in similar systems. However, it was also noticed that the thermal behaviour of this system (and indeed the Clindamycin incorporated preparation) was simpler than that found in the HEMA/Styrene swollen in 1.5M antibiotic solution preparations (Figure IV (18)). The presence of antibiotic and water in the monomer mixture before polymerisation seemed to result in simpler peak

FIGURE IV (22)

MELTING ENDOTHERM OF MAA/ACM/STY
WATER SWOLLEN HYDROGEL

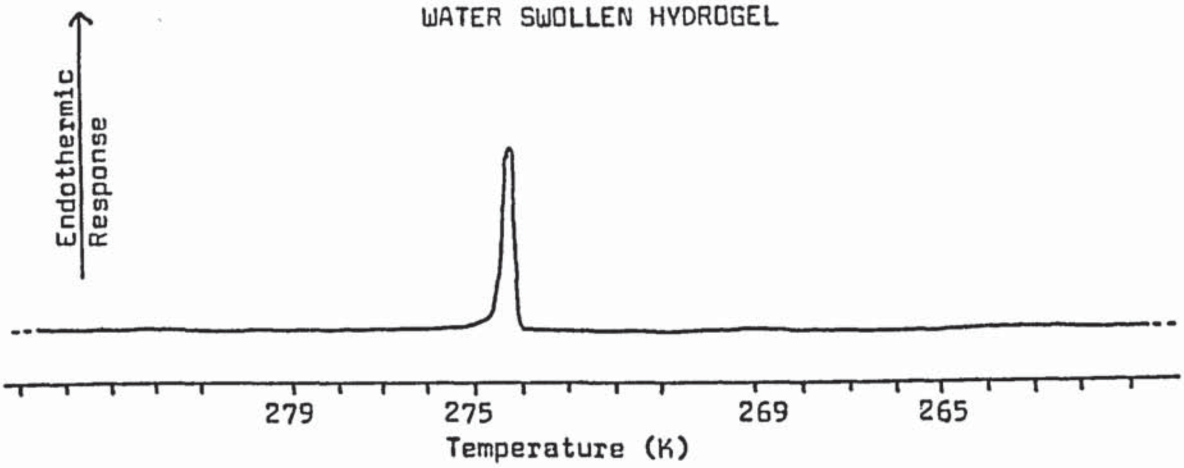
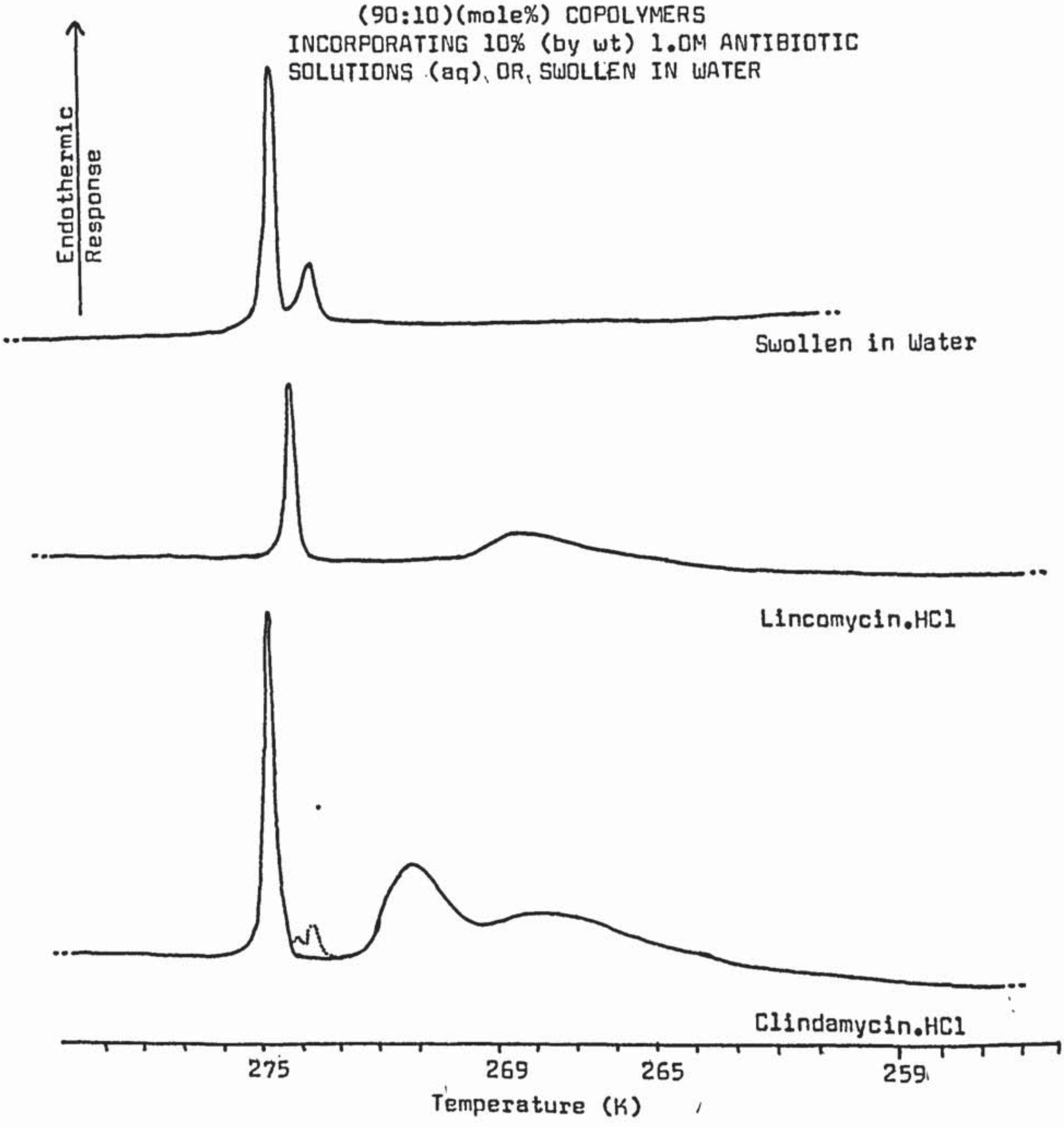


FIGURE IV (23)

MELTING ENDOTHERMS OF HEMA/STYRENE
(90:10)(mole%) COPOLYMERS
INCORPORATING 10% (by wt) 1.0M ANTIBIOTIC
SOLUTIONS (aq) OR SWOLLEN IN WATER



structures than were found for polymerised systems swollen in antibiotic solutions. This was presumably the result of a decrease in the extent of water structuring by the antibiotics in antibiotic incorporated preparations due to interaction between the antibiotic and the monomers before polymerisation. In this particular system the differences in the E.W.C's of the two different types of preparation were not large except for Clindamycin which resulted in an E.W.C. of 34.6% when Clindamycin solution was incorporated but 42.3% when the preparation was swollen in 1.5M Clindamycin solution.

The overall effect of antibiotic incorporation seems to have been to increase monomer unit/antibiotic interactions before monomer unit/water interactions were established thus resulting in a less complex water structured system.

Returning to Figure IV (23), the D.S.C. trace of the Clindamycin incorporated preparation shows a transition at 275K associated with the melting of pure water in the system. The dotted lines illustrate the melting of imperfect crystalline water fractions that were occasionally observed (as mentioned earlier). This preparation also displayed a transition at 268K as seen for the Lincomycin incorporated preparation as well as a transition at 271K (not observed for the Lincomycin incorporated preparation). This followed similar observations on the 1.5M antibiotic solution swollen forms of these preparations where a 271.5K transition was found for the Clindamycin solution swollen preparation. This was interpreted here as being the same "depressed" water peak as seen in the antibiotic solution swollen preparations and the absence of such a transition in both types of Lincomycin preparations could be attributed to their lower E.W.C's (and freezing water contents) than the corresponding Clindamycin preparations.

The above antibiotic incorporated HEMA/Styrene preparations not only provided further evidence of both antibiotics having the same inherent water structuring effects when in suitable environments but also suggested increased antibiotic/monomer unit interactions in antibiotic solution incorporated systems than in antibiotic solution swollen systems.

IV.6.iii Antibiotic Incorporated MAA/ACM Preparations

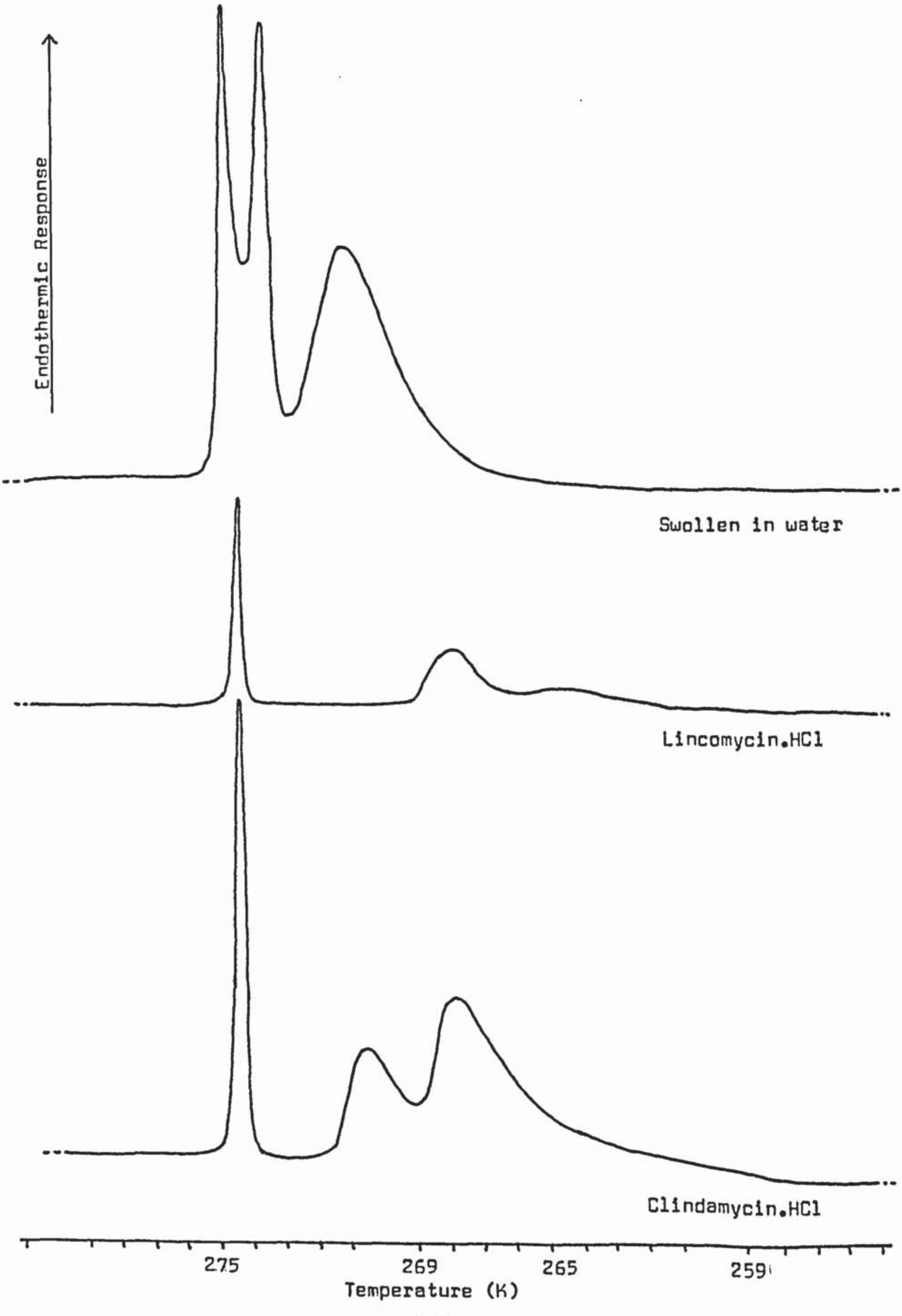
The D.S.C. traces obtained from analysis of MAA/ACM preparations swollen in water or with 10% 1.0M antibiotic solution incorporated can be seen in Figure IV (24).

The water swollen preparation displayed peaks at about 275K and 274K corresponding to the melting of pure water and imperfectly crystalline water fractions respectively. However, an additional transition was also found at 271K, the interpretation of which was more involved. Transitions in antibiotic containing systems at about 271K have previously been designated "depressed" water fractions this being supported by the extent of the depressions observed for a range of concentrations of aqueous antibiotic solutions.

However, such an assignment here would not seem justified since although a similar 271K peak was found for the Clindamycin incorporated preparation, no such transition was found for the Lincomycin incorporated preparation. Both the antibiotic incorporated preparations displayed transitions at 268K, these being assigned to "restricted" water fractions of the type previously seen to melt at about this temperature. However, the Lincomycin incorporated preparation also displayed a transition at about 264.5K. This corresponded to the temperatures of transitions seen

FIGURE IV (24)

MELTING ENDOTHERMS OF MAA/ACM COPOLYMERS INCORPORATING 10% (by wt) 1.0M ANTIBIOTIC SOLUTIONS (aq) OR SWOLLEN IN WATER



in other systems earlier, for example pACM preparations swollen in 1.5M antibiotic solutions (Figure IV (17)) and an aqueous solution of acrylamide monomer (Figure IV (10)). The same transition may well have been present for the Clindamycin incorporated MAA/ACM preparation but obscured by the onset of the 268K transition. The 264.5K transition may therefore be the melting of another small structured water fraction. The 271K transition, if not a depressed water fraction, may well be yet another structured water state within the continuum of water states already proposed. If this were the case, the absence of this transition in the Lincomycin incorporated preparation but its presence in the Clindamycin incorporated preparation once more illustrates the different types of water structure these antibiotics produce depending on the nature of their environment. The study of the thermal behaviour of a range of different MAA/ACM compositions would enable the positive identification of the 271K transition since whilst a depressed water fraction would melt at a temperature depending on sample composition, structured water fractions have already been seen to melt at fairly constant and readily identifiable temperatures. The presence of depressed water fraction sizes of the order water swollen > Clindamycin incorporated > Lincomycin incorporated = zero would seem likely considering the similar trend of the freezing water contents of these preparations even though their E.W.C's were similar (Chapter III).

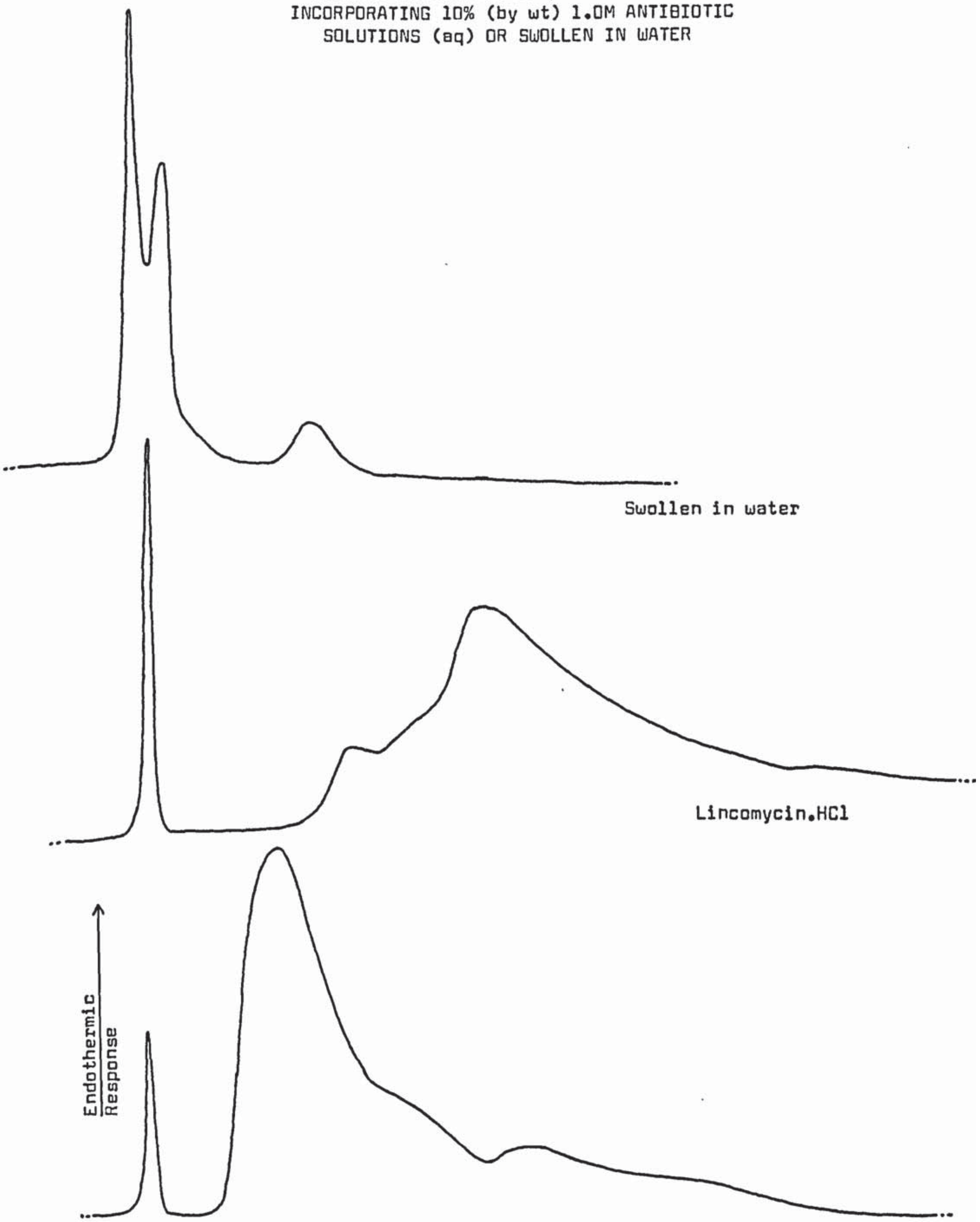
IV.6.iv <u>Antibiotic Incorporated pHEMA Preparations</u>

The D.S.C. traces obtained from the analyses of water swollen and 10% (by wt) 1.0M antibiotic solution (aq) incorporated pHEMA preparations are shown in Figure IV (25).

The 275K and 274K transitions observed for the water swollen preparations

FIGURE IV (25)

MELTING ENDOTHERMS OF pHEMA HYDROGELS INCORPORATING 10% (by wt) 1.0M ANTIBIOTIC SOLUTIONS (aq) OR SWOLLEN IN WATER



were identified as the melting of pure water and slightly imperfect crystalline water fractions respectively. The 269K transition also found was considered to reflect some of the inherent water structuring capabilities of the matrix itself, also evident in aqueous HEMA monomer solutions (Figure IV (12).

The Clindamycin incorporated preparation displayed a large transition at 270K. This would correspond to the melting of a depressed water fraction, a process not observed at this temperature for the Lincomycin incorporated preparation. This was explained again (as for the similar observations on MAA/ACM preparations) in terms of the higher freezing water content of the Clindamycin incorporated preparation (20.2%) than the Lincomycin incorporated preparation (10.1%). However, since the Lincomycin incorporated preparation in this case had a significant freezing water content (compared with the 1% or 2% found in previous Lincomycin incorporated preparations) the higher effective concentration of antibiotic in this freezing water fraction than in the Clindamycin incorporated preparation resulted in a smaller but lower temperature (further depressed) depressed water peak than was found with the Clindamycin incorporated preparation. This peak was found at 263.5K superimposed on other structured water fraction transitions. The Lincomycin incorporated preparations displayed a 268K transition corresponding to previously identified restricted water which in the case of the Clindamycin preparation was obscured by the depressed water transition onset. Both antibiotic incorporated preparations showed substantial shoulders at 265K to 266K a temperature seen earlier to be characteristic of the melting of small structured water fractions. A transition was also observed at 262K for the Clindamycin incorporated preparation although it would have been obscured by the depressed water peak in the case of the Lincomycin incorporated preparation. The only previously observed transition at about this temperature was an exothermic one at 263K for pACM swollen in 1.5M Lincomycin solution (aq). It may well be that this temperature represents another temperature at which mildly stabilised water fractions melt. However, the Clindamycin incorporated preparations also showed a minor transition at about 256K and the Lincomycin incorporated preparations showed small transitions at 253K. The temperatures of these transitions were the lowest of any preparations studied during the course of this research and since they were not "characteristic" temperatures for the melting of structured water fractions their consideration as further structured water fractions within the continuum of water states can only be justified after further study.

Throughout these studies a number of readily identifiable transition temperatures were observed. These were considered as "characteristic" temperatures for the melting of particular types of structured water. Not all these transitions were observed in each case though, differences in the transitions seen for similar preparations with different antibiotics introduced, frequently being recorded. This led to the suggestion that both Lincomycin and Clindamycin have the same inherent water structuring ability. However, which facets of these abilities are actually demonstrated in any particular system depends on the nature of the preparation itself and the effects can be different for each of the antibiotics.

It was difficult to draw a general trend of the types of water structuring effects seen for each antibiotic when in certain E.W.C. and freezing/non-freezing water content systems. However, there appeared to be a transition point found for pHEMA and MAA/ACM preparations where the nature of the complex antibiotic/water/polymer competitive interactions reached a peak and this led to the multiple peak nature of the D.S.C. traces seen. This type of interaction was perhaps the most finely balanced for the antibiotic incorporated pHEMA preparations for which the determination of the freezing/non-freezing water composition of the preparations was subject to a large variation. This variation was found not only from sample to sample but from run to run on the same sample and was considered indicative of the finely balanced complex competitive interactions in the pHEMA preparations (see Chapter III).

The implications of the water structuring capabilities of these antibiotics are obvious. The antibiotics can themselves alter the freezing water and non-freezing water composition of a given system and the water structuring affects of the antibiotics can be manifest in different ways depending on the nature of the system itself. If the system considered were a cell or membrane transport system then any cellular process controlled or dependant upon water structure for its integrity could be severely disrupted by alteration of water structure by the antibiotics. The fact that this could occur for one antibiotic and not the other would allow the differences in the extents of irritation of the two antibiotics to be accounted for.

IV.6.v D.S.C. Analysis of Dehydrated and Rehydrated Hydrogels

Further evidence illustrating the essential role of water in the multiple peak thermal behaviour of systems studied earlier in this chapter was obtained from D.S.C. studies of dehydrated samples of preparations which demonstrated multiple peak formation when in the hydrated state. The samples of the preparations were dried in the manner used during the determination of E.W.C., as described in Chapter II.

The D.S.C. traces for all the dehydrated samples studied showed no deviation from the baseline over the temperature range employed. The preparations studied in the dehydrated state included pHEMA, HEMA/Styrene, MAA/ACM and MAA/ACM/STY polymers either swollen in water or with 10% 1.0M aqueous antibiotic solution incorporated.

The observed lack of any transition illustrates the importance of the presence of water in the preparations for the display of multiple peak thermal behaviour.

A number of samples that had been dehydrated were also rehydrated in a minimum of distilled water (typically 1.0 ml). Even after rehydrating for only 24 hours samples such as MAA/ACM incorporating Lincomycin or Clindamycin regained a large proportion of the freezing water content and multiple peak thermal behaviour that had been found with the original hydrated sample. However, it was observed from these limited studies that the thermal transition profiles were less diverse than in the original hydrated samples. These effects were probably due to the disturbance of the balance of interactions in the preparations as a result of the dehydration/rehydration process. This area seemed worthy of further investigation.

IV.7 D.S.C. Cooling Studies of Antibiotic Preparations and Some Observations on Sodium Chloride Preparations

D.S.C. studies can also be made of the crystallisation processes occurring in samples during cooling. The process can be controlled and calibrated in the same manner as for sample heating. However, D.S.C. studies in the cooling mode, especially for hydrogel systems, are poorly documented and transition interpretations uncertain. It must be stressed that the studies presented here require expansion before the assignments and theories presented can be confirmed.

IV.7.1 D.S.C. Cooling Studies on Aqueous Antibiotic Solutions

The D.S.C. traces obtained during cooling studies on distilled water and 0.1M aqueous solutions of Lincomycin and Clindamycin are shown in Figure IV (26), Figure IV (27) and Figure IV (28) respectively.

Previous cooling studies carried out by Pedley and Tighe[10] on hydrated equimolar copolymers of methacrylic acid and acrylamide have already been discussed and their results shown in Figure IV (2). Other observations of relevance here include studies of the homogeneous nucleation of water and before considering Figures IV (26) to IV (28) it is worth discussing these studies.

The equilibrium freezing of an aqueous system requires, among other things, that an ice crystal nucleus be made available at the equilibrium freezing point of the system. The use of emulsification procedures[37,38] has made possible the study of the homogeneous nucleation temperature of water ie the temperature at which water undergoes spontaneous crystallisation in the absence of any impurity (the crystallisation of water in the presence of an impurity is known as heterogeneous nucleation).

MacKenzie[37] found the homogeneous nucleation temperature of water to be -38.3°C and aqueous solutions of solutes were found to undercool well below the homogeneous nucleation temperature of water.[37,152] For example, a 40% (by wt) aqueous solution of sucrose had a "homogeneous" nucleation temperature of -51.8°C. A fact also considered by Franks.[153,154] The general nature of such cooling traces follow the form shown in

FIGURE IV (26)

FREEZING EXOTHERM OF WATER

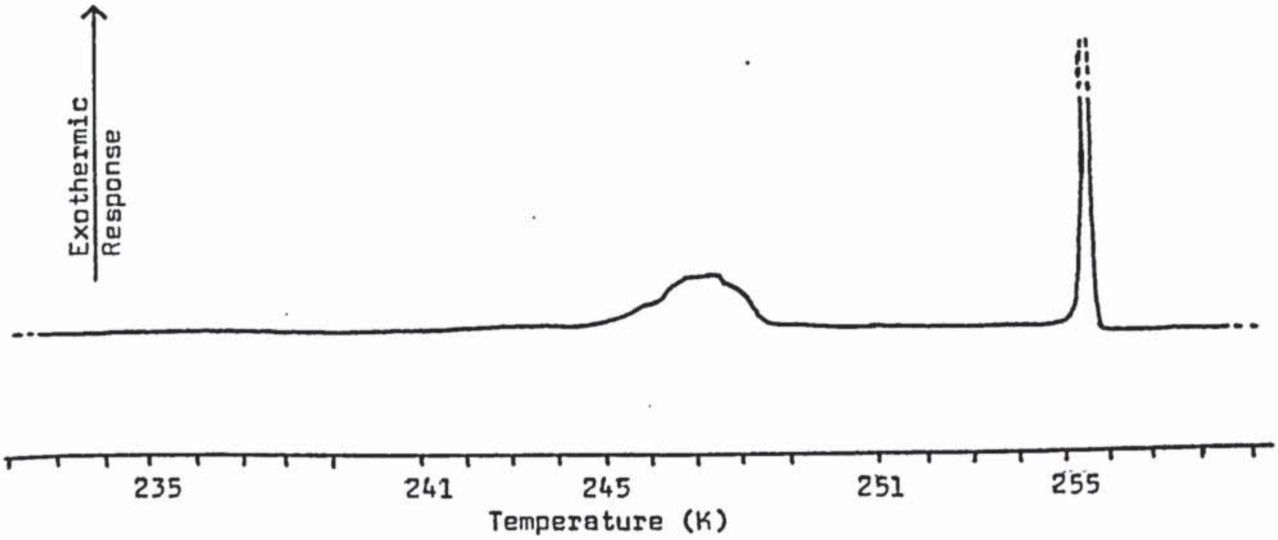


FIGURE IV (27)

FREEZING EXOTHERMS OF 0.1M LINCOMYCIN.HCl SOLUTION (aq) AFTER SUCCESSIVE TEMPERATURE CYCLES

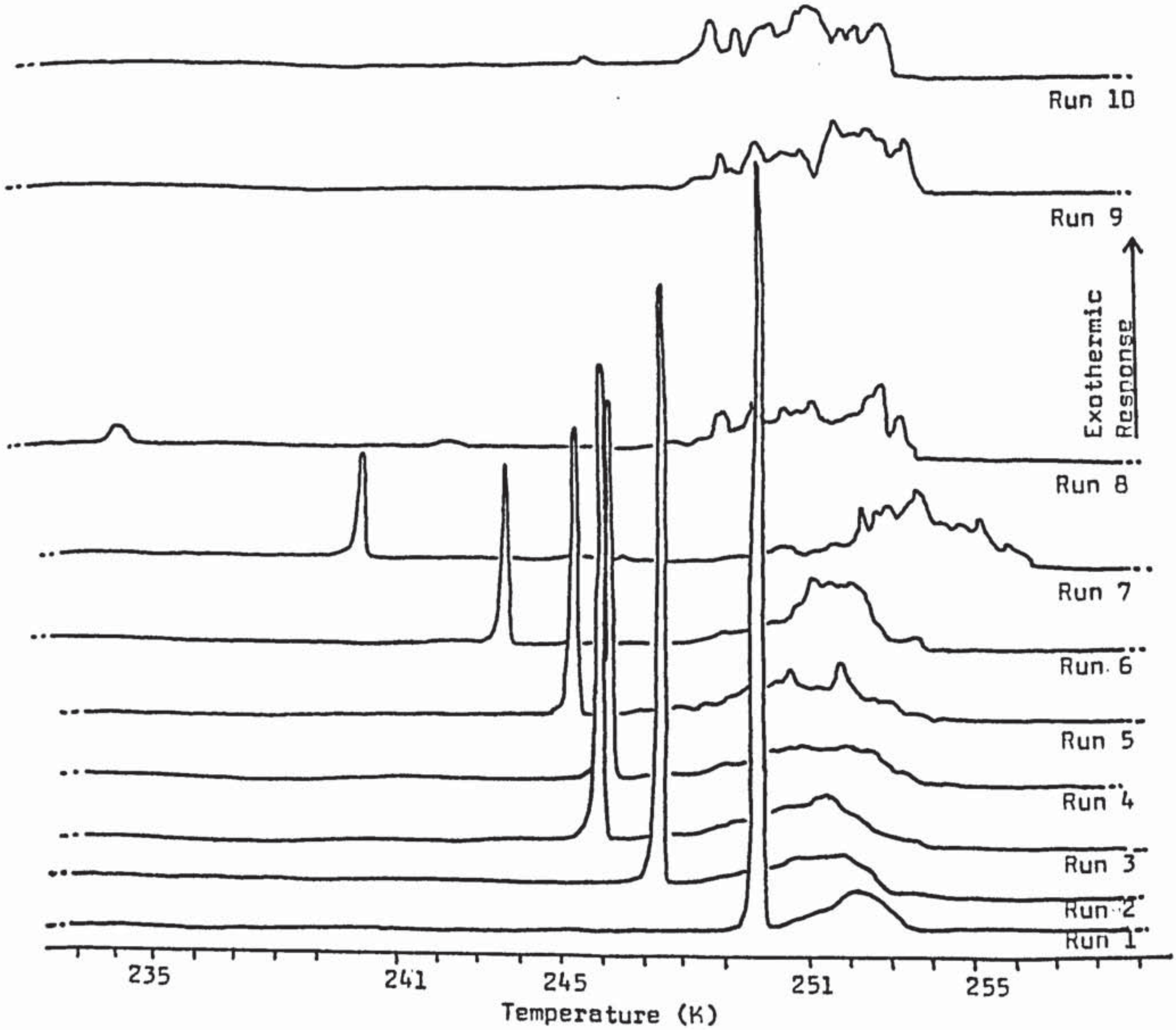


FIGURE IV (28)

FREEZING EXOTHERMS OF 0.1M CLINDAMYCIN.HCl
SOLUTION (aq) AFTER SUCCESSIVE TEMPERATURE CYCLES

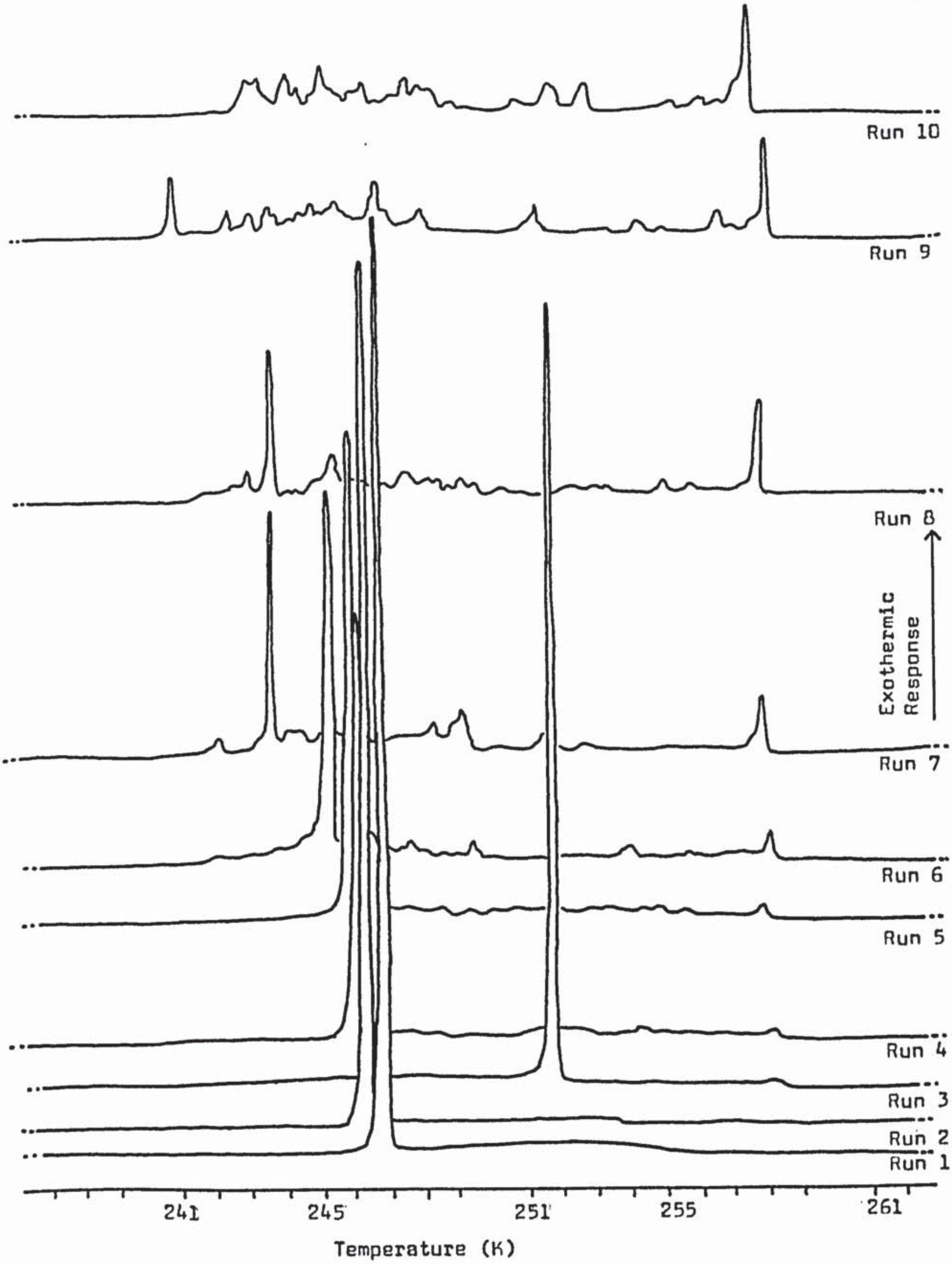
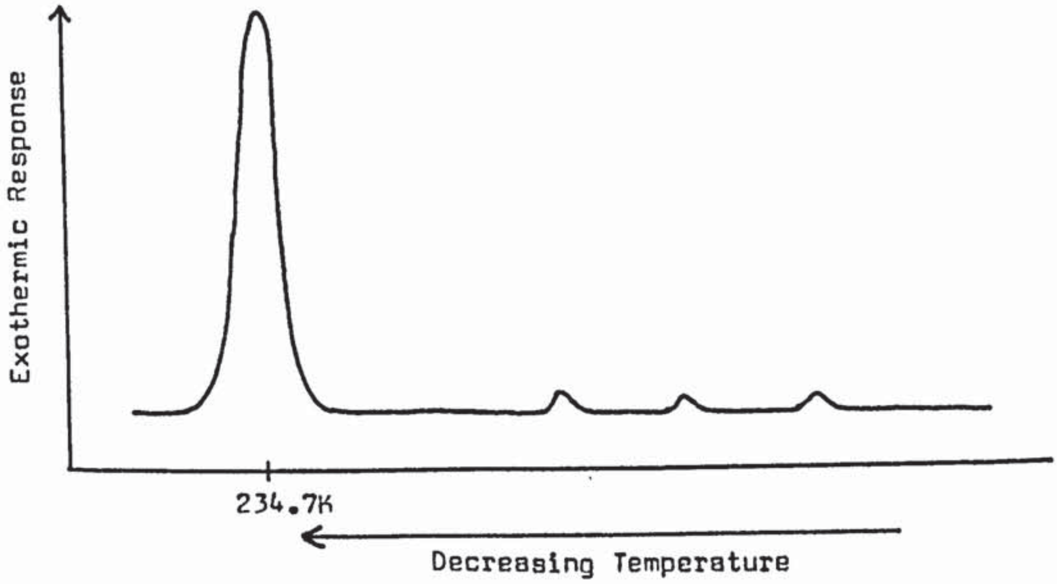


Figure IV (29). The transition at $-38.5^{\circ}C/234.7K$ represents the homogeneous nucleation of water. The small peaks occurring randomly during the cooling of the sample prior to homogeneous nucleation represent the crystallisation of water in a small number of the emulsified droplets in which the crystallisation was seeded by impurities. However, the finite number of impurities in an infinite number of emulsified droplets allows the majority of water in the impurity-free droplets to undergo simultaneous crystallisation at the homogeneous nucleation temperature of water.

Referring back to Figure IV (26), the freezing exotherm of water, the main water crystallisation peak at about 256K correlated well with that previously assigned to the freezing of pure water in hydrogels.[10] However, occasionally a small "fine structure" similar to that seen in Figure IV (2) was found for water samples. This was presumably due to sample contamination and impurities.

FIGURE IV (29)

TYPICAL HOMOGENEOUS NUCLEATION OF WATER



The Figures IV (27) and IV (28) show that for the aqueous antibiotic solutions the "fine structure" was generally superimposed onto or at a slightly lower temperature than the "pure" water crystallisation peak, although considerable variation was found, in some cases the fine structure appearing before the main crystallisation peak.

The affect of temperature cycling on these systems can also be seen in these figures. The general effect was to increase the "fine structure" component at the expense of the pure water peak. After many temperature cycles the fine structure became spread over a wide temperature range and was composed of many small sharp transitions (especially in the case of aqueous Clindamycin solutions). The presence of these apparently random small transitions was reminiscent of similar structures reported during homogeneous nucleation studies of water. Studies on more concentrated aqueous solutions of the antibiotics (0.25M) yielded similar results.

In all of these cooling studies both the antibiotics seemed to be showing similar effects on the freezing of water in the solutions. In both cases a pure water crystallisation temperature was found at a fairly constant temperature of about 245K to 250K with an associated fine structure due to the crystallisation of impure water fractions, or more likely, "restricted" water fractions. Such structures were found at lower temperatures than the pure water transitions because of their "reluctancy" to freeze.

However, D.S.C. studies of many systems showed earlier that the pure water component increased with temperature cycling when the sample was heated. The cooling studies suggested that the pure water component decreases on temperature cycling. There must be some major reorganisation processes occurring in such samples during temperature cycling that leave us with something of a paradox.

The observed pure water peak decrease and simultaneous broadening of fine structure on cooling would suggest gradually less specifically identifiable forces influencing the crystallisation processes. It was possible that this process was associated with small "compartmentalised" amounts of water influenced by the antibiotics, the number of these increasing as their average size decreased during temperature cycling as the pure water peak decreased (as seen when cooling the samples). However, because the temperature cycling had led to more and more water having similar properties ie the extent of the diversification of water structures in the sample had decreased, when the sample was heated more and more water melted at the same temperature, ie the pure water peak increased (as seen when heating the samples). When re-cooled the reorganisation within the samples continued toward a more homogeneous dispersion of water and antibiotic.

During the cooling process the pure water peak was also observed to move to progressively lower temperatures. (Figures IV (27) and IV (28)). Such a process could be accounted for in the above model if at the initial stage of temperature cycling small domains of pure water existed in the samples protected in some way from being initiated into crystallisation by the antibiotics due to their structured water layers. As temperature cycling continues and the system becomes more homogeneous the pure water domains become smaller but the deeper structured water layers between these domains and the antibiotics result in later and later initiation of crystallisation of the pure water domains and so the freezing of the pure water occurs at lower and lower temperatures. This process might be analogous to the unexplained depression of homogeneous nucleation temperature of water by solutes in the water phase of emulsions.[37] The lowest temperature cooling transition observed occurred at 234K (Figure IV (27)), this was the position of the pure water transition of an aqueous Lincomycin solution after eight temperature cycles. By this stage the peak was quite small and was not detected on the subsequent cooling run. The final temperature of this peak (234K) correlates well with the homogeneous nucleation temperature of water[37] and might suggest that the explanation for the cooling behaviour trends above was a reasonable one. However, further research was required before the above speculative theory could be substantiated.

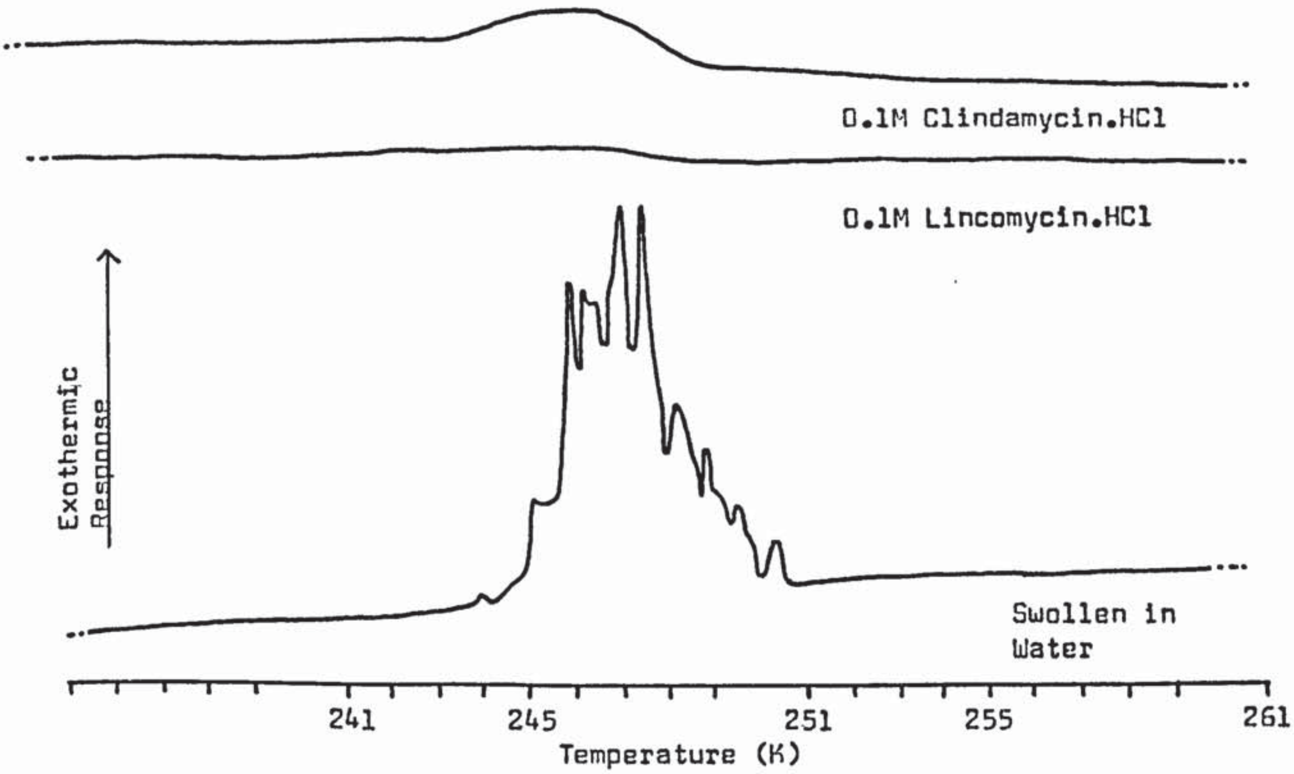
IV.7.ii D.S.C. Cooling Studies on Antibiotics in Hydrogel Preparations

D.S.C. cooling traces for HEMA/Styrene copolymers swollen in water or 0.1M aqueous antibiotic solutions can be seen in Figure IV (30). Figure IV (30) shows that no pure water transitions were observed. This was because there was a very small amount of freezing water in these systems and the freezing water that was present produced the "fine structures" seen. The fine structures of the water swollen preparations were more clearly resolved into a number of closely situated sharp peaks than the comparatively smooth transitions of the antibiotic solution swollen preparations, the resolution increasing on temperature cycling.

However, the results of these studies did not contribute much to the understanding of the crystallisation processes involved, with the exception of evidence of the fine structures remaining in a narrower temperature band than was found during the solution cooling studies after temperature cycling. The physical constraint of the matrix presumably retarded some of the reorganisation processes. Far more studies on the cooling of other hydrogel preparations are necessary before the nature of the effects producing the crystallisation observations reported here can be clarified.

FIGURE IV (30)

FREEZING EXOTHERMS OF HEMA/STYRENE (90:10)(mole%) COPOLYMERS SWOLLEN IN VARIOUS SOLUTIONS (aq)

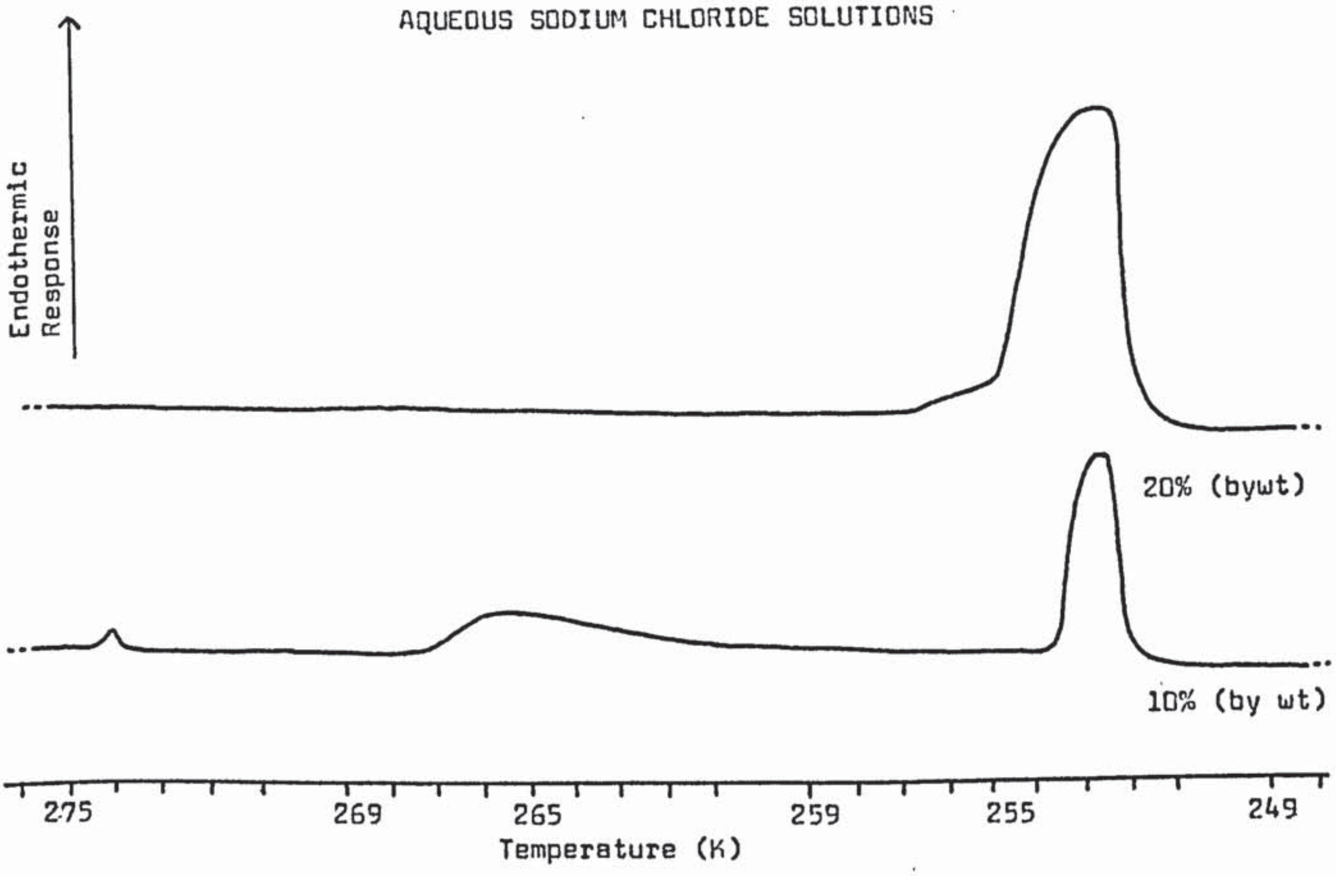


IV.7.iii D.S.C. Studies on Sodium Chloride Systems

The melting endotherms of some aqueous sodium chloride solutions can be seen in Figure IV (31). This clearly illustrates the simpler aqueous solution melting behaviour of sodium chloride than seen for the antibiotics. The 10% (by wt) aqueous solution of sodium chloride surprisingly showed trace amounts of "pure" water at 274K, not seen for the 20% (by wt) solution. Both solutions showed a large endothermic transition at 252.5K corresponding to the melting of the crystalline eutectic of such samples as reported by Homshaw[135] (-21.4°). The transitions seen at about 265.5K and 255.5K (shoulder on eutectic peak) for the 10% (by wt) and 20% (by wt) aqueous sodium chloride solutions respectively agree well with the expected depressions of the freezing

FIGURE IV (31)

MELTING ENDOTHERMS OF CONCENTRATED
AQUEOUS SODIUM CHLORIDE SOLUTIONS



points of -6.6° (266.6K) and -16.5° (256.7K) respectively.[155]

The D.S.C. trace obtained from the cooling of a 10% (by wt) aqueous sodium chloride solution can be seen in Figure IV (32). The position of the main "pure" water freezing exotherm was at about 248K. Since the corresponding peaks of pure water samples were found at 255K to 256K this corresponded fairly well with the expected depression of -6.6° (about 248.5K). A small transition at about 230K was also seen corresponding well with the expected position of the eutectic formation.

The D.S.C. traces obtained from heating pACM preparations swollen in 0.1M and 1.0M aqueous sodium chloride solutions can be seen in Figure IV (33). The sample swollen in 0.1M sodium chloride solution

FIGURE IV (32)

FREEZING EXOTHERM OF A
10% (by wt) AQUEOUS SODIUM CHLORIDE SOLUTION

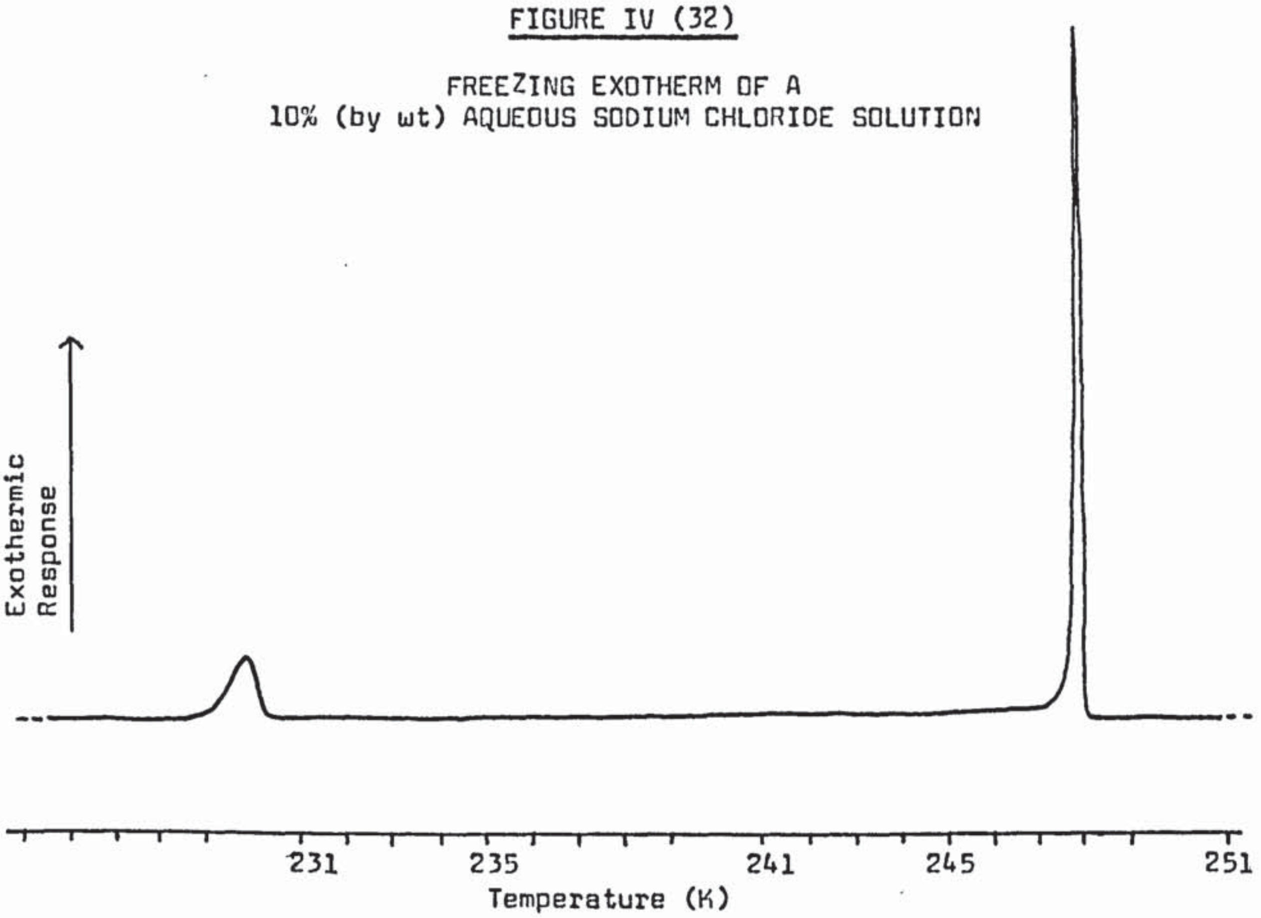
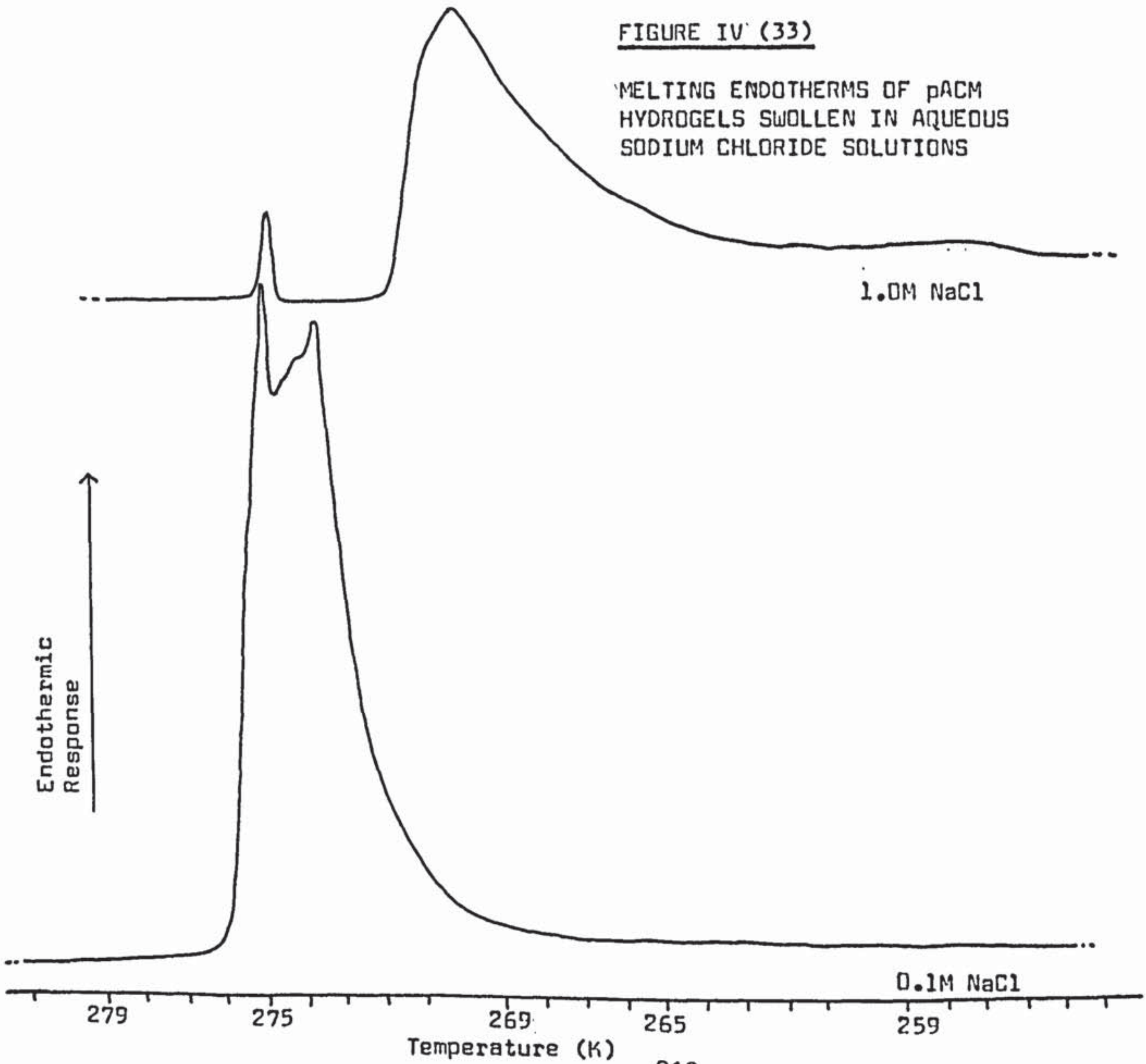


FIGURE IV (33)

MELTING ENDOTHERMS OF pACM
HYDROGELS SWOLLEN IN AQUEOUS
SODIUM CHLORIDE SOLUTIONS



did not show behaviour appreciably different from that seen for pACM swollen in 0.1M solutions of other solutes (Figure IV (15)). However, the samples swollen in 1.0M sodium chloride solution displayed very characteristic D.S.C. trace profiles. A small peak at about 275K corresponding to the melting of a small pure water fraction in the system could be identified. A large transition at about 270K corresponded with the expected depression of most of the remaining water in the sample and a small transition at about 257.5K corresponding roughly to the melting of the sodium chloride/water eutectic was also just discernible.

The melting endotherm of a HEMA/Styrene copolymer swollen in 0.1M aqueous sodium chloride solution can be seen in Figure IV (34). The thermal behaviour observed was not significantly different from that seen during studies of corresponding water swollen, and various other solution swollen HEMA/Styrene preparations (Figure IV (16)) but the temperature range studied did not include the melting of the sodium chloride/water eutectic.

IV.8 Brief Summary of D.S.C. Observations and Interpretations

Although the various sections of this chapter have themselves been summarised a brief overall summary is appropriate. The multiple peak thermal behaviour reported in this chapter was of a nature not widely reported in the literature. Comparable observations on hydrogel systems have only been made by Ahad[139] and Lee, Jhon and Andrade[140] as well as members of this research group[10,16] to the author's knowledge although the work of Homshaw must also be noted.[135] Molyneux[156] commented on the scarcity of polymer/water studies in general in 1975 but although progress may have been rapid in this general field, hydrogel/water/

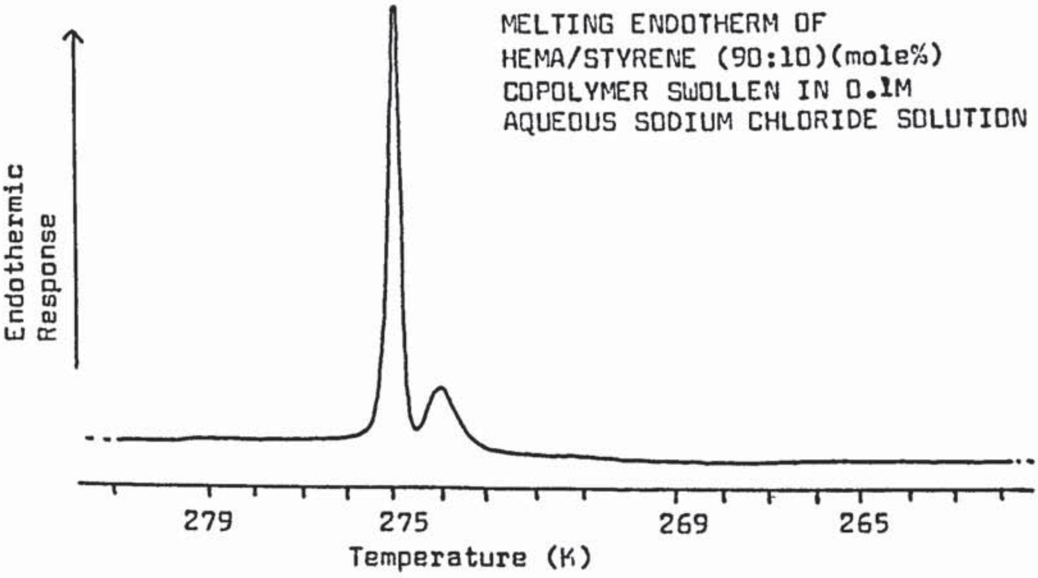


FIGURE IV (34)

MELTING ENDOTHERM OF HEMA/STYRENE (90:10)(mole%) COPOLYMER SWOLLEN IN 0.1M AQUEOUS SODIUM CHLORIDE SOLUTION

solute interactions of the type described here are not well documented. The lack of previously reported multiple peak formations may partly be explained by the tendancy in previous work to use faster heating rates and rather less sensitive instrumentation than were used here. This would result in the melting endotherms having poorly resolved shoulders which might easily be overlooked.

Alternative explanations of the peak structures, such as eutectic formation have been discussed and all but discounted in this chapter, and much work has been shown which backs up the water structuring hypothesis.

Recent work by Grossman, Tirrell and Vogl[157] showed simpler but similar melting endotherm structures, also commenting that the interpretation of such behaviour could include a change in the thermal conductivity of the sample during melting as suggested to them by Franks, as well as water structuring contributions.

These problems apart, the studies described in this chapter suggest that Lincomycin and Clindamycin have the same inherent water structuring properties but that these are demonstrated to different extents in different environments.

The next logical stage of this research was therefore to study systems in which the water structuring properties of the antibiotics might exert effects that could be compared with their properties in biological systems. This work has been described in the following chapters.

CHAPTER V

PERMEABILITY STUDIES I - SYSTEM CHARACTERISATION AND ANTIBIOTIC PERMEATION STUDIES

V.1 Introduction

The research presented so far in this thesis has demonstrated a number of fundamental differences in the physical-chemical properties of Lincomycin and Clindamycin, not least of which was their differing water structuring abilities. This chapter is concerned with the transport properties of the antibiotics themselves across hydrogel membranes of different types of water environment.

Some previous studies on the effects of membrane structure and pore size have already been discussed within the context of hydrogel characterisation. Despite these studies the role of the nature of water in such preparations and its organisation in determining transport phenomena is largely unexplained. Refojo[115] concluded from his studies on the water permeability of hydrogels that for high E.W.C. preparations (60% E.W.C.) transport was predominantly viscous flow whereas in lower E.W.C. preparations diffusion seemed to be more important.

Hydrophilic solutes and hydrophobic solutes have been postulated to possess different permeation mechanisms. Yasuda et al[121] developed a "free volume" model for hydrophilic solute permeation based on the following assumptions;

(i) The effective free volume for solute diffusion corresponds to the free volume of the aqueous phase,

(ii) The solute diffuses through "fluctuating pores" by successive jumps, and

(iii) The solute permeates through aqueous regions, solute-solute interactions are minimal.

Kim et al[158,159,160] reported from hydrophilic and hydrophobic solute transport studies that the water content and extent of cross-linking of hydrogels had the most profound influences on permeability.

In general, solutes can be considered to permeate by either "pore" or "partition" type mechanisms. Porous flux of a solute can be considered to be associated with the bulk water regions of hydrogels whereas partition flux is associated with the polymer matrix and interfacial and bound water components of the hydrogel.

The studies of Kim et al[158] on pHEMA reached the following conclusions;

(i) Hydrophilic solutes permeated via a pore mechanism and the partition coefficients depended on solute molecular size. Bulk water in hydrogels was mainly used for transport and solute permeability increased as the E.W.C. of the hydrogel increased.

(ii) Hydrophobic solutes permeated by either pore or partition mechanisms and diffusion coefficients were lower than for hydrophilic solutes.

There have been other attempts to develop theoretical frameworks on which the analysis of transport properties of network polymers can be based for example Kulkarni and Mashelkar[161,162]. Recently, Edmonds[163,164] developed a model for membrane ion channels based on ordered water structures, however there is as yet no direct evidence that such ordered water channels exist.

There are a number of studies of drug permeation across hydrogel membranes reported in the literature. However, the majority of these are concerned with the development of zero-order, sustained release drug delivery systems.[165,166,167] Studies reported by Spacek and Kubin[168] and Ratner and Miller[123] illustrate the hemodialysis applications of hydrogel materials. Many studies of the permeation of pharmaceutical compounds across polymeric membranes have been reported but few of these are of direct relevance to this research.[169,90,170,171,159,172,160]

V.2 Characterisation of Transport Processes

V.2.1 General Transport Parameters

The end result of a transport process is the appearance of the species under consideration at the other side of the barrier. The permeability coefficient, P, takes into account physico-chemical interactions between the material of the membrane and the solute and can be considered as the product of the diffusion coefficient, D, and the partition coefficient, k_D, as shown in Equation V.1.

$$P = Dk_D \qquad \text{Equation V.1}$$

where the diffusion coefficient reflects the intrinsic mobility of the transported substance inside the membrane and the partition coefficient expresses the solubility of the solute within the membrane. This can be defined as in Equation V.2.

$$k_D = Cg/C \qquad \text{Equation V.2}$$

where Cg and C are the equilibrium concentrations of a given substance inside the swollen hydrogel and in its environment respectively.

If solute movement follows known laws then the quantity of material which moves across the membrane, Q, can be expressed by Equation V.3.

$$Q = (P\Delta p/h)\,At \qquad \text{Equation V.3}$$

where Δp is the pressure difference between the input and output sides of the membrane, A is the cross-sectional area, t is the time involved, h is the membrane thickness and P is the permeability coefficient. Implicit in Equation V.3 is a rate factor since time is included as well as a quantitative term related to the amount of penetrant which can be accommodated by the membrane.

Transport behaviour can be considered under three types of conditions. Under steady-state conditions Fick's first law of diffusion states that the rate, dA/dt, of diffusion of A moles per unit time is proportional to the surface area of diffusion, $S(cm^2)$, and the concentration gradient (the rate of change of concentration C' with distance x in cm, dC'/dx) in an isotropic medium.

$$dA/dt = D' \, SdC'/dx \qquad \text{Equation V.4}$$

where D' is the intrinsic diffusion coefficient.

The experimental steady state conditions specify that the concentration gradient dC'/dx is constant, so that at any two planes in the isotropic medium where $x = 0$ at C'_2 and $x = X$ at C'_1

$$dA/dt = D'S(C'_2 - C'_1)/X \qquad \text{Equation V.5}$$

where X = membrane thickness (cm)

C'_1 = concentration of desorbing solution

C'_2 = concentration of diffusing solution.

The steady-state equation (Equation V.5) states that the rate is constant with time for constant concentrations C'_2 and C'_1.

However, quasi-steady-state diffusion differs from steady state diffusion in that the concentrations of the diffusing and desorbing solutions are not held constant. The concentration, C_2, of the diffusing solution decreases as the concentration, C_1, of the desorbing solution increases and the concentrations of both solutions approach the same equilibrium values with time. In such cases it is possible to obtain a value for the permeability coefficient from the slopes of plots of $\log(C_2-C_1)/C_0$ versus time, where C_0 = initial donor solution concentration.

Whereas in steady-state transport and quasi-steady-state transport it is the constant concentration gradient alone and both the concentration gradient and rate of approach of solution concentrations to equilibrium respectively that are rate determining, in non steady-state diffusion the rate determining factor is the rate of diffusion in the membrane.

There is usually some initial non steady-state contribution discernible at the start of a permeability experiment. This is the "time lag" which occurs until the amounts of solute leaving and entering the membrane become equal.

Comprehensive accounts of the mathematical treatment of results have been presented by Garret and Chemburker,[169] Daynes[173] and Barrer.[174]

Various authors have chosen to treat their results in differing manners according to the particular type of system being used. This led to a wide range of permeability coefficient units according to the way in which the "pressure difference" or concentration terms had been expressed (see below). Such observations led the author to select the method of Flynn and Smith[90] and Flynn and Roseman[170] since the permeability cell used in transport studies across polymer membranes closely resembled that used during these studies. The units of permeability coefficient used ($cm^2\ sec^{-1}$) were also found in the studies of Zentner et al[171,159,172,160] on the permeation of certain pharmaceutical compounds through hydrogels.

Flynn and Roseman[170] found that during the course of their experiments the receptor solution concentration never exceeded 10% of the most dilute donor concentration. Therefore "sink conditions" existed, the excess solute in the donor compartment or the presence of an external reservoir, assuring that essentially a constant donor concentration existed. The steady-state slopes obtained for transport were converted to total steady-state flux, Q_T, in milligrams per second. The permeability coefficient was thus defined as shown in Equation V.6.

$$P = Q_T \times h/AC_0 \qquad \text{Equation V.6}$$

where h = membrane thickness

A = cross-sectional area

C_0 = concentration in milligrams per millilitre in the applied phase

As has already been mentioned, the units of permeability coefficient are found to vary from author to author. However, any permeability equation must include terms expressing the amount of substance transported, the thickness of the membrane, the cross-sectional area of the membrane and the initial solute concentration. The units in Equation V.6 were therefore, Q_T = mg sec^{-1}, h = cm, A = cm^2 and C_0 = mg cm^{-3}. These units are often condensed, which means that the final units, cm^2 sec^{-1}, do not supply any information on the nature of the components used. These units have also been used by Kopecek, Vacik and Lim[124] as well as Flynn and Smith,[90] Flynn and Roseman[170] and Zentner et al.[171,159,172,160]

The permeability apparatus was assembled as described in Chapter II. Before, the assessment of the permeability characteristics of the antibiotics through various hydrogel membranes could be made, it was necessary to characterise the fluid circulation and stirring rate parameters of the apparatus.

V.2.ii Effect of Fluid Circulation System on Permeability

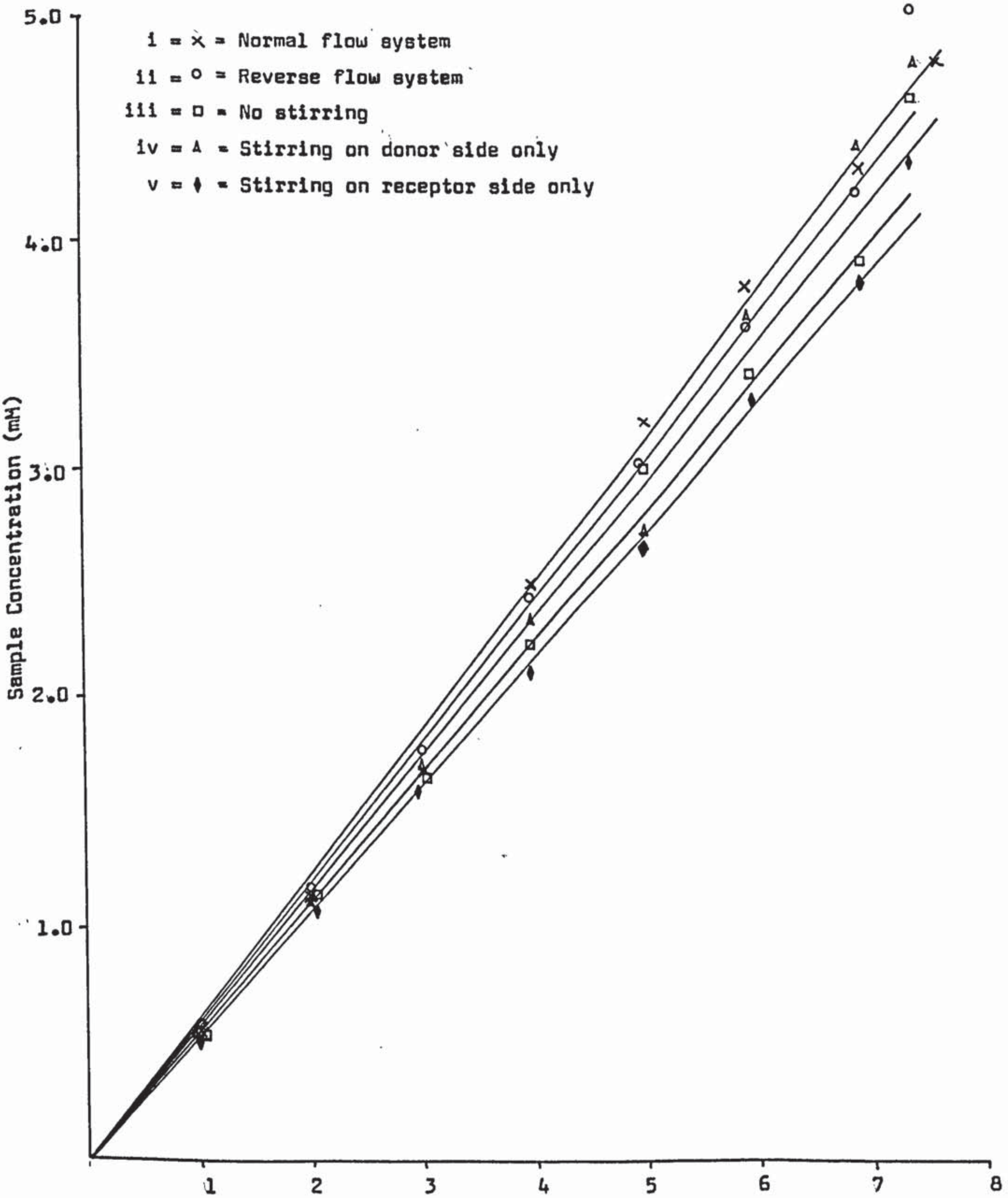
Flynn and Smith[90] carried out an extensive investigation of the characteristics of a vertical membrane equicompartment permeability cell not dissimilar to that used during these studies. Their system showed little variation when fluid flow through the system was reversed and also found that the stirring rate for the 10 cm^2 diffusional area was not critical above 30 rpm, even though stirring rates of 120 rpm were available.

Kumamoto[175] also investigated the effects of unstirred layers under conditions of both stirring and non-stirring of both donor and receptor solutions. He effectively demonstrated that transport rates were the same for conditions where both solutions were stirred and where the receptor solution only was stirred. However, there were significant drops in transport under conditions where neither solution was stirred and where only the donor solution was stirred. These results showed that the boundary layer on the receptor solution side was more important in affecting transport than that on the donor solution side.

In order to establish the effectiveness of fluid circulation in the system used during these studies the permeability characteristics of a pHEMA (water swollen) membrane to aqueous 0.25M potassium chloride solutions under various conditions was established. The results are presented in Figure V (1)(i) both solutions stirred, normal flow, (ii) both solutions stirred, reverse flow, (iii) no stirring at all, (iv) donor solution only stirred, and (v) receptor solution only stirred. It was found that only very small changes in permeation were found for the above changes in the fluid circulation systems. In general, successive runs on the same membrane under identical conditions resulted in a variation of about ± 5% for the value of the permeability coefficient. This was slightly better than had been achieved by Flynn and Smith[90] and since

FIGURE V (1)

AFFECT OF STIRRING SYSTEM AND FLUID CONDUCTION ON THE PERMEABILITY OF WATER SWOLLEN pHEMA TO 0.25M POTASSIUM CHLORIDE SOLUTION (aq) AT 37°C



the proportionating pump was circulating fluid at a rate of 90 ml/min in all the above experiments it would seem that effects due to boundary layers[175] had been greatly reduced by efficient fluid circulation alone, without the need for stirring.

V.2.iii Effect of Sampling Rate on Permeability

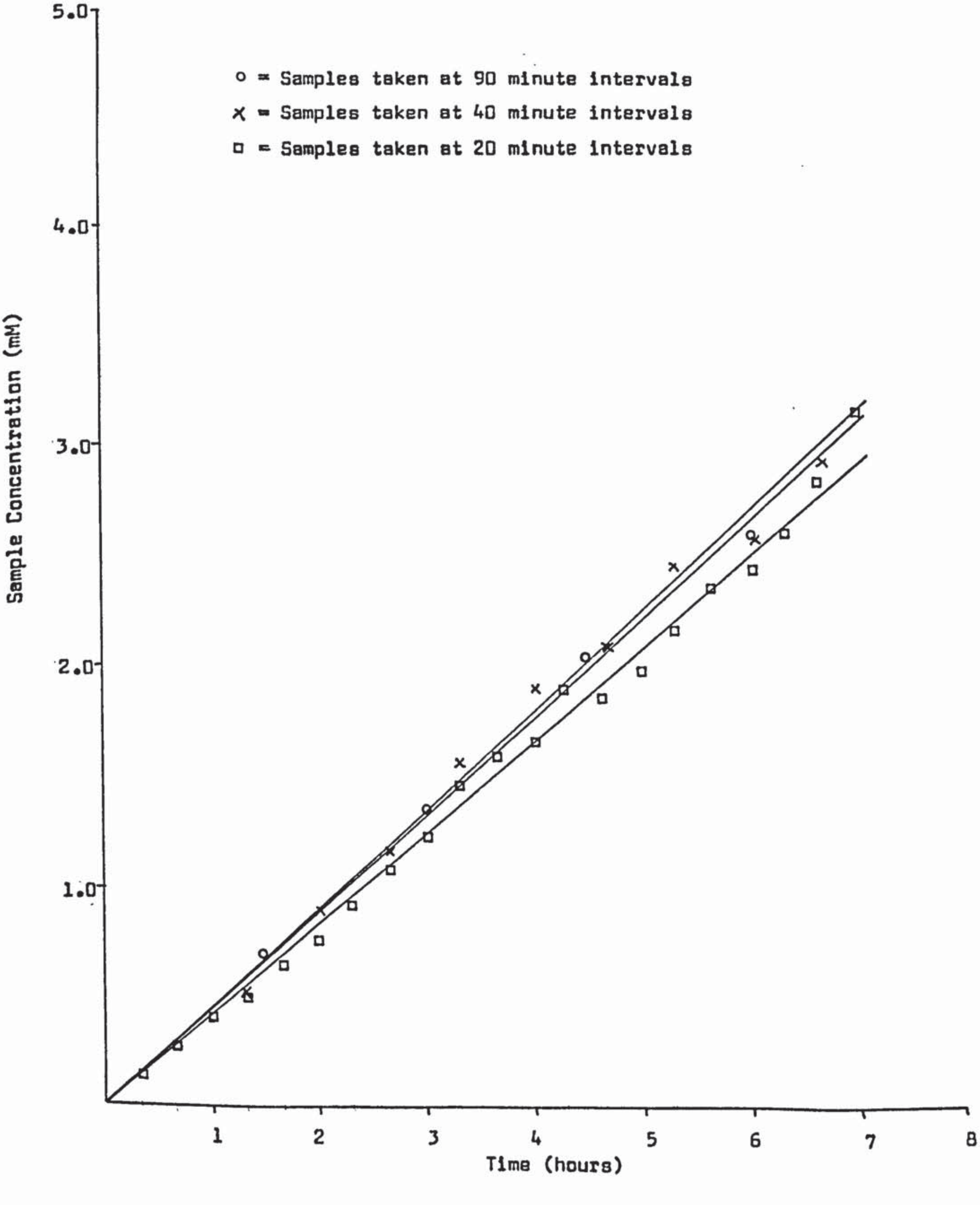
Since sample sizes of 1 ml were generally taken throughout the course of this work from a fluid volume of 130 ml on each side of the membrane, it was necessary to investigate the effect of the gradual depletion of receptor solution volume on permeability.

From Figure V (2) it can be seen that very little difference in potassium chloride transport was found when the sampling rate was doubled and even trebled. The order of permeability coefficients determined of 90 min > 40 min > 20 min was as would be expected but the variation was less than ± 3.5%. Such a finding was surprising considering that for the case of 1 ml samples every 20 minutes the volume removed from the receptor solution by the end of the experiment was of the order of 15% of the original volume or about 20% of the final volume. These observations were encouraging and so the fluid circulation rate of 90 ml/min and stirring rate of about 250 rpm were maintained throughout all these studies.

Variations in permeability coefficients for runs under identical conditions but with new membranes in each case were generally larger than the values reported above. The major factor responsible being membrane thickness. Because the membranes used during these studies were not from commercial sources, the variation in thickness must be considered to be relatively large. Nevertheless, the performance of the permeability apparatus as a whole was considered satisfactory for the intended studies.

FIGURE V (2)

AFFECT OF SAMPLING RATE ON THE PERMEABILITY OF WATER SWOLLEN pHEMA TO 0.25M POTASSIUM CHLORIDE SOLUTION (aq) AT 37°C



V.3 Permeability Studies on Lincomycins

Studies on the permeation of aqueous solutions of Lincomycin and Clindamycin across water swollen pHEMA, HEMA/STY, MAA/ACM and MAA/ACM/STY membranes did not detect the transport of either antibiotic over the 8 hour experimental periods used. However, these results were of use in the later determinations of the pore size "cut-off" points for the compounds. Permeation studies of antibiotics across pACM (water swollen) membranes were more successful.

V.3.1 Effect of Solute Concentration on Antibiotic Permeation Over a Range of Temperatures

The permeability characteristics of 0.10M, 0.25M and 0.50M aqueous solutions of both Lincomycin and Clindamycin were studied using water swollen pACM membranes (E.W.C. = 89.3%). Determinations were made at 23°C, 37°C and 45°C. Figures V (3), V (4) and V (5) show the changes in permeability observed for different initial solution concentrations of Lincomycin at 23°C, 37°C and 45°C respectively. Figures V (6), V (7) and V (8) show the corresponding observations found for Clindamycin solutions.

The expected trend of greater transport rate with higher initial solution concentration was observed in each case. However, particularly noticeable were the differences in the permeation of the two antibiotics at 23°C the rate of Clindamycin transport being markedly lower than Lincomycin transport for all concentrations at this temperature. At higher temperatures transport rates of the two compounds were comparable. Direct comparisons can only be made by consideration of the permeability coefficient values as presented in Table V.A.

The data presented in Table V.A can be treated in a number of ways. A plot of permeability coefficient against initial solute concentration can

FIGURE V (3)

PERMEATION OF AQUEOUS LINCOMYCIN SOLUTIONS ACROSS WATER SWOLLEN pACM MEMBRANES AT 23^{o}C

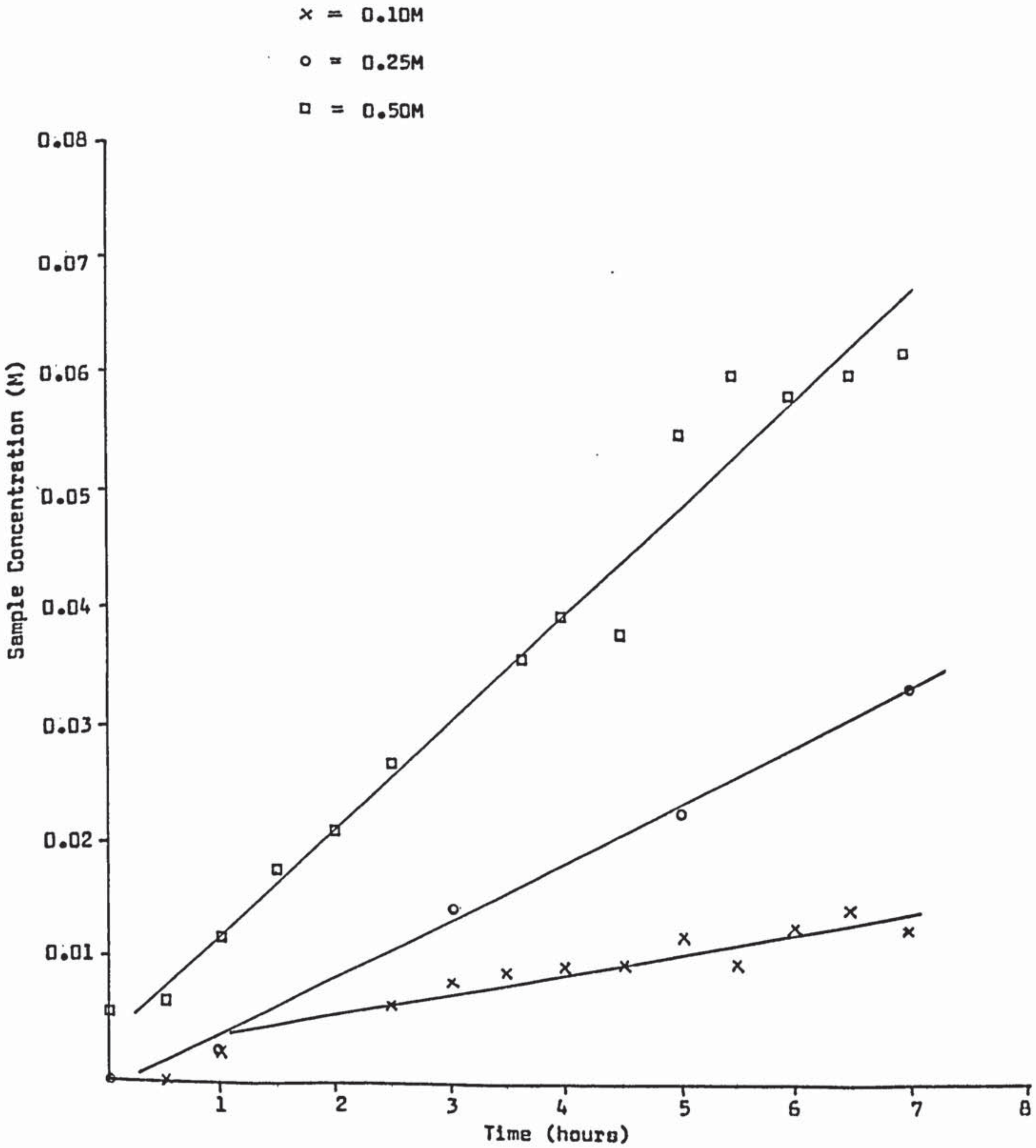


FIGURE V (4)

PERMEATION OF AQUEOUS LINCOMYCIN SOLUTIONS ACROSS WATER SWOLLEN pACM MEMBRANES AT 37°C

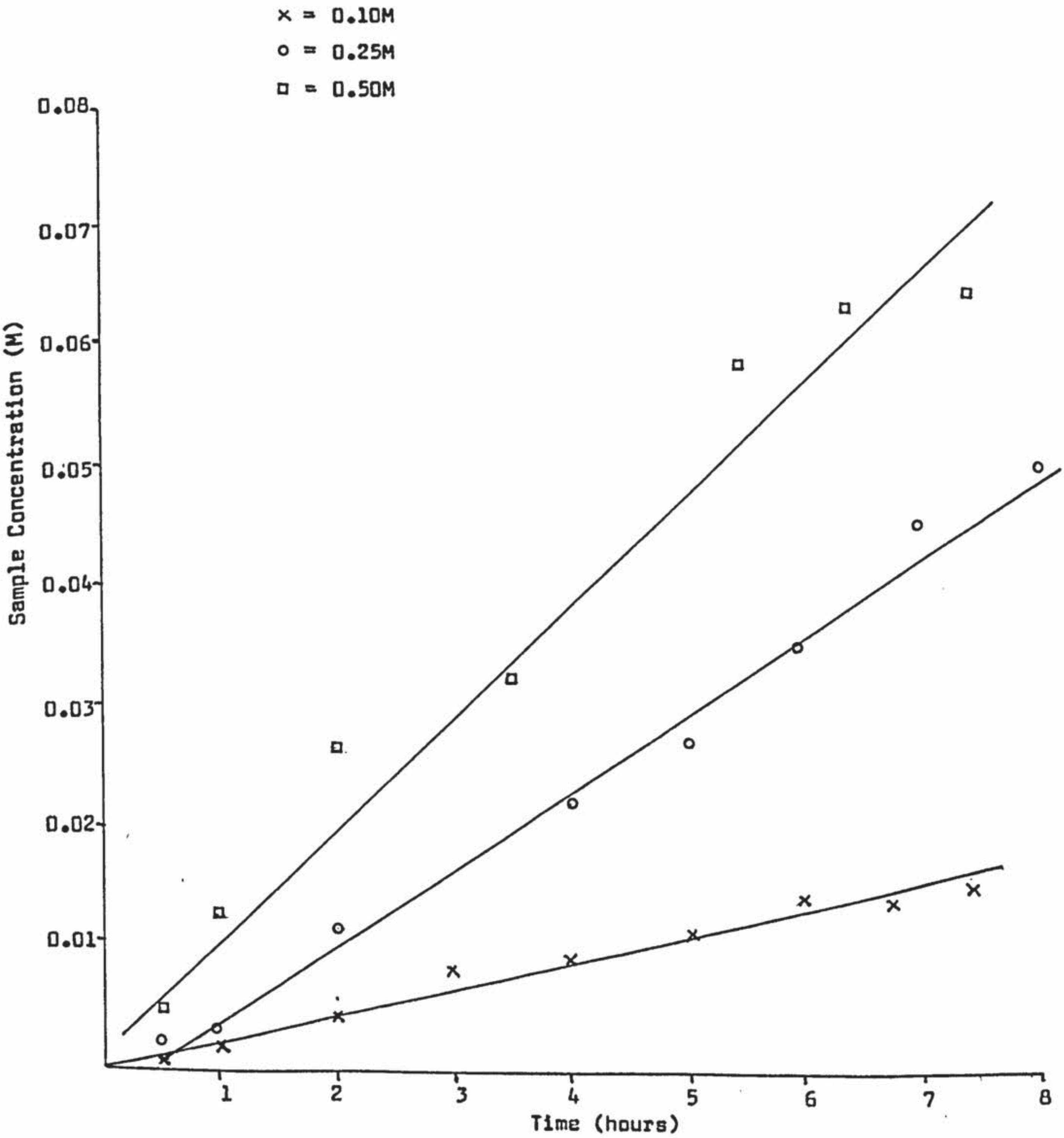
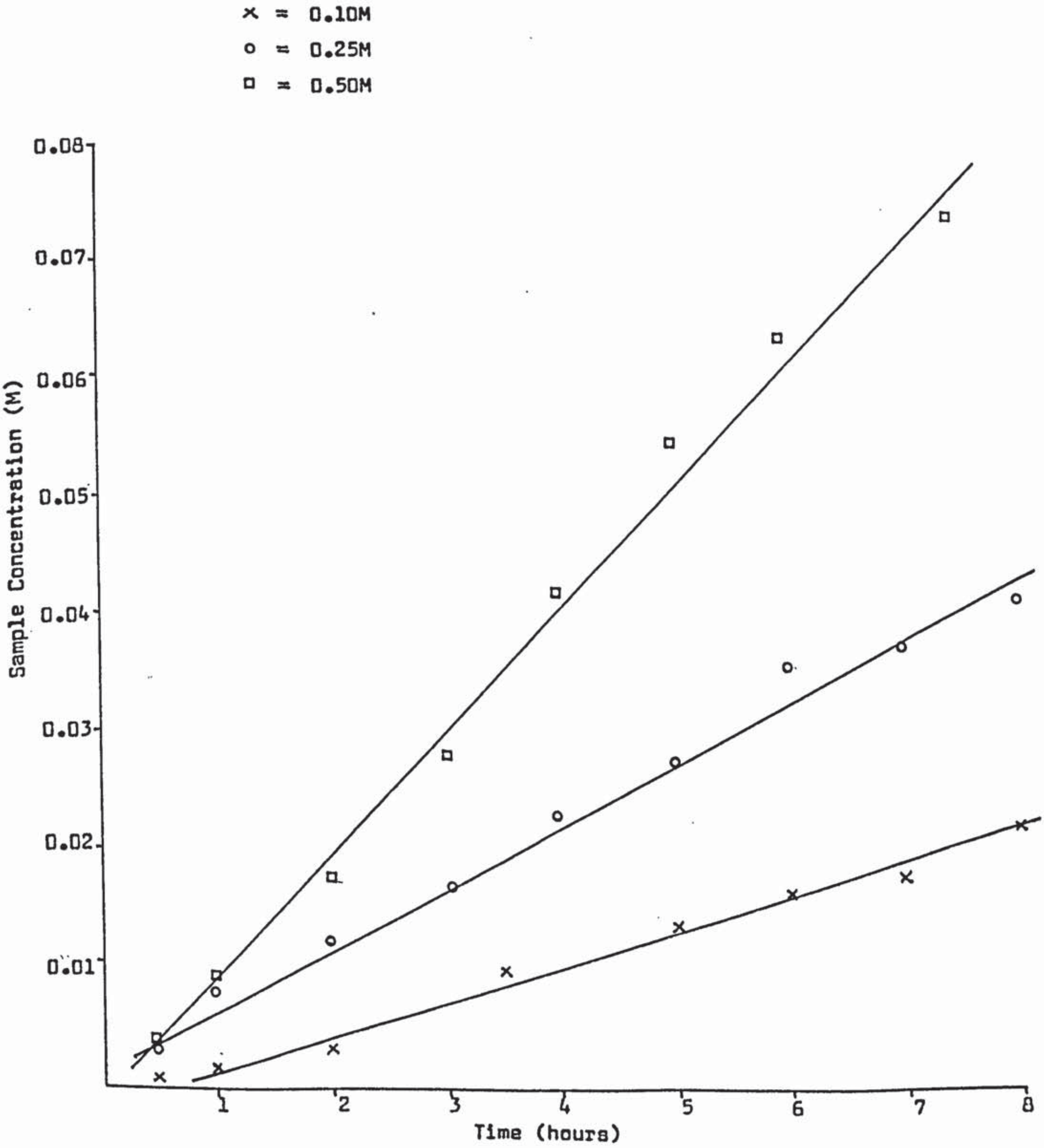


FIGURE V (5)

PERMEATION OF AQUEOUS LINCOMYCIN SOLUTIONS ACROSS WATER SWOLLEN pACM MEMBRANES AT 45°C



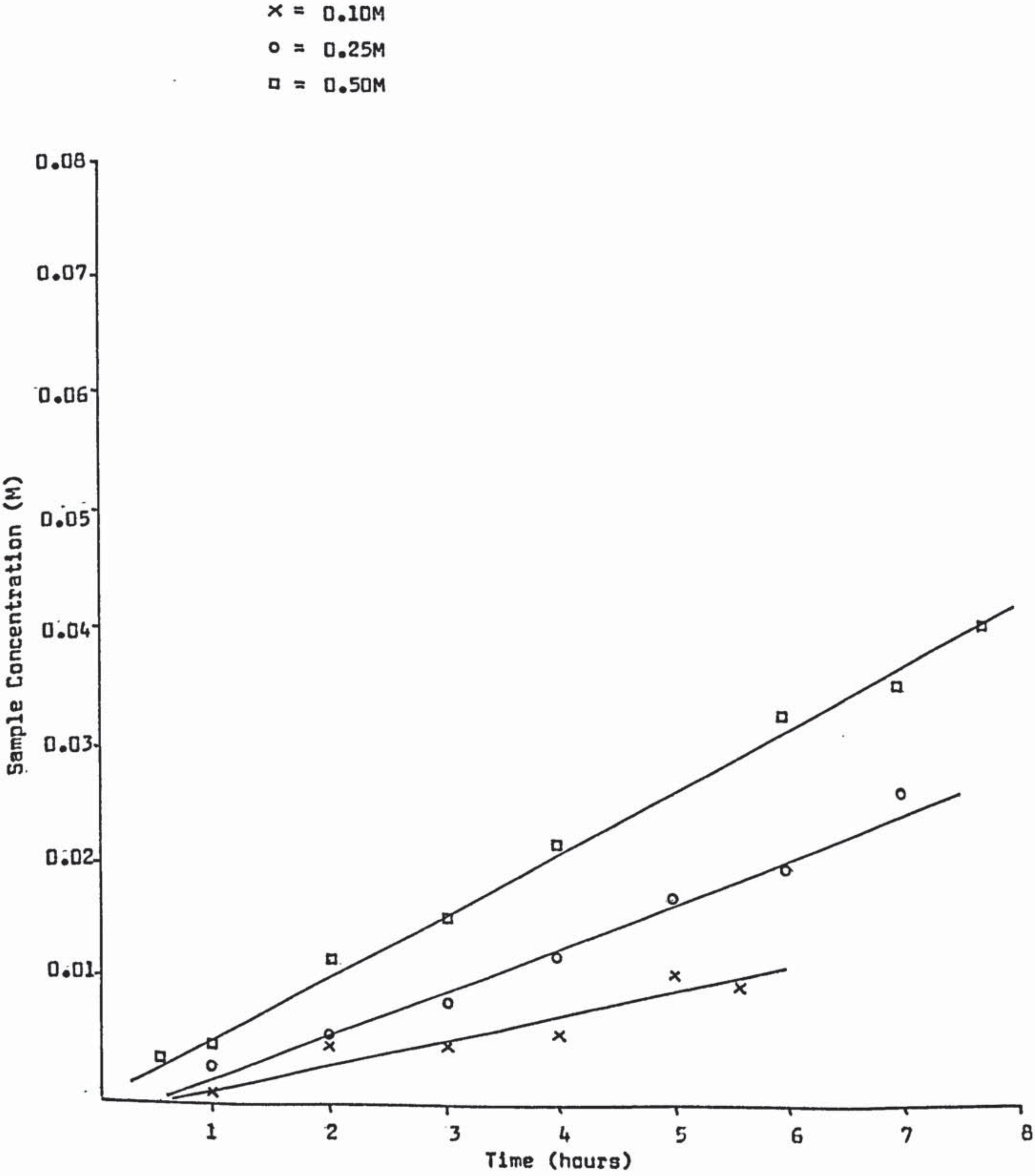


FIGURE V (6)

PERMEATION OF AQUEOUS CLINDAMYCIN SOLUTIONS ACROSS WATER SWOLLEN pACM MEMBRANES AT 23°C

FIGURE V (7)

PERMEATION OF AQUEOUS CLINDAMYCIN SOLUTIONS ACROSS WATER SWOLLEN pACM MEMBRANES AT 37°C

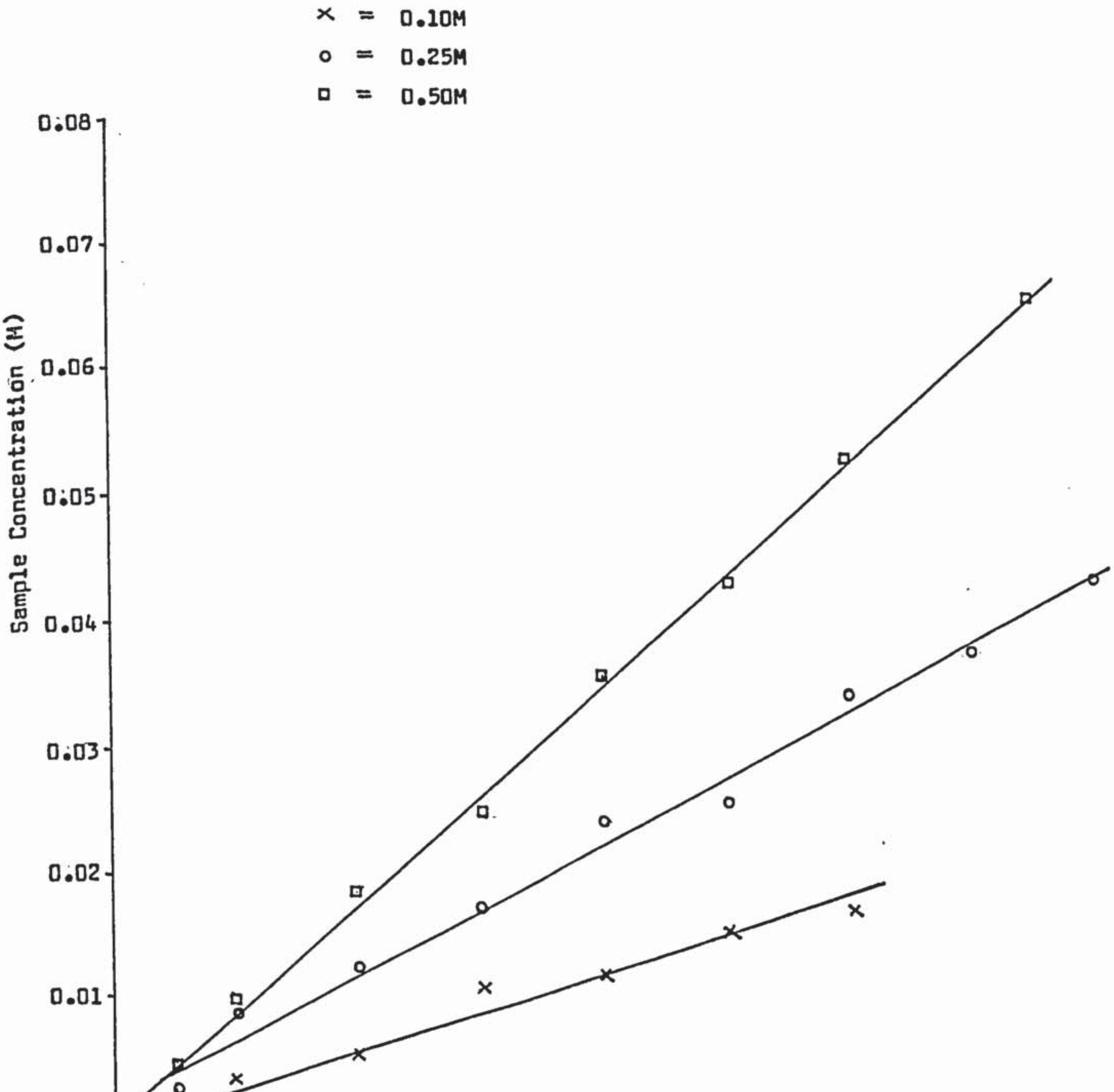


FIGURE V (8)

PERMEATION OF AQUEOUS CLINDAMYCIN SOLUTIONS ACROSS WATER SWOLLEN pACM MEMBRANES AT 45°C

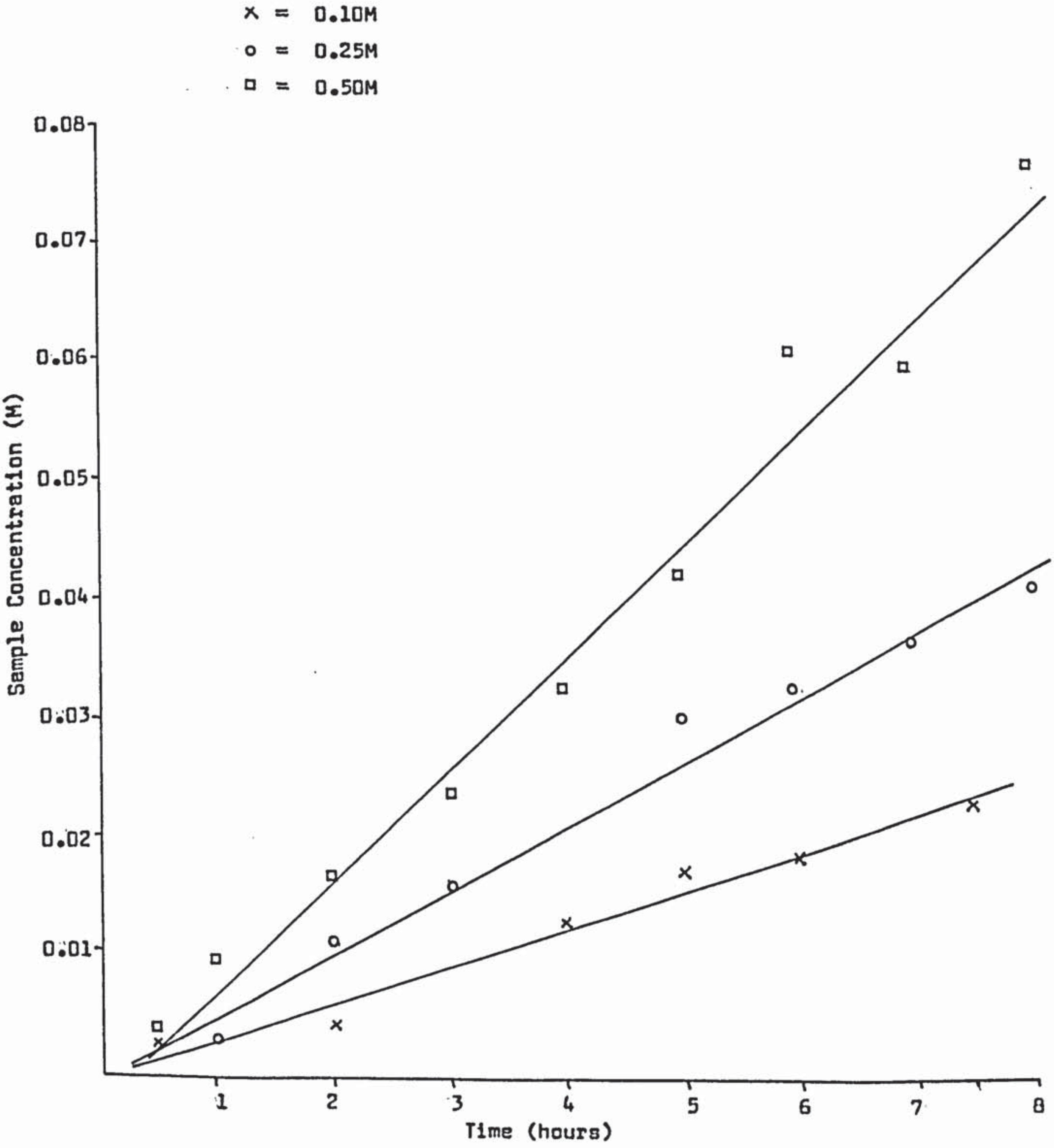


TABLE V.A

PERMEABILITY COEFFICIENTS FOR ANTIBIOTIC TRANSPORT ACROSS WATER SWOLLEN pACM MEMBRANES

	Temperature (^oC)	Initial Solute Concentration (M)	Permeability Coefficient P (cm^2 sec^{-1})
Lincomycin	23	0.50 0.25 0.10	27.5×10^{-7} 36.4×10^{-7} 33.3×10^{-7}
	37	0.50 0.25 0.10	35.2×10^{-7} 50.0×10^{-7} 34.4×10^{-7}
	45	0.50 0.25 0.10	39.6×10^{-7} 38.3×10^{-7} 56.8×10^{-7}
Clindamycin	23	0.50 0.25 0.10	18.4×10^{-7} 24.7×10^{-7} 33.7×10^{-7}
	37	0.50 0.25 0.10	34.2×10^{-7} 41.0×10^{-7} 60.4×10^{-7}
	45	0.50 0.25 0.10	36.9×10^{-7} 38.3×10^{-7} 65.3×10^{-7}

be used to demonstrate Fickian behaviour for solute transport. A plot of the logarithm of permeability coefficient against reciprocal temperature enables the activation energy of the transport process to be determined and consideration of the original permeability data allows first order rate constants to be determined.

The activation energies for the transport processes are readily calculable from the data obtained over the temperature range studied using the Arrhenius equation. The logarithmic form of this equation can be seen in

Equation V.7.

$$\log k = \log A - E/2.303\ RT$$ Equation V.7

where k = rate of reaction (permeability coefficient)

A = frequency factor

E = Activation energy for the process

R = Gas constant

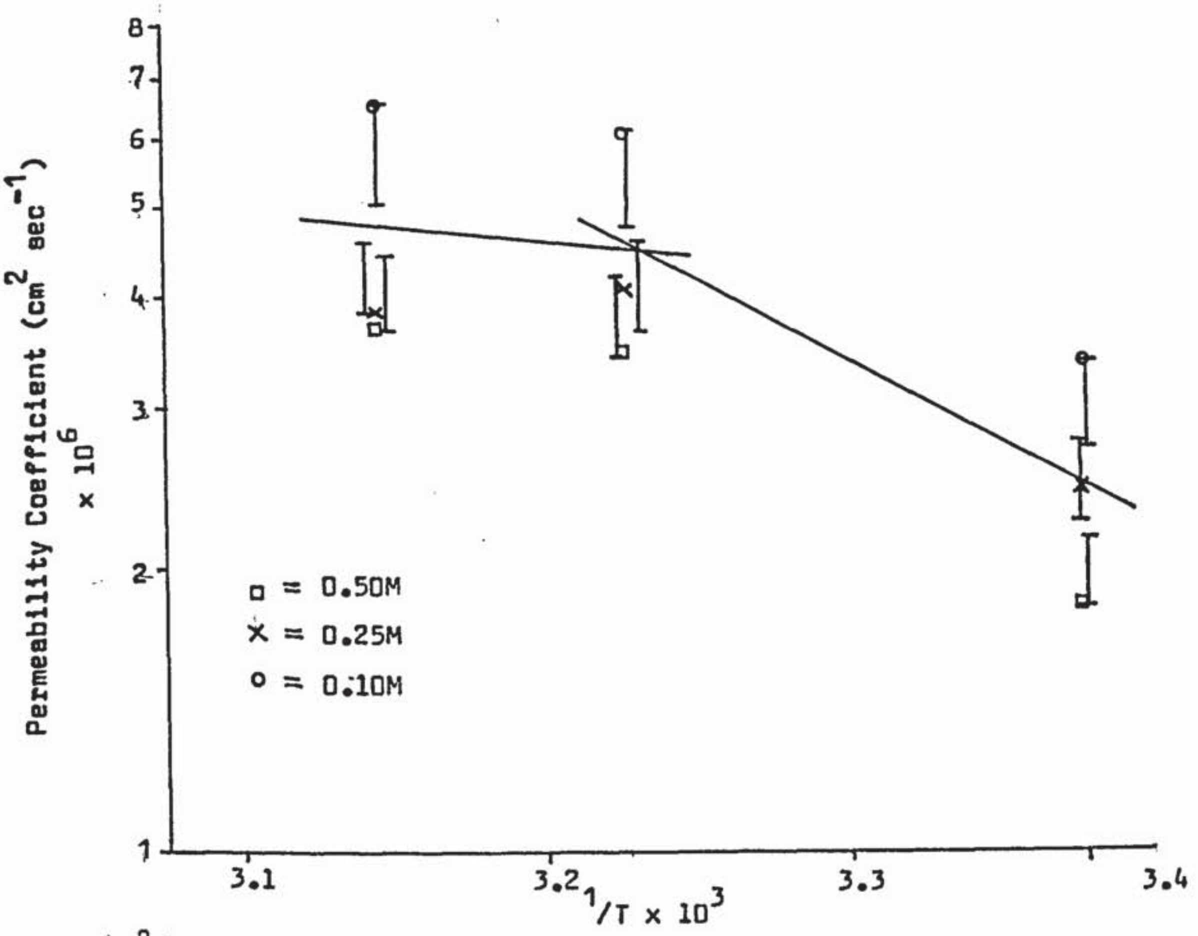
T = Temperature (K)

The slope of an Arrhenius plot of logP against $^{1}/T$ is equal to $-E/2.303\ RT$. Figure V (9) shows such plots for Clindamycin and Figure V (10) for Lincomycin for aqueous solution concentrations of 0.50M, 0.25M and 0.10M in each case.

Figure V (10) clearly shows the amount of scatter for values of the permeability coefficient of Lincomycin over the range of concentrations and temperature used. However, selective positioning of ± 10% error bars for the data allowed the linearity of the permeability coefficients with reciprocal temperature to be shown. The permeability data for Clindamycin were treated in a similar manner (Figure V (9)), but as can be seen there was a distinct change of slope below 37°C for each concentration used even with the use of ± 10% error bars. In fact minimum error bars of about ± 20% would be required for the Clindamycin data to demonstrate the type of linearity seen for Lincomycin. Such error limits would be unacceptable and unreasonable. It was therefore concluded that the change of slope seen for Clindamycin was a valid observation. Such abrupt changes in slope are not altogether unknown. Permeability experiments on polymers above and below their glass transition temperatures (T_g) have been shown to possess such characteristics,[176] although such an explanation was not appropriate here.

FIGURE V (9)

ARRHENIUS PLOT FOR CLINDAMYCIN PERMEABILITY DATA



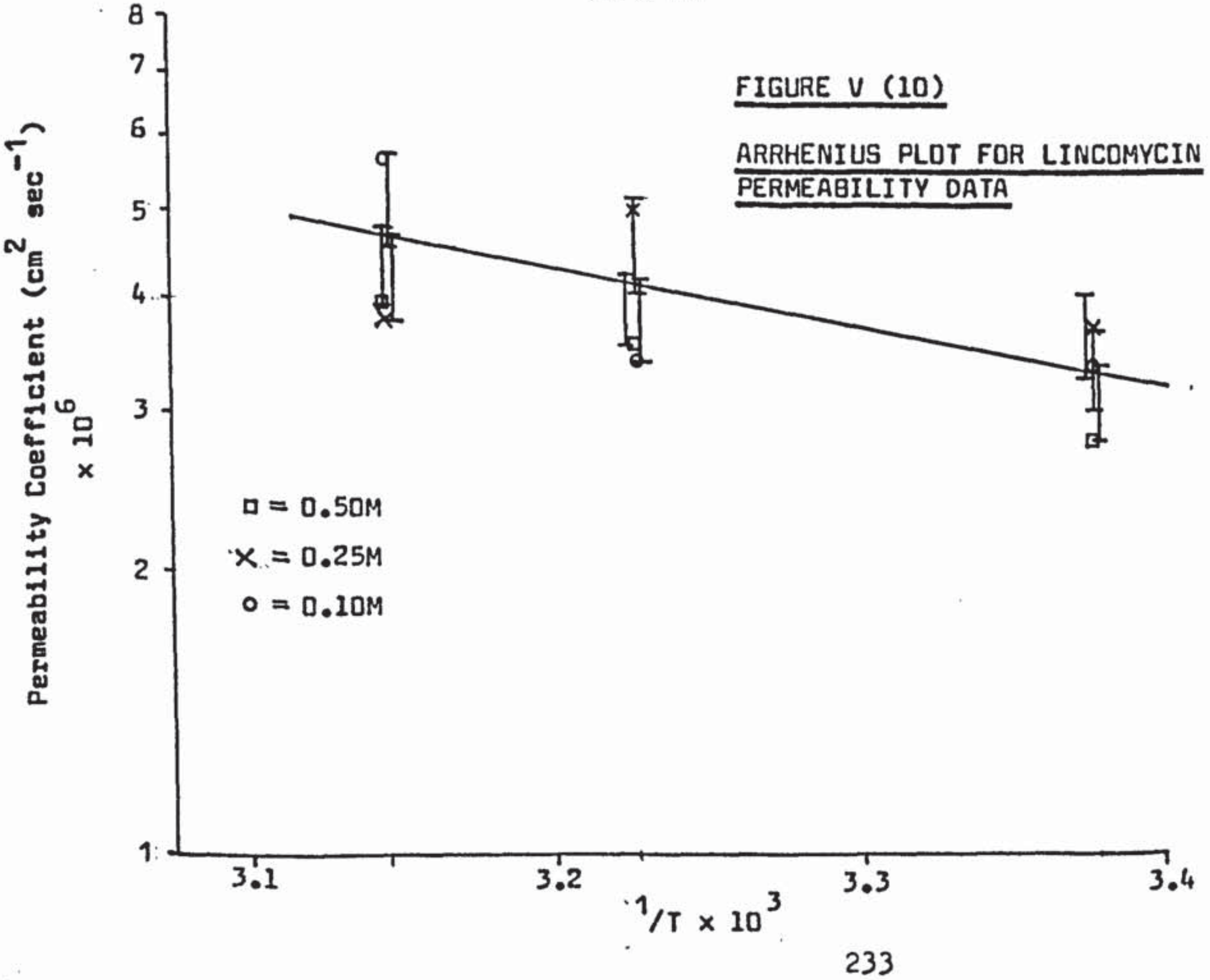


FIGURE V (10)

ARRHENIUS PLOT FOR LINCOMYCIN PERMEABILITY DATA

The values of the activation energies calculated for Lincomycin (± 10% error limits) and Clindamycin (± 20% error limits, so as to eliminate the change in slope) were 2.92 K cal mol^{-1} (12.24K J mol^{-1}) and 5.01 K cal mol^{-1} (20.96 K J mol^{-1}) respectively. These values were in agreement with previously reported values for the transport of various solutes across hydrogel membranes of 9.22 to 4.62 K cal mol^{-1} for potassium chloride transport,[177] 3.55 to 4.17 K cal mol^{-1} for urea and 3.09 to 4.13 K cal mol for sodium chloride transport.[123]

It can be seen from Figures V (9) and V (10) the slope of the Clindamycin arrhenius plot above 37°C was comparable to the slope found for Lincomycin over the whole temperature range. The deviation of the Clindamycin plot below 37°C could be correlated with an increase in activation energy, so that changes in temperature have a greater influence on Clindamycin permeability between 23°C and 37°C than between 37°C and 45°C.

The data can also be presented in the form of a plot of permeability coefficient versus initial solute concentration as shown in Figures V (11) and V (12) for Lincomycin and Clindamycin respectively. The use of error limits of ± 10% (selectively positioned) show that both antibiotics can be seen to behave in a Fickian manner except that the permeability coefficients for Clindamycin increase with decreasing solution concentration. This might well reflect something of the association of Clindamycin in concentrated solutions, discussed in Chapter III, in that the larger associated particles have a lower rate of transport than the individual molecules.

Calculations of first order rate constants can be made from the original data on the basis of the time taken to complete 25% of the reaction in each case. However, such a treatment does not allow for differences in

FIGURE V (11)

PLOTS OF PERMEABILITY COEFFICIENT VERSUS DONOR SOLUTION CONCENTRATION AT VARIOUS TEMPERATURES FOR AQUEOUS LINCOMYCIN SOLUTIONS

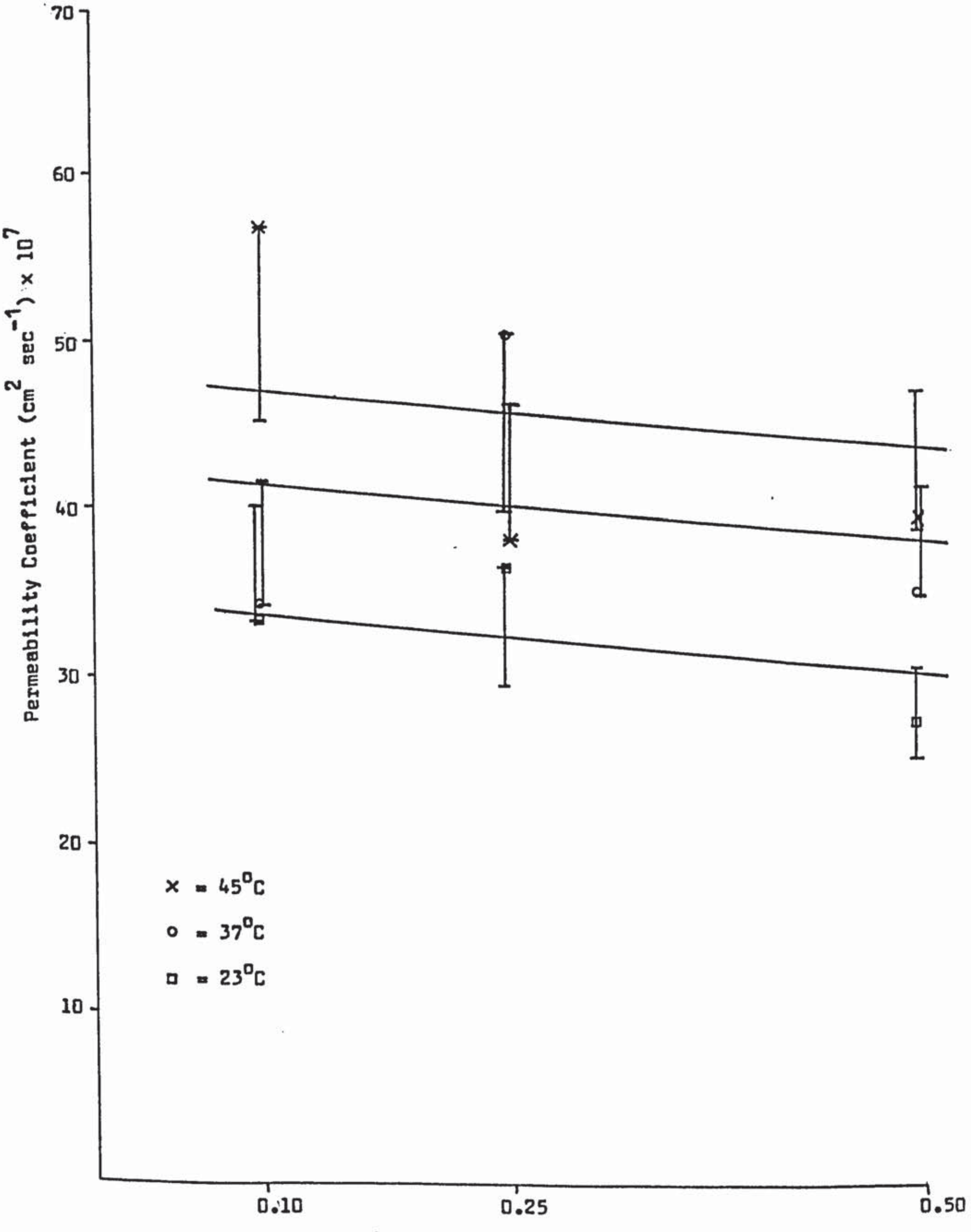
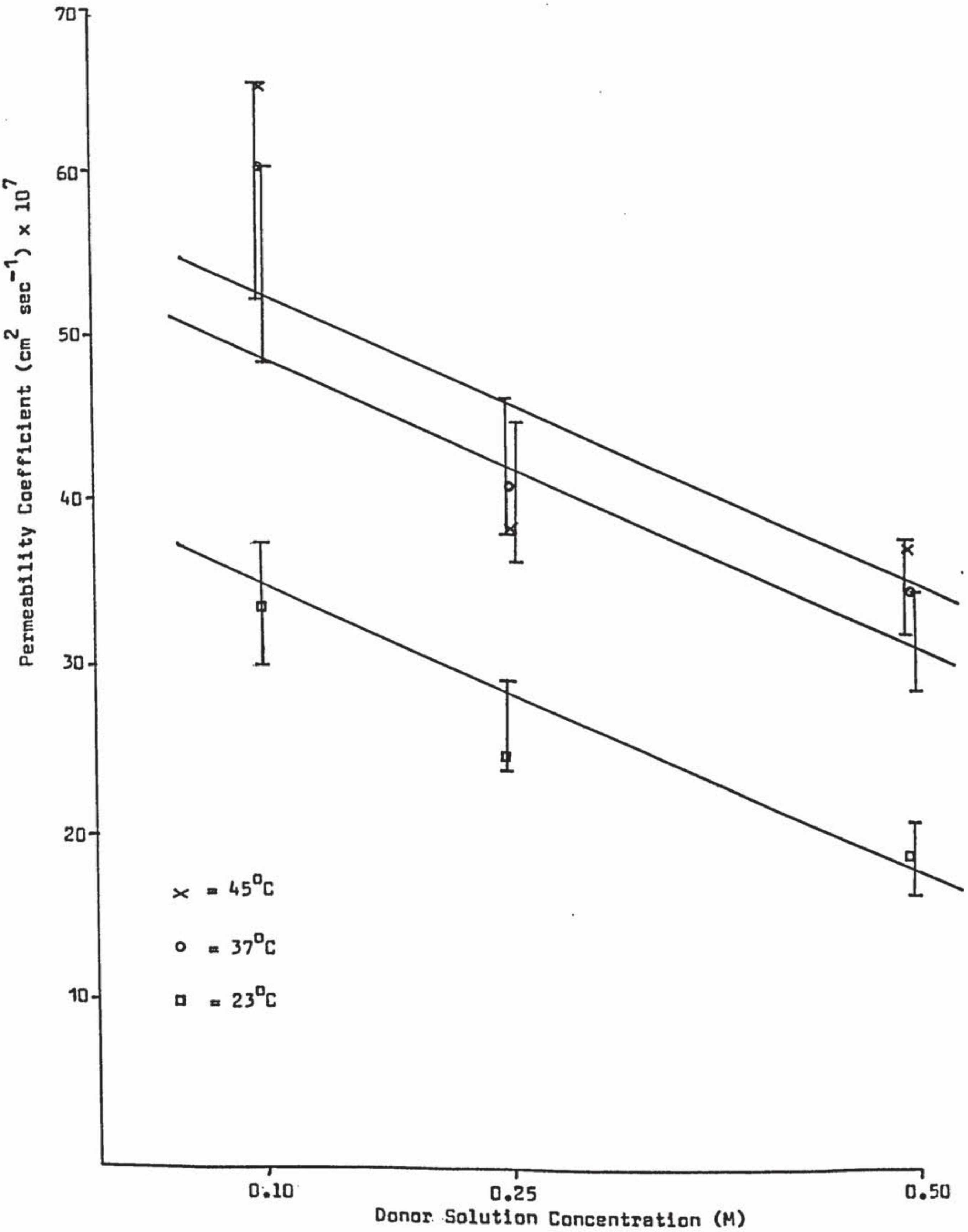


FIGURE V (12)

PLOTS OF PERMEABILITY COEFFICIENT VERSUS DONOR SOLUTION CONCENTRATION AT VARIOUS TEMPERATURES FOR AQUEOUS CLINDAMYCIN SOLUTIONS



transport rate due to different membrane thicknesses in different experiments. This factor could account for the lack of any significant increase in first order rate constant with increasing temperature. However, a slight increase in rate constant was found with increasing initial solution concentration for Clindamycin (and not for Lincomycin) as seen in the example of rate constant values shown in Table V.B. This change was however not considered significant bearing in mind the errors involved.

TABLE V.B

1st ORDER RATE CONSTANTS AT 37°C

	Solute Concentration (M)	1st Order Rate Constant
Lincomycin	0.50 0.25 0.10	1.5×10^{-5} sec^{-1} 1.5×10^{-5} sec^{-1} 1.3×10^{-5} sec^{-1}
Clindamycin	0.50 0.25 0.10	2.2×10^{-5} sec^{-1} 1.5×10^{-5} sec^{-1} 1.2×10^{-5} sec^{-1}

From the overall permeability data Lincomycin can be considered to have behaved in a broadly Fickian manner but Clindamycin showed deviations from this behaviour at low temperature and high concentration. On this basis the systems studied must be considered to involve several complex and possibly competitive interactions, for example, interactions between polymer matrix and water and water and antibiotic. Such interactions could also be considered to be influenced by both temperature and solute concentration.

However the concept of intermolecular association of Clindamycin at high concentrations would explain the reduction in transport observed. The

FIGURE V (13)

AFFECT OF % (by wt) MBACM CROSS-LINKING AGENT ON THE PERMEATION OF 0.1M LINCOMYCIN (aq) ACROSS WATER SWOLLEN pACM MEMBRANES AT 37°C

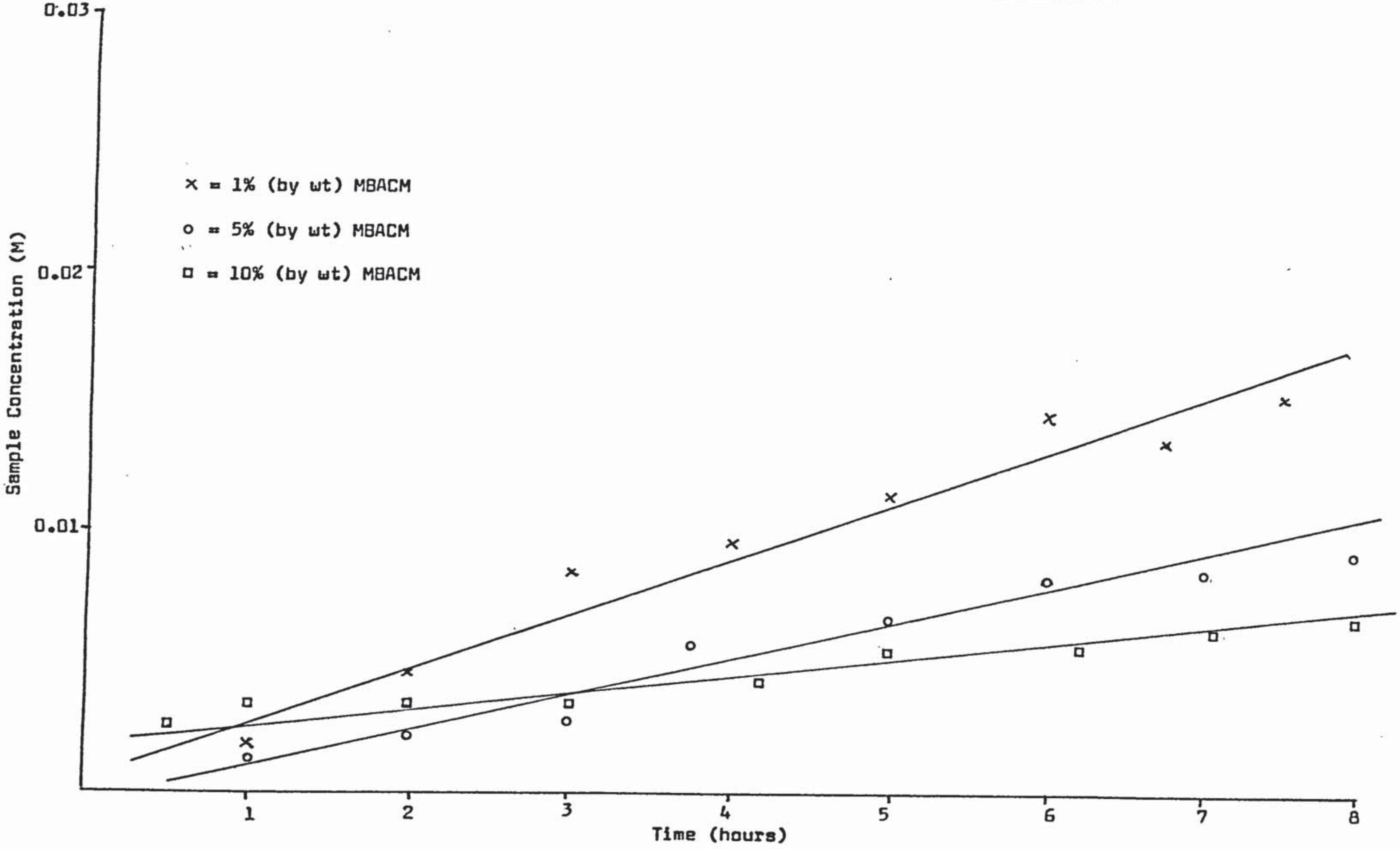
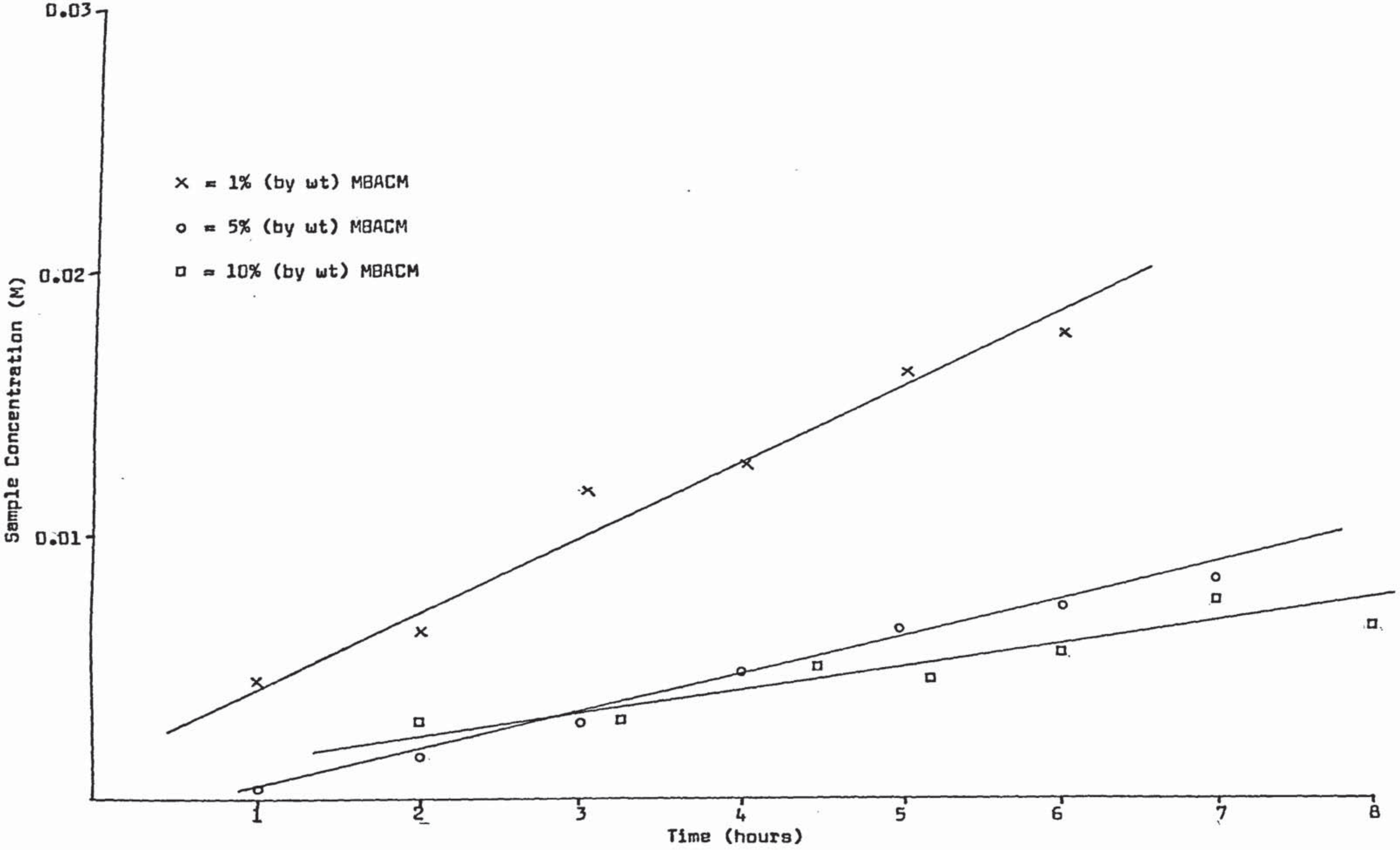


FIGURE V (14)

AFFECT OF % (by wt) MBACM CROSS-LINKING AGENT ON THE PERMEATION OF 0.1M CLINDAMYCIN (aq) ACROSS WATER SWOLLEN pACM MEMBRANES AT 37°C



extent of the association could also be considered to be greater at lower temperatures than at high temperatures where entropic effects would be greater. This process would also have to be reconciled with the larger increase in Clindamycin solubility with temperature relative to Lincomycin solubility (reported in Chapter III). However, the solubility differences between these compounds when in the aqueous phase of hydrogel membranes are not likely to have been rate determining.

The large errors involved in these studies and the small permeability differences between the antibiotics also reflect the fact that any specific interactions occurring would not necessarily be optimised in the high E.W.C. pACM preparations used. The permeability experiments reported here also need to be extended both in the range of solution concentrations and experimental temperatures studied before further conclusions can be drawn.

V.3.ii The Affect of the Extent of Cross-Linking of pACM Hydrogels on the Permeation of Lincomycins

A series of water swollen pACM membranes were prepared using either 1%, 5% or 10% (by wt) MBACM cross-linking agent. The E.W.C's of these preparations were 89.3%, 82.0% and 74.8% respectively. The permeation of 0.1M aqueous solutions of Lincomycin and Clindamycin across these membranes are shown in Figures V (13) and V (14) respectively.

It can be seen that an increase in the extent of cross-linking led to a decrease in transport for both antibiotics. This was as might have been expected and paralleled the similar observations of Zentner on progesterone transport across pHEMA membranes.[159] The values of permeability coefficients determined here are shown in Table V.C.

TABLE V.C

PERMEABILITY COEFFICIENTS FOR ANTIBIOTIC TRANSPORT ACROSS pACM MEMBRANES OF DIFFERENT CROSS-LINKING AGENT COMPOSITIONS AT 37°C

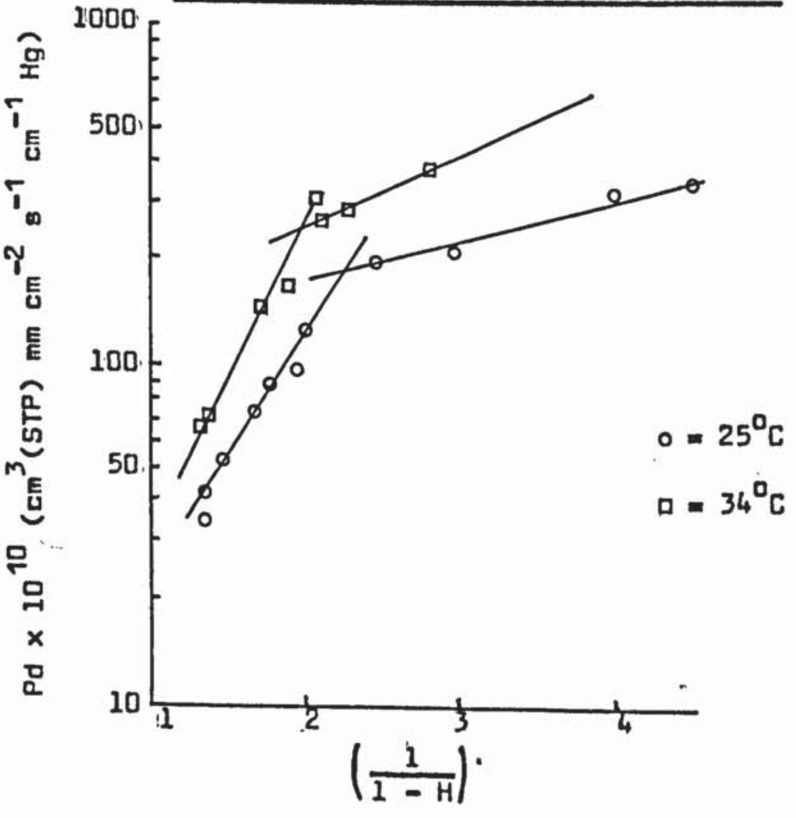
	% (by wt) MBACM	Permeability Coefficient ($cm^2\ sec^{-1}$)
Lincomycin (0.1M)	1.0	34.4×10^{-7}
	5.0	21.8×10^{-7}
	10.0	9.8×10^{-7}
Clindamycin (0.1M)	1.0	60.4×10^{-7}
	5.0	23.5×10^{-7}
	10.0	13.8×10^{-7}

A plot of logP versus ($^1/1-H$), where H is a function of membrane hydration equal to E.W.C./100, allows the estimation of the pore sizes at which the water structure lining the pores and the hydration shells of the antibiotics interact to progressively greater extents until the antibiotics cannot permeate the membrane, ie the "cut-off" point is reached. The technique has been used previously[178] as shown in Figure V (15) where the logarithm of dissolved oxygen permeability was plotted against ($^1/1-H$). This showed a sharp discontinuity of oxygen transport corresponding to pore radii of 4.9 Å and 5.7 Å at 34°C and 25°C respectively.

A similar plot for the data obtained from the affect of extent of cross-linking (which affects E.W.C.) on antibiotic permeability is shown in Figure V (16). From the limited data the slope at high hydration can be drawn in. However, the change in slope at lower hydration has been estimated bearing in mind the gradual interaction of water structures described earlier and the "cut-off" plot characteristics of NaCl, KCl and $CaCl_2$ presented in Chapter VI. The minimum hydration value for "cut-off" could also be estimated from the observed lack of antibiotic permeation across water swollen pHEMA, HEMA/STY, MAA/ACM and MAA/ACM/STY membranes mentioned earlier. The estimated "cut-off" points for the two antibiotics were not dissimilar for the 0.1M solutions used, suggesting that association of Clindamycin was not important at these low concentrations. The results of high concentration solution studies may prove more interesting. Studies also have to be conducted using membranes of (^{1}/1-H) values of 2 to 3 (about 50% to 60% E.W.C.) in order to accurately determine the "cut-off" points for the antibiotics.

FIGURE V (15)

AFFECT OF CRITICAL PORE SIZE ON DISSOLVED OXYGEN PERMEABILITY COEFFICIENTS AT 25°C AND 34°C FOR A RANGE OF WATER CONTENT PREPARATIONS

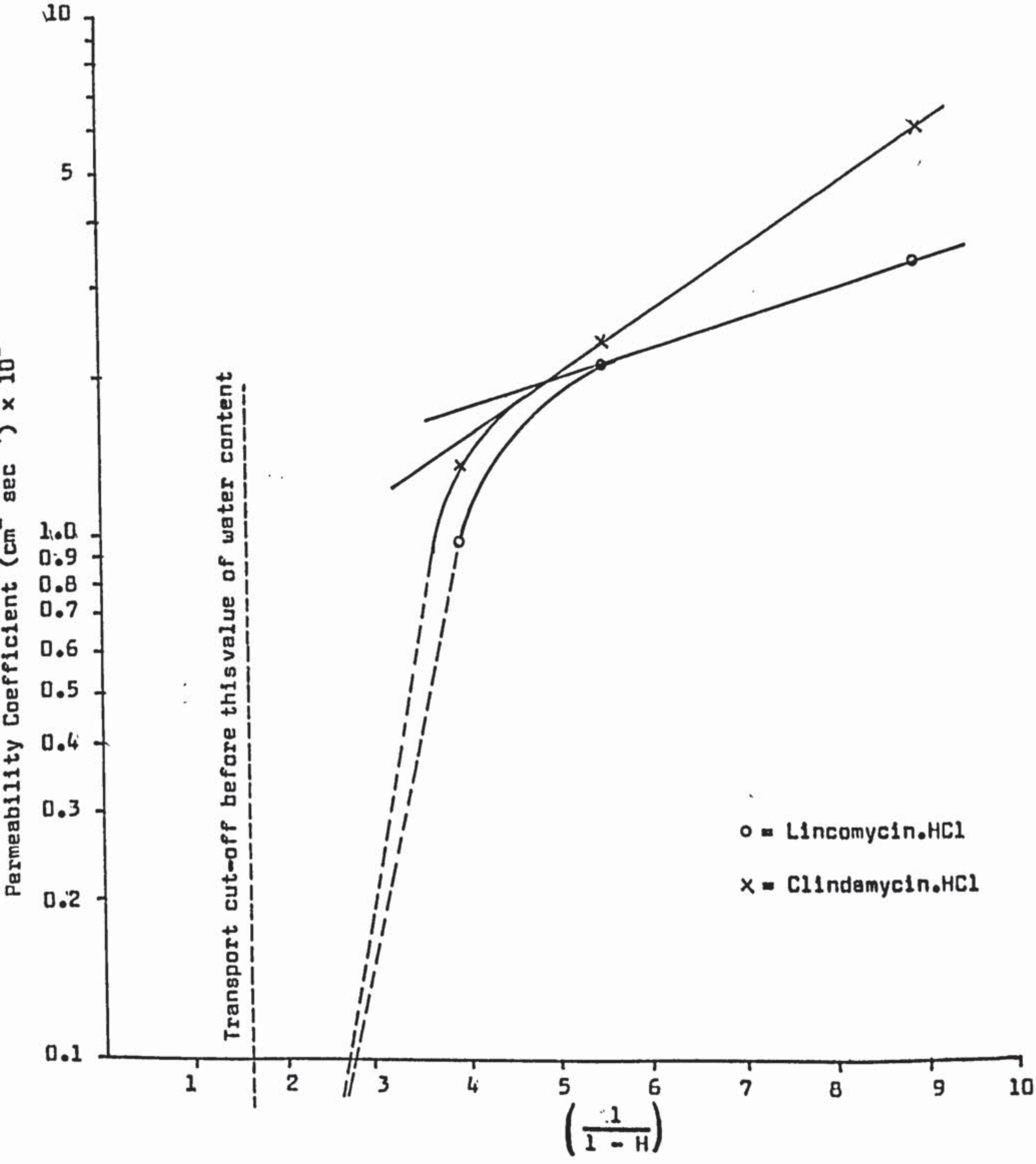


V.3.iii The Affect of Membrane Thickness on the Permeation of Lincomycins

Since the equation defining the permeability coefficient included a correction for membrane thickness, studies of antibiotic transport across

FIGURE V (16)

AFFECT OF CRITICAL PORE SIZE ON THE PERMEATION OF 0.1M AQUEOUS SOLUTIONS OF LINCOMYCIN AND CLINDAMYCIN ACROSS VARIOUS WATER CONTENT HYDROGELS



membranes of varying thicknesses should show a linear dependance of permeability coefficient with membrane thickness of zero slope.

A series of pACM (water swollen) membranes were prepared of different thicknesses by successive addition of gaskets to the mould. Hence 1 x t, 2 x t, 3 x t and 4 x t membrane thicknesses were achieved by using 1, 2, 3 and 4 gaskets respectively. The swollen preparations covered a range of 0.05 cm to 0.24 cm in thickness. The transport of 0.1M aqueous solutions of Lincomycin and Clindamycin across a range of membrane thicknesses at 37°C are summarised in Figures V (17) and V (18) respectively. The data pertaining to these experiments are shown in Table V.D and plots of $^1/P$ versus $^1/h$ are shown in Figure V (19).

TABLE V.D

PERMEABILITY DATA FROM EXPERIMENTS OF AFFECT OF MEMBRANE THICKNESS ON ANTIBIOTIC TRANSPORT

	$^1/h$ (cm^{-1})	P (cm^2 sec^{-1})	$^1/P$ (cm^{-2} sec)
0.1M Lincomycin			
1 x t	17.11	34.4×10^{-7}	29.1×10^4
2 x t	8.75	39.6×10^{-7}	25.3×10^4
3 x t	5.62	39.6×10^{-7}	25.3×10^4
4 x t	4.14	32.0×10^{-7}	31.2×10^4
0.1M Clindamycin			
1 x t	16.40	60.4×10^{-7}	16.6×10^4
2 x t	7.87	67.0×10^{-7}	14.9×10^4
3 x t	5.62	53.0×10^{-7}	18.9×10^4
4 x t	4.14	66.9×10^{-7}	15.0×10^4

The use of selectively positioned error bars in Figure V (19) shows that zero slope can be obtained for both compounds by using minimum error limits of ± 7.5%. Thus the plots showed that membrane thickness had been well accounted for in the calculation of permeability coefficients.

FIGURE V (17)

AFFECT OF MEMBRANE THICKNESS (t) ON THE PERMEATION OF 0.1M AQUEOUS LINCOMYCIN SOLUTIONS ACROSS WATER SWOLLEN pACM MEMBRANES AT 37°C

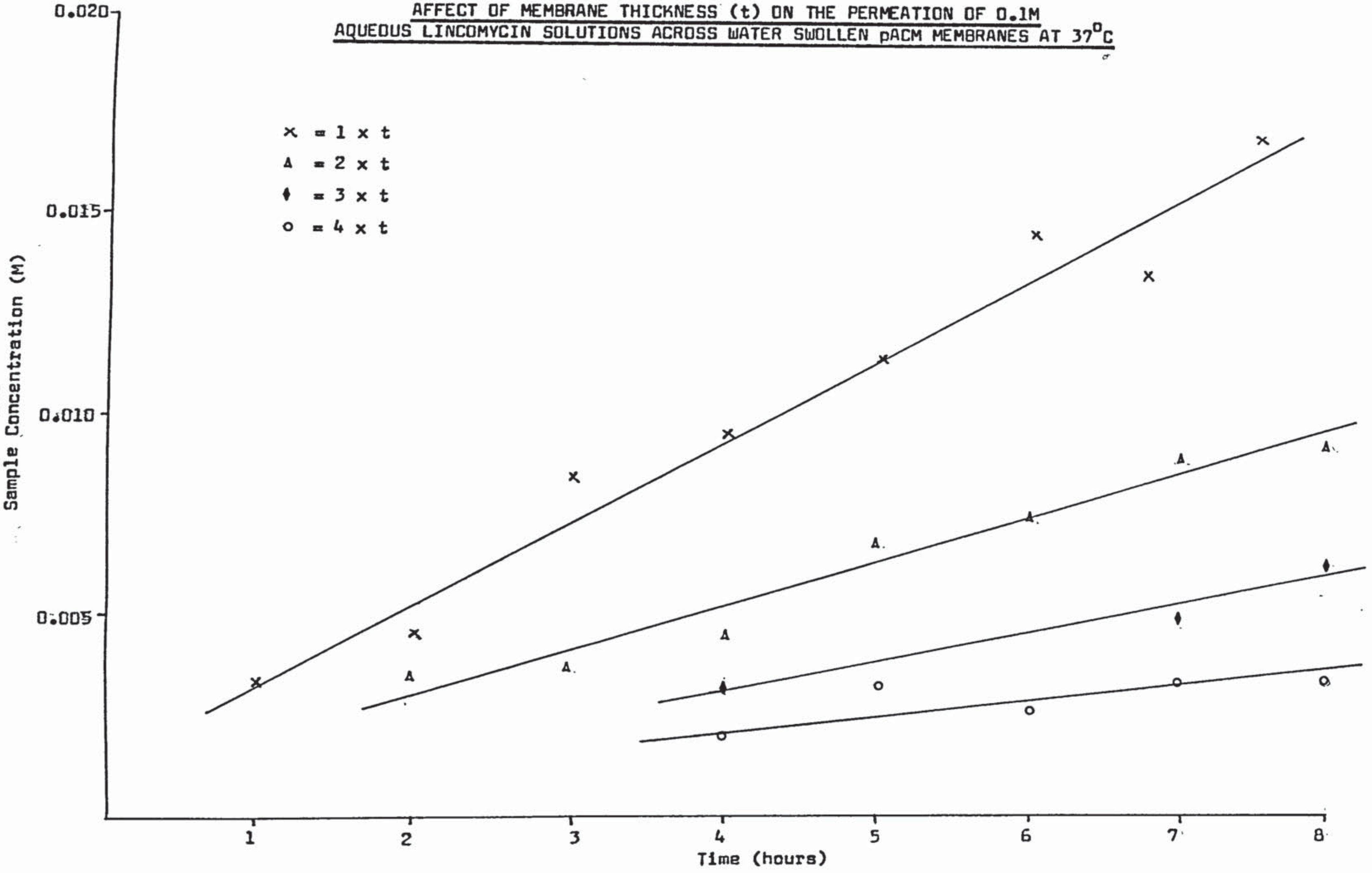


FIGURE V (18)

AFFECT OF MEMBRANE THICKNESS (t) ON THE PERMEATION OF 0.1M AQUEOUS CLINDAMYCIN SOLUTIONS ACROSS WATER SWOLLEN pACM MEMBRANES AT 37°C

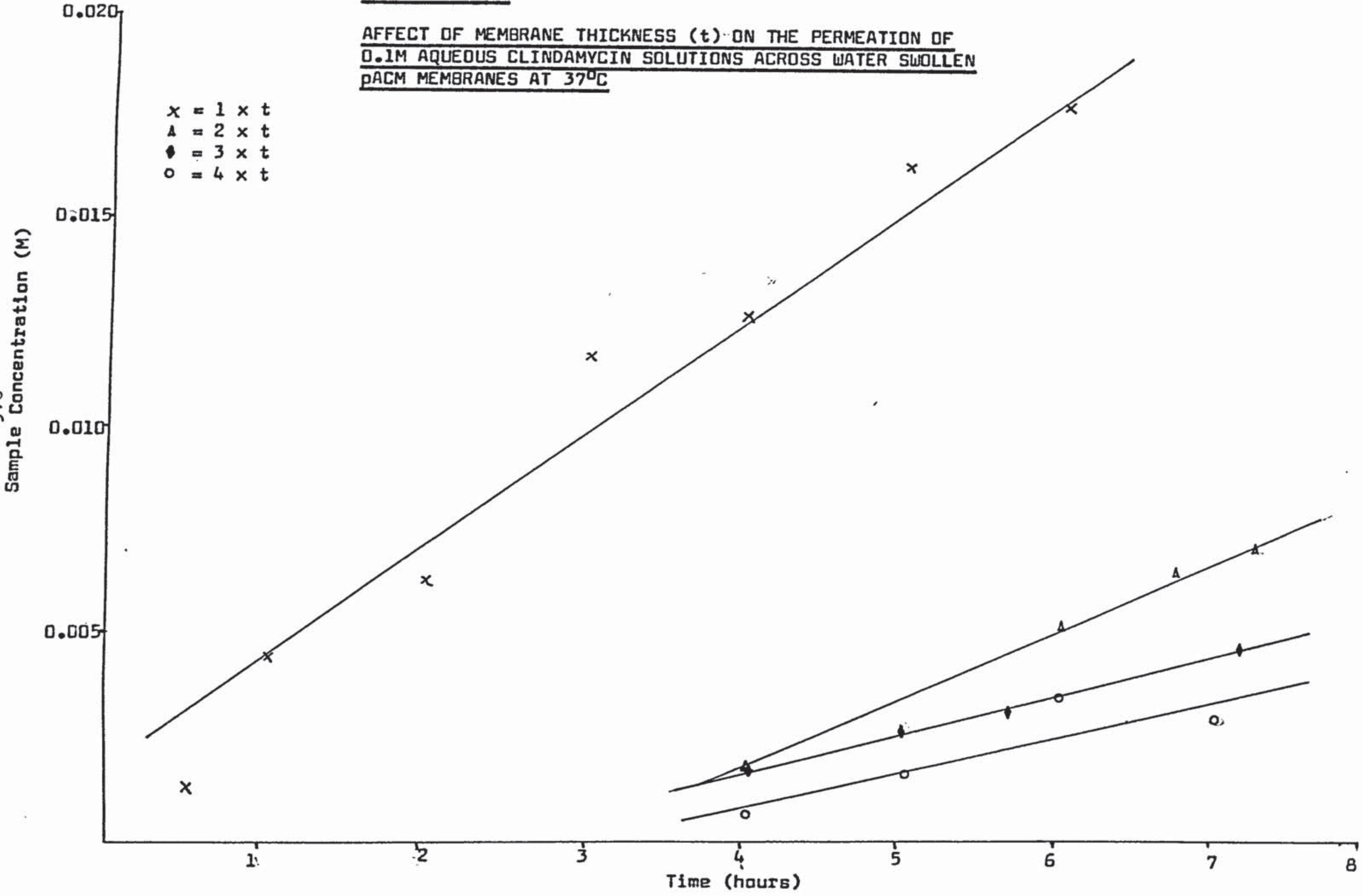
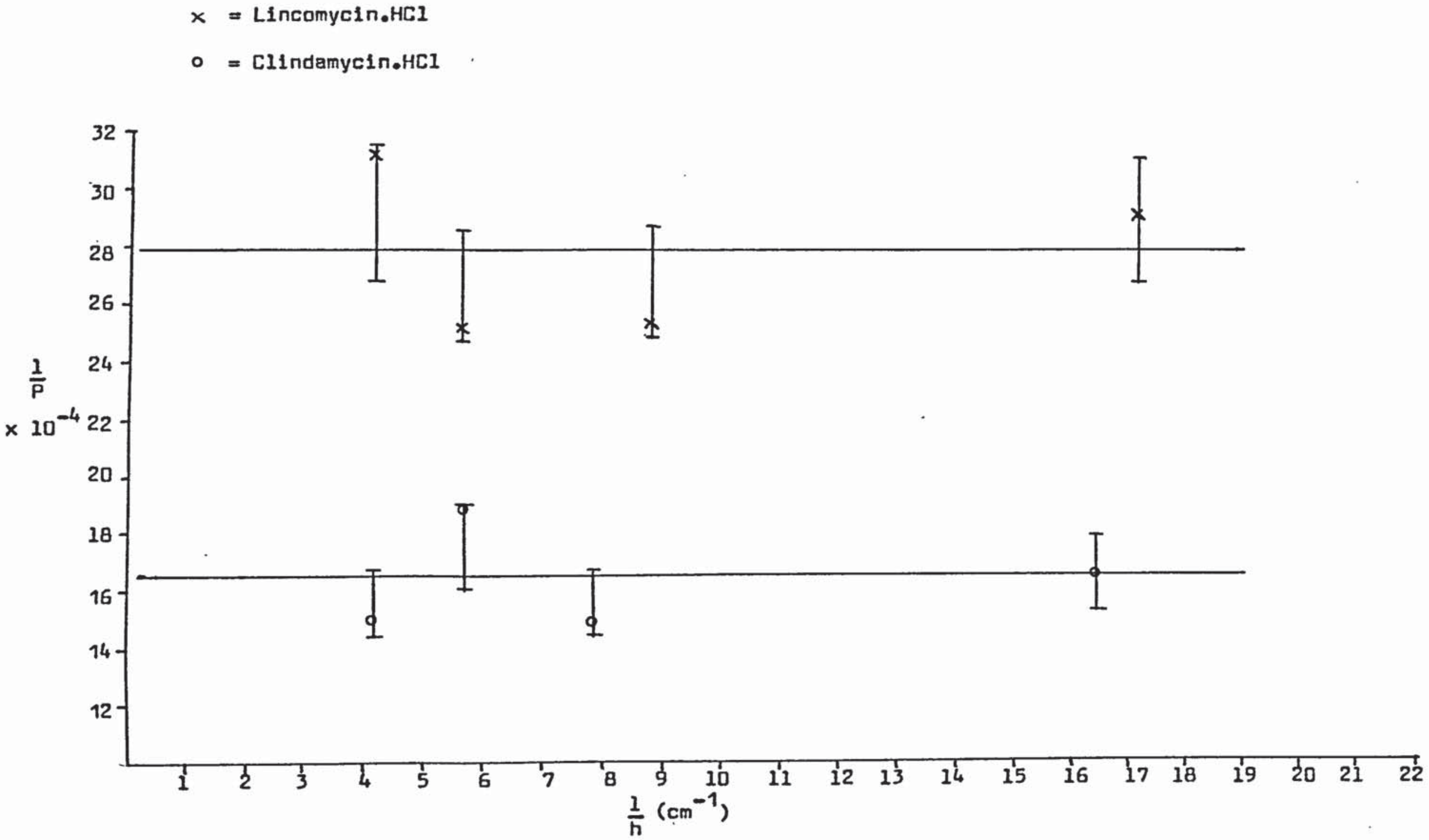


FIGURE V (19)

PLOTS OF $^1/P$ VERSUS $^1/h$ FOR THE PERMEATION OF AQUEOUS 0.1M SOLUTIONS OF LINCOMYCIN AND CLINDAMYCIN ACROSS WATER SWOLLEN pACM MEMBRANES AT 37°C



This paralleled the findings of Flynn and Roseman.[170]

Figure V (19) would also seem to highlight an overall difference in the rates of transport of the two antibiotics, Clindamycin appearing to permeate faster under these conditions than Lincomycin. Since associative affects at the low concentrations used are not considered to be significant at this temperature (37°C), the results might indicate something of the interactions of antibiotic and matrix water structures, the extent of interaction presumably being lower for Clindamycin than for Lincomycin. Alternatively the higher transport of Clindamycin in these high E.W.C. preparations might reflect its greater solubility than Lincomycin at this temperature.

Because of the complexity of the antibiotic-matrix, antibiotic-water and matrix-water competitive interactions no single factor can be isolated as being responsible for the differences in transport behaviour of the antibiotics. The range of solution concentrations, experimental temperatures and membrane water contents used needs to be expanded in order to ascertain the factors influencing the transport processes.

The next logical step in this research was to study the transport of some biologically significant solutes across antibiotic incorporated hydrogel membranes with a view to explaining the types of process influenced by the antibiotics which could lead to the reported differences in irritation of the two compounds. This work is presented in Chapter VI.

CHAPTER VI

PERMEABILITY STUDIES II - SOLUTE PERMEATION STUDIES ACROSS ANTIBIOTIC INCORPORATED HYDROGELS

VI.1 Introduction

This chapter deals with permeability studies extended to include the transport of various solutes across various hydrogel membrane preparations into which the antibiotics had been incorporated during polymerisation. The effects may be of relevance to the irritation processes observed in biological systems. The studies commenced with the investigation of sodium chloride, potassium chloride and calcium chloride transport behaviour and were later extended to cover other biologically significant solutes.

VI.2 Solute Permeation Across Water Swollen Hydrogel Membranes

Before the effects of antibiotic incorporation into hydrogel membranes on the transport of various solutes could be studied it was necessary to characterise the transport of these solutes through water swollen membranes.

Studies on the transport of individual 0.25M aqueous solutions of sodium chloride, potassium chloride and calcium chloride as well as combined solutions of all three of the above solutes 0.25M with respect to each, were carried out, although later studies were confined to the transport of the combined solute solutions.

The sample concentrations of the above solutes were determined by flame photometry as described in Chapter III and assuming co-transport of chloride ions with the cations so as to maintain electrochemical neutrality.

VI.2.i Solute Permeation Across Water Swollen pACM Membranes

The permeability of a water swollen pACM membrane (E.W.C. = 89.3%) to a combined aqueous solution 0.25M with respect to NaCl, KCl and $CaCl_2$ at 37°C can be seen in Figure VI (1). This showed that the order of rates of transport was KCl $>$ NaCl $\gg$ $CaCl_2$. It was apparent that even in such a large pore size, high E.W.C. membrane some differentiation of transport on the basis of hydration cosphere size was present, the hydration cosphere sizes increasing in the order $K^+ < Na^+ < Ca^{2+}$. It was also seen that with such high transport rates the permeability plot tailed off with time as donor and receptor solution solute concentrations approached equilibrium. The permeability coefficients determined from this experiment were used to estimate the pore size "cut-off" points for NaCl, KCl and $CaCl_2$ transport (see later) in the manner used for the antibiotics in Chapter V.

VI.2.ii Solute Permeation Across Water Swollen pHEMA Membranes

The permeability of a water swollen pHEMA membrane (E.W.C. = 35.5%) to a combined aqueous solution 0.25M with respect to NaCl, KCl and $CaCl_2$ at 37°C can be seen in Figure VI (2). This showed that once again the order of rates of transport was KCl $>$ NaCl $\gg$ $CaCl_2$. This trend was continued throughout the majority of the permeability studies carried out during this research.

The permeation of separate 0.25M solutions of KCl, NaCl and $CaCl_2$ across the same membrane composition at 37°C had slightly different characteristics (Figure VI (3)). The order of rates of transport of NaCl $>$ KCl $>$ $CaCl_2$ was found and although KCl and $CaCl_2$ transport was linear with time, NaCl transport tailed off even though donor and receptor solution NaCl concentrations were far from equilibrium. Because these findings were

FIGURE VI (1)

PERMEATION OF A COMBINED AQUEOUS SOLUTION 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ ACROSS A WATER SWOLLEN pACM MEMBRANE AT 37°C

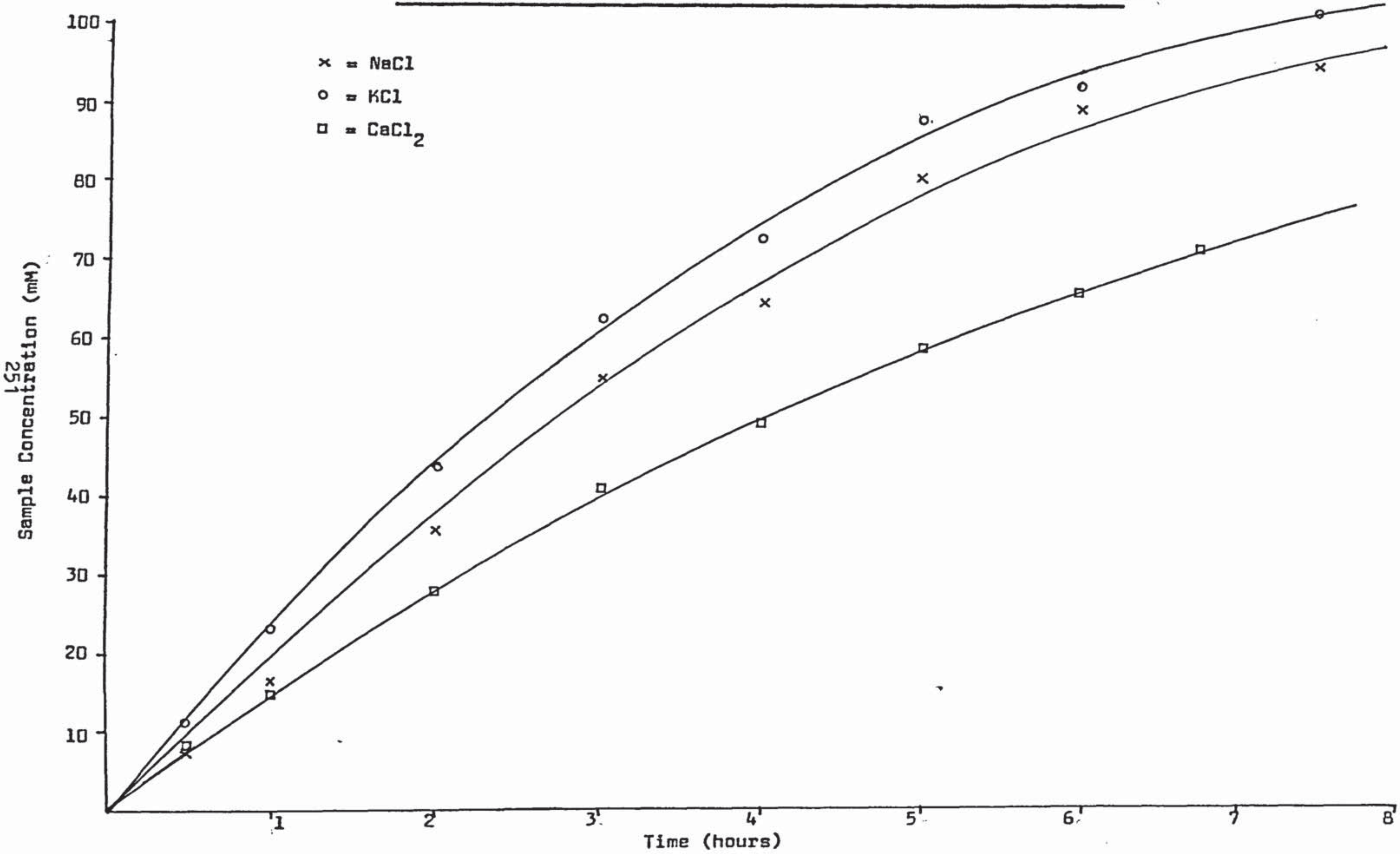


FIGURE VI (2)

PERMEATION OF A COMBINED AQUEOUS SOLUTION 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ ACROSS A WATER SWOLLEN pHEMA MEMBRANE AT 37^{0}C

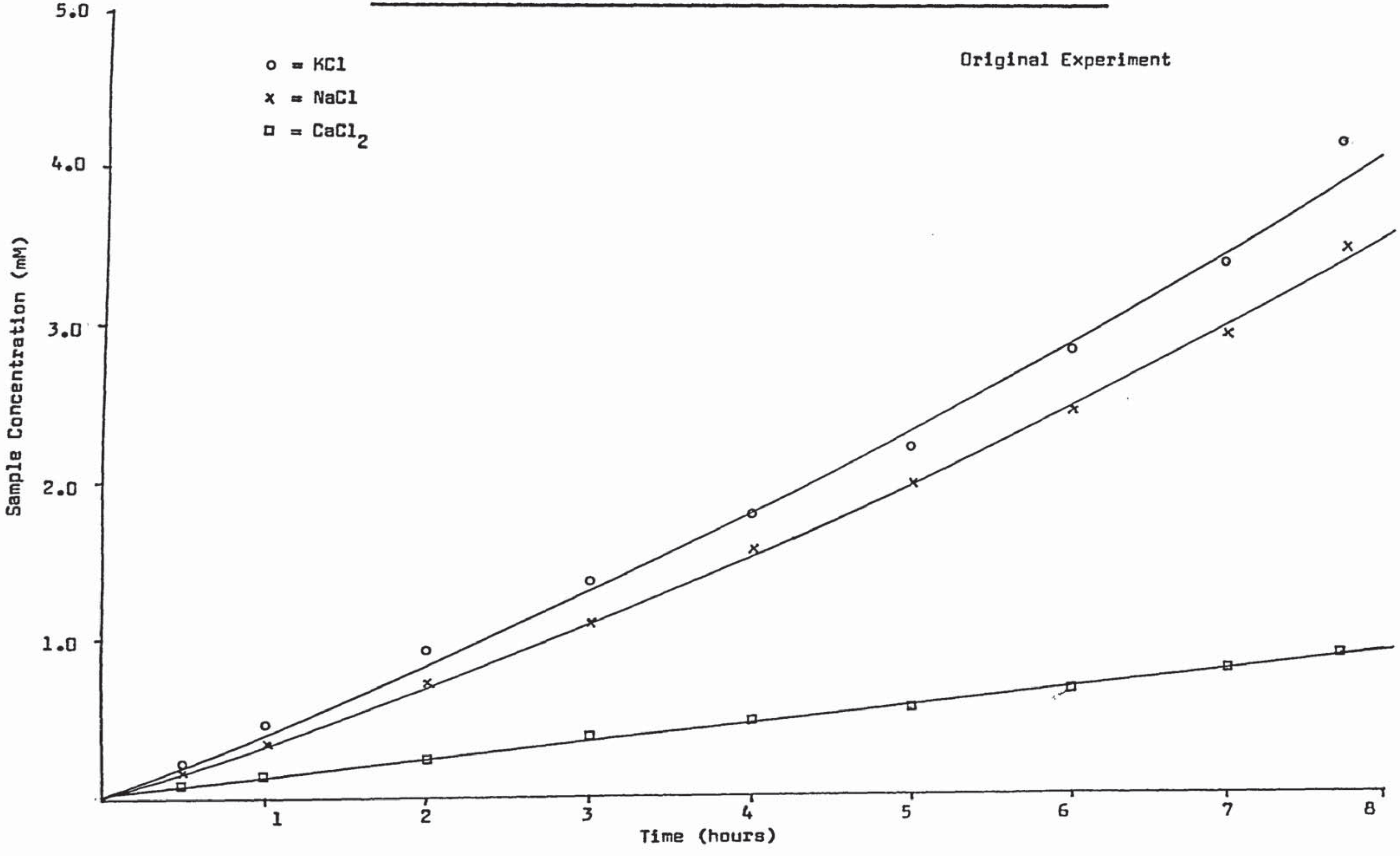
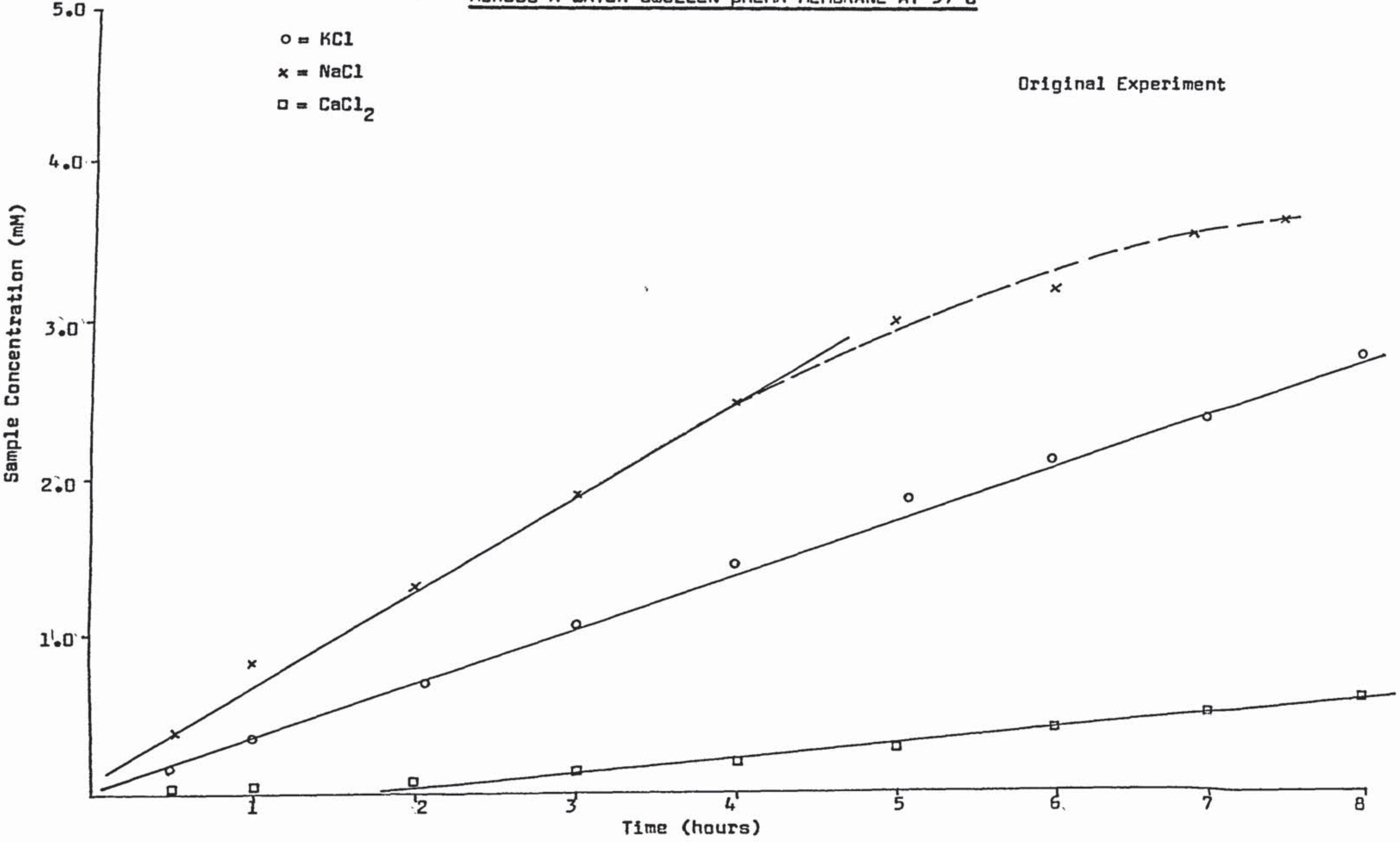


FIGURE VI (3)

PERMEATION OF SEPARATE 0.25M AQUEOUS SOLUTIONS OF NaCl, KCl and $CaCl_2$ ACROSS A WATER SWOLLEN pHEMA MEMBRANE AT 37°C



contrary to the behaviour found throughout this work the experiments were repeated. Figure VI (4) shows the repeated permeability run of a water swollen pHEMA membrane to a combined aqueous solution 0.25M with respect to NaCl, KCl, and $CaCl_2$ at 37°C. The order of rates of transport was KCl > NaCl > $CaCl_2$ as was found before and had been expected. However, the repeated permeability of a water swollen pHEMA membrane to separate 0.25M aqueous solutions of NaCl, KCl and $CaCl_2$ at 37°C once more showed certain peculiarities (Figure VI (5)). Although the overall order of rates of transport was KCl > NaCl > $CaCl_2$, as expected, this time KCl transport was found to tail off with time. These experiments were also repeated at 45°C. The results for the permeation of a combined aqueous solution 0.25M with respect to NaCl, KCl and $CaCl_2$ across a water swollen pHEMA membrane at 45°C are shown in Figure VI (6). This showed the expected KCl > NaCl ≫ $CaCl_2$ transport rate order but suggested that the rates of NaCl and KCl transport were very similar. These observations were paralleled in the results of the permeation of separate 0.25M aqueous solutions of NaCl, KCl and $CaCl_2$ across a water swollen pHEMA membrane at 45°C (Figure VI (7)). Figures VI (6) and VI (7) also showed that although $CaCl_2$ transport remained at the same level, NaCl and KCl transport were slightly greater in separate solute solution experiments than in combined solute solution experiments. This could be explained in terms of the lack of competition for transport between solutes in separate solutions leading to increased transport, although such an effect was not so clear at lower temperatures.

It was also noted that increasing experimental temperature from 37°C to 45°C reduced the overall solute transport rates (see Table A later). This was explained by the observed minima in water content of pHEMA at about 60°C,[78] the lower E.W.C. of pHEMA at 45°C than at 37°C leading to reduced overall transport.

FIGURE VI (4)

PERMEATION OF A COMBINED AQUEOUS SOLUTION 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ ACROSS A WATER SWOLLEN pHEMA MEMBRANE AT 37°C

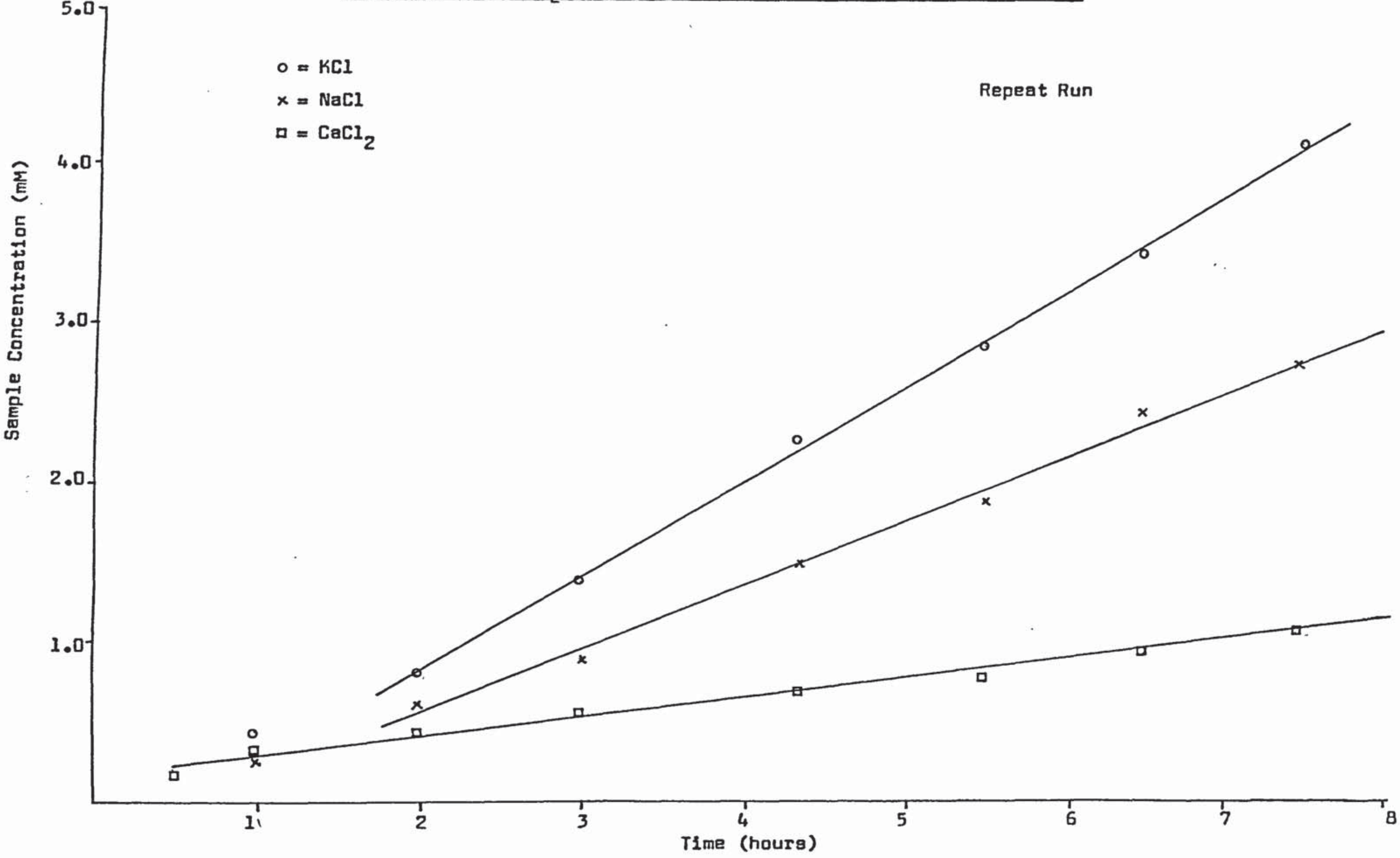


FIGURE VI (5)

PERMEATION OF SEPARATE 0.25M AQUEOUS SOLUTIONS OF NaCl, KCl and $CaCl_2$

ACROSS A WATER SWOLLEN pHEMA MEMBRANE AT 37°C

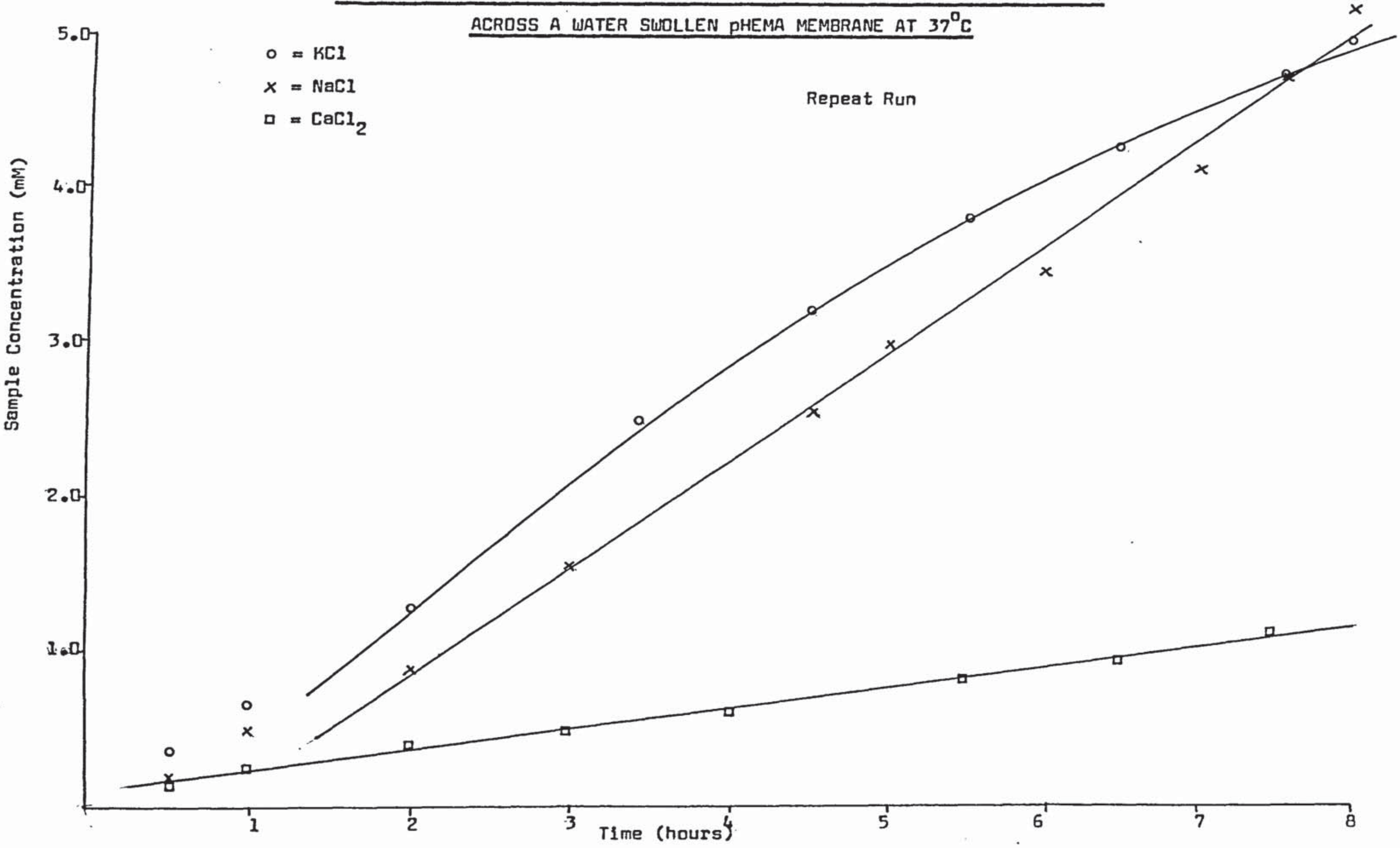


FIGURE VI (6)

PERMEATION OF A COMBINED AQUEOUS SOLUTION 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ ACROSS A WATER SWOLLEN pHEMA MEMBRANE AT 45°C

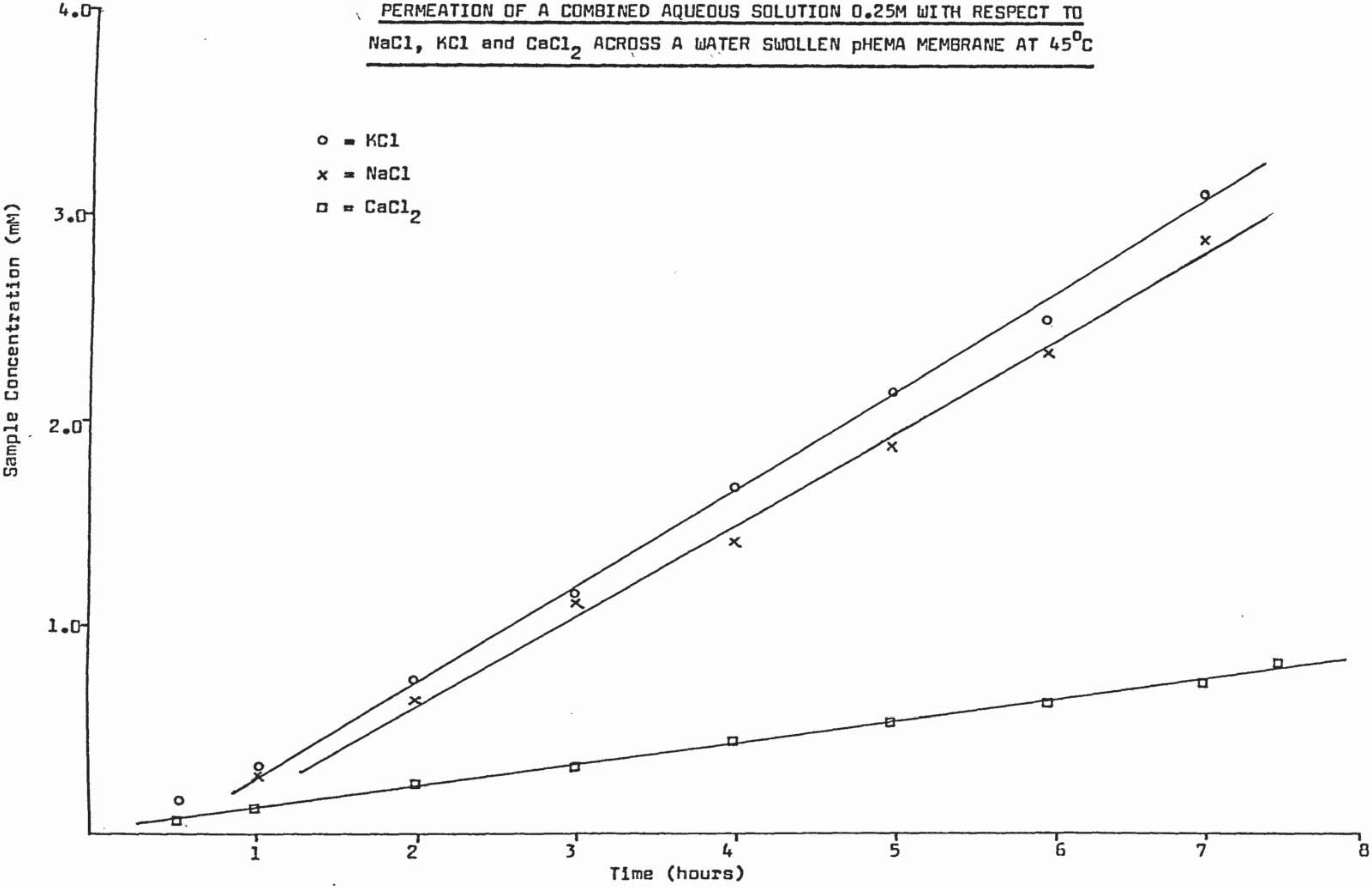
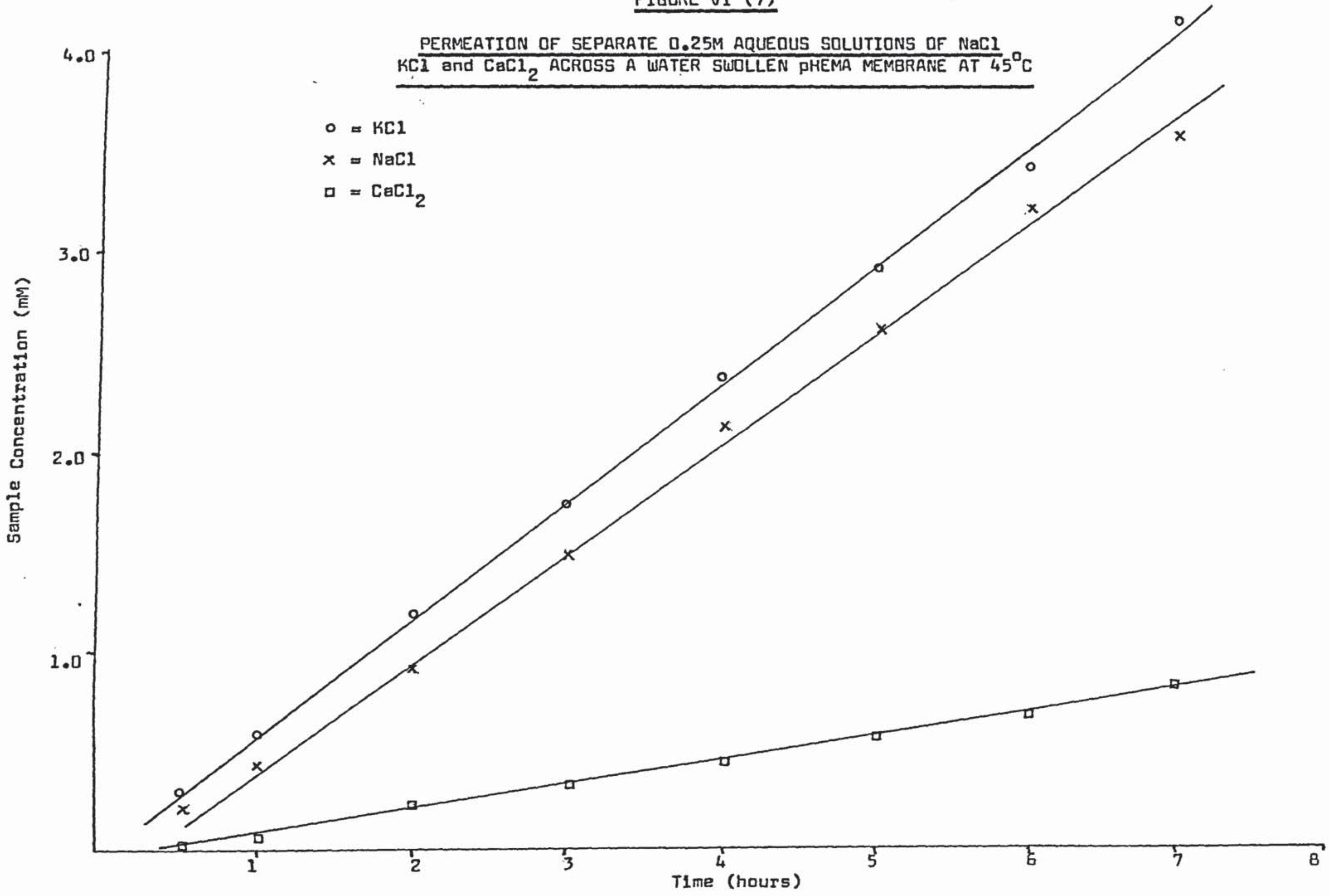


FIGURE VI (7)

PERMEATION OF SEPARATE 0.25M AQUEOUS SOLUTIONS OF NaCl KCl and $CaCl_2$ ACROSS A WATER SWOLLEN pHEMA MEMBRANE AT 45°C



The peculiarities of individual solute solution transport across pHEMA at 37^{o}C might have resulted from small amounts of absorbed solutes in the membrane which were able to affect the transport of the next solute studied. Such effects may have been due to the water structure "making" or "breaking" capabilities of the solutes themselves in this particular water environment although there was no evidence of such behaviour in the other systems studied.

It must also be pointed out that combined solutions 0.25M with respect to NaCl, KCl and $CaCl_2$ are of greater tonicity than the individual solute solutions and thus can be expected to deswell hydrogel membranes to a greater extent than separate solutions would. This in turn would influence solute transport rates making the comparison of combined and separate solute solution transport rates more complex.

VI.2.iii Solute Permeation Across Water Swollen HEMA/Styrene Membranes

Figure VI (8) shows the permeability of water swollen HEMA/Styrene (E.W.C. = 23.1) to a combined aqueous solution 0.25M with respect to NaCl, KCl and $CaCl_2$ at 37^{o}C. The transport of separate 0.25M solutions of NaCl, KCl and $CaCl_2$ can be seen in Figure VI (9). The most obvious features of these plots were that no $CaCl_2$ transport was detected and that the lower E.W.C. of these membranes as compared to pHEMA and pACM resulted in a further reduction in NaCl and KCl transport. The complete exclusion of $CaCl_2$ was due to the large hydrated size of Ca^{2+} (see later). It was also noted that the difference between KCl and NaCl transport rates was greatest in the separate solute solution studies (due to lack of competition for transport) KCl transport being faster than NaCl transport.

The experiments were also carried out at 45^{o}C and the results of the

FIGURE VI (8)

PERMEATION OF A COMBINED AQUEOUS SOLUTION 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ ACROSS A WATER SWOLLEN HEMA/STYRENE (90:10) (mole %) MEMBRANE AT 37°C

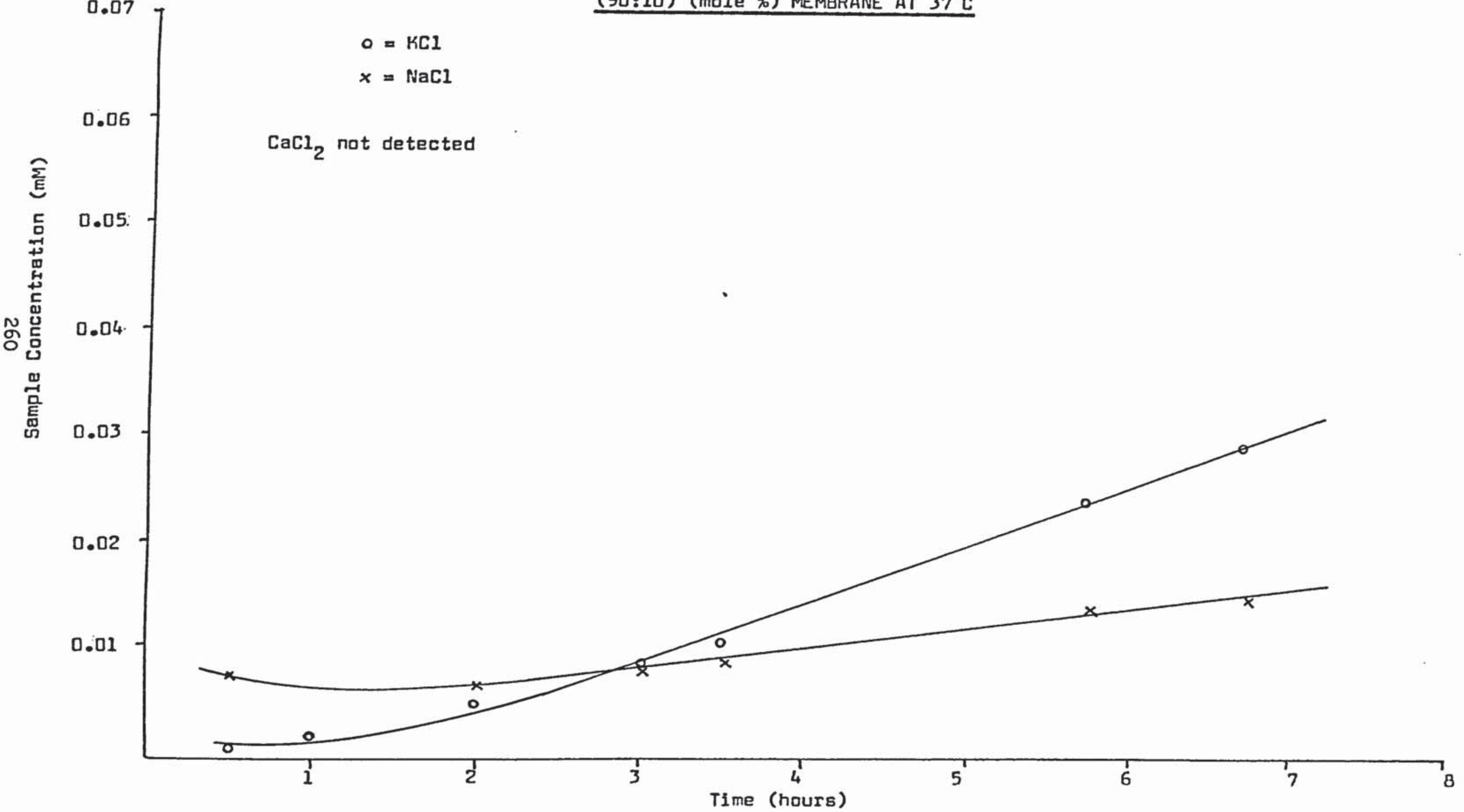


FIGURE VI (9)

PERMEATION OF SEPARATE 0.25M AQUEOUS SOLUTIONS OF NaCl, KCl and $CaCl_2$ ACROSS A WATER SWOLLEN HEMA/STYRENE (90:10) (mole %) MEMBRANE AT 37°C

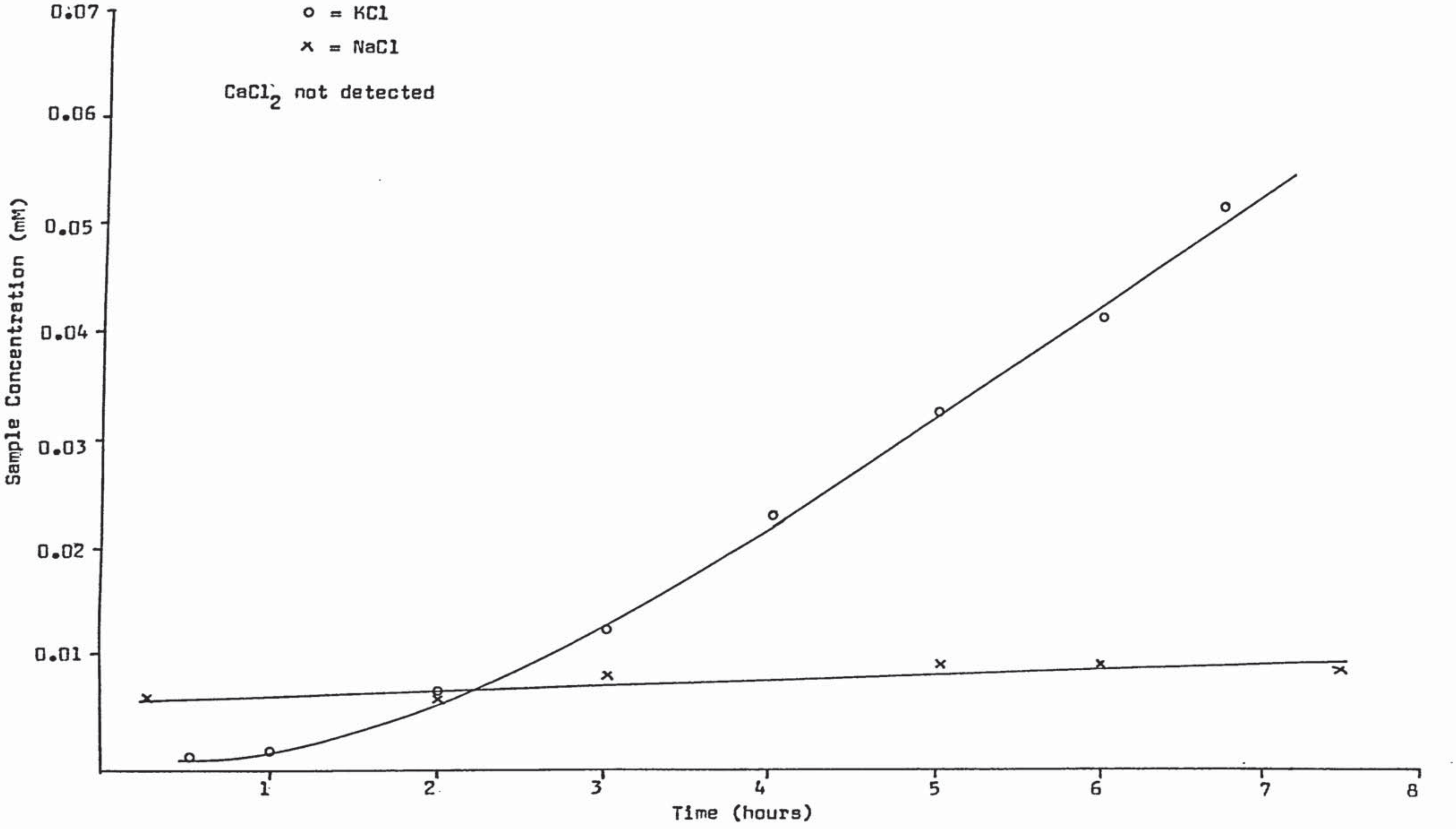


FIGURE VI (10)

PERMEATION OF A COMBINED AQUEOUS SOLUTION 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ ACROSS A WATER SWOLLEN HEMA/STYRENE (90:10) (mole %) MEMBRANE AT 45°C

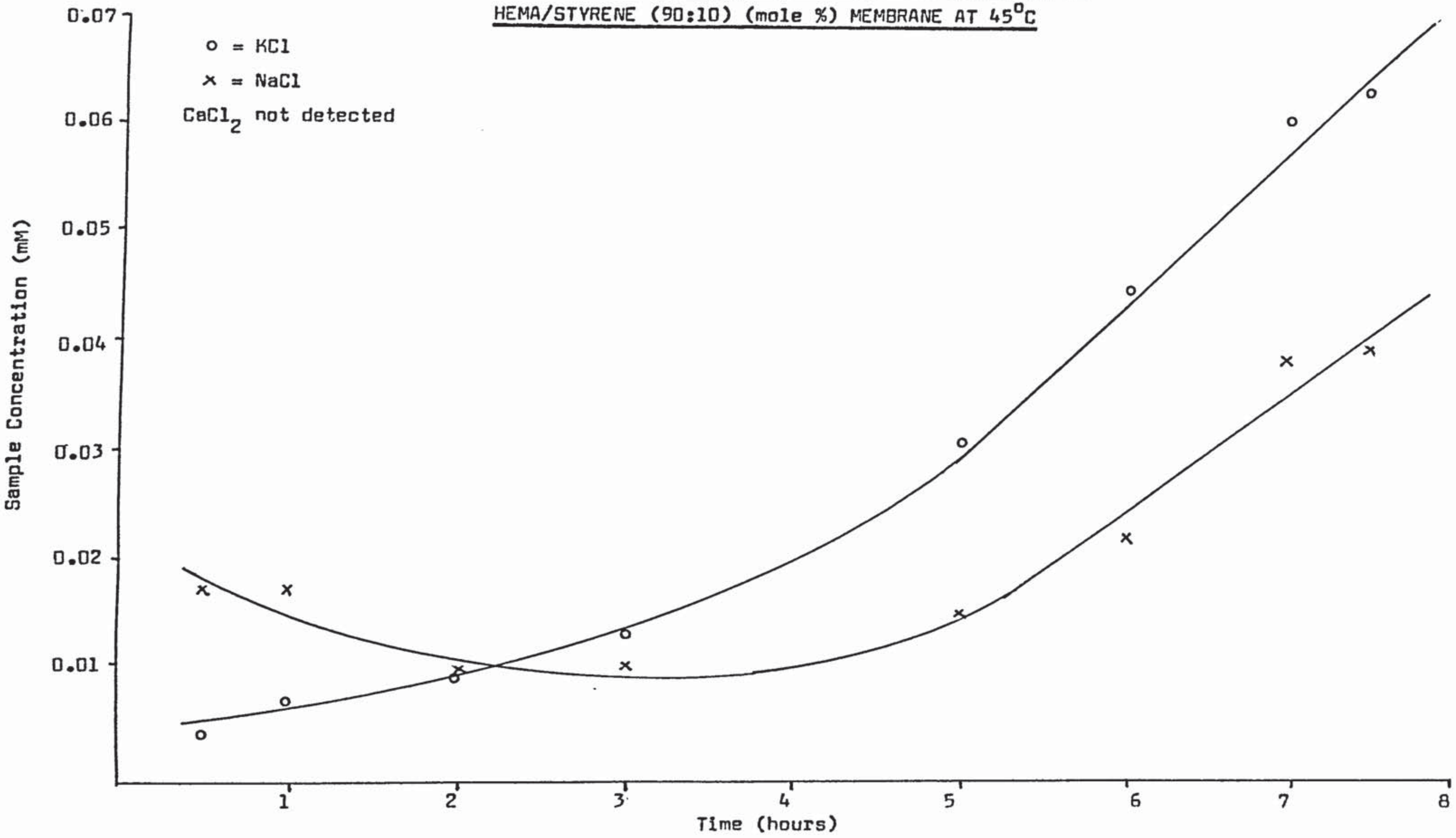
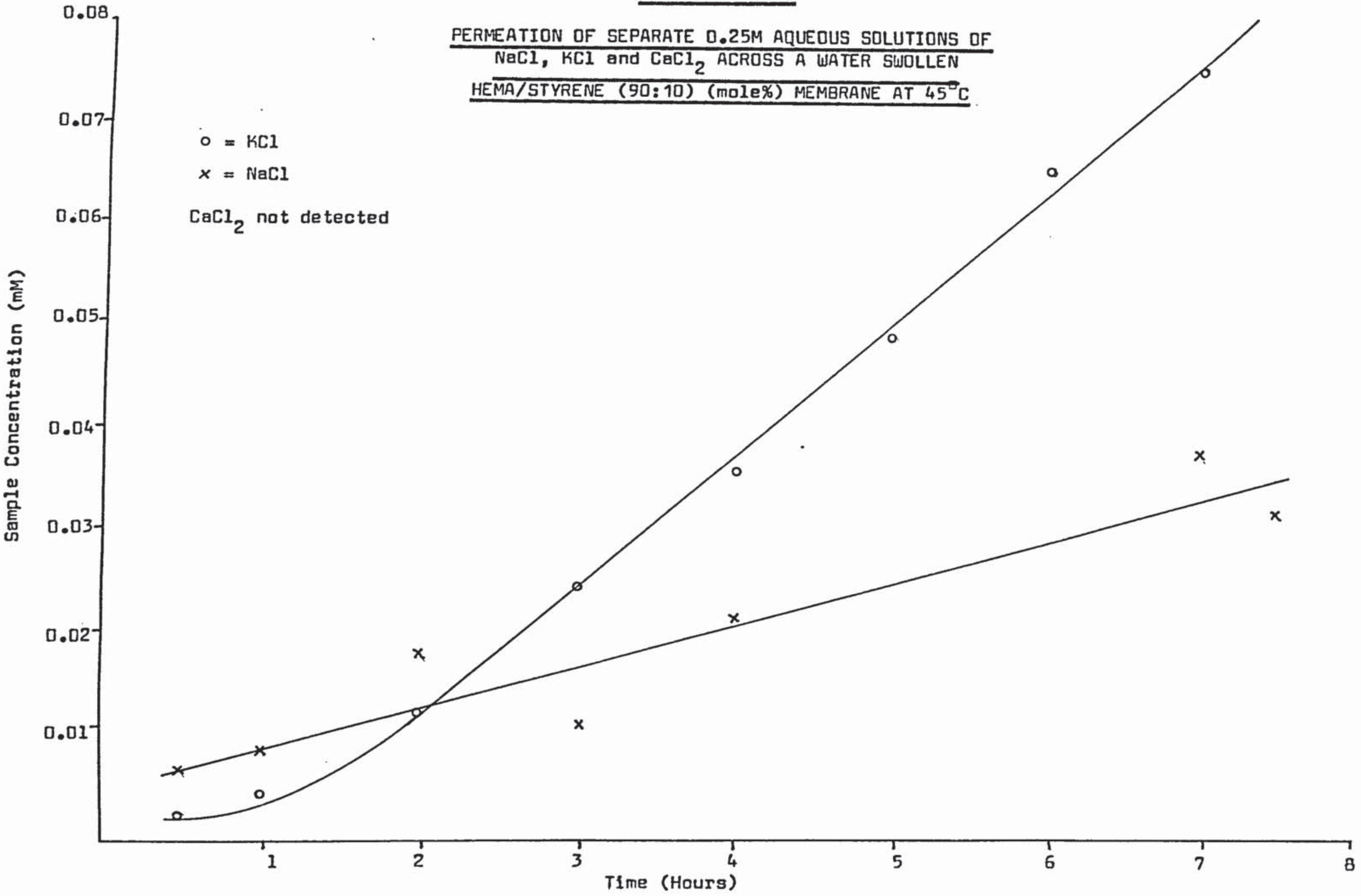


FIGURE VI (11)

PERMEATION OF SEPARATE 0.25M AQUEOUS SOLUTIONS OF NaCl, KCl and $CaCl_2$ ACROSS A WATER SWOLLEN HEMA/STYRENE (90:10) (mole%) MEMBRANE AT 45°C



combined solute solution studies can be seen in Figure VI (10) while the results of the separate solute solution studies can be seen in Figure VI (11). These experiments again showed that KCl transport was greater than NaCl transport, no $CaCl_2$ transport being detected. The difference in rates of NaCl and KCl transport was once again greatest in the "non-competitive" separate solute solution experiments, and transport rates increased with increasing experimental temperature.

The problems of high Na^+ contamination in the initial stages of permeability runs, an observation highlighted by the high sensitivity used in sample analysis for these particular systems, could also be seen from these results.

The ability to exclude calcium transport whilst allowing sodium and potassium transport, as well as to some extent differentiating sodium and potassium transport, on the basis of hydrated ionic size has far reaching implications in fields such as dialysis and the design of permselective membranes for sensors.

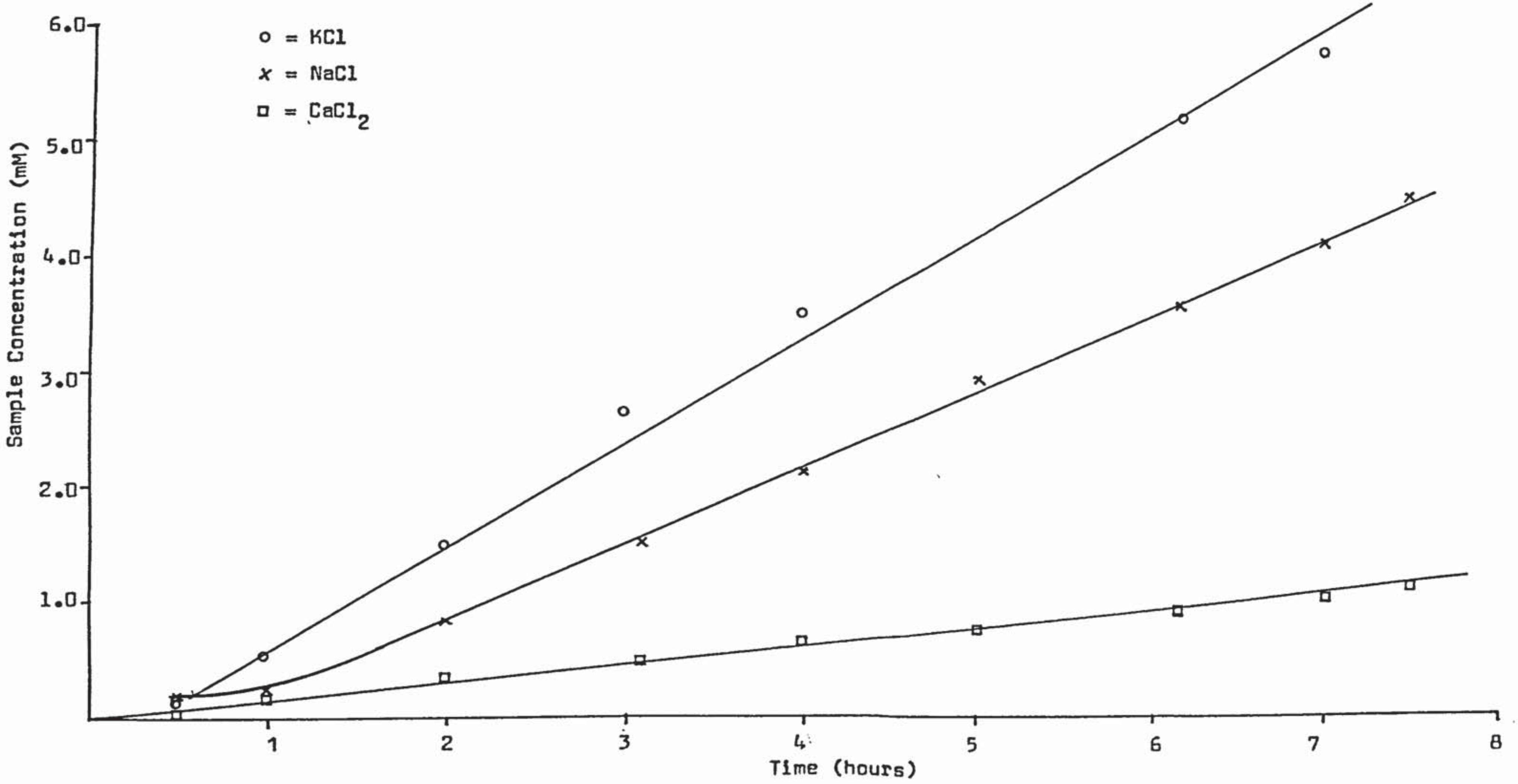
VI.2.iv Solute Permeation Across Water Swollen MAA/ACM Membranes

The permeability of a water swollen MAA/ACM membrane (E.W.C. = 37.6) to a combined aqueous solution 0.25M with respect to NaCl, KCl and $CaCl_2$ at 37^0C can be seen in Figure VI (12).

Although these plots seemed to demonstrate the expected order of transport ($KCl > NaCl > CaCl_2$) and linearity with time this type of composition could be affected by factors which could influence its permeability characteristics. It has been noted in Chapter III that the incorporation of ionogenic groups such as MAA into pHEMA hydrogels reduces NaCl permeability whereas ionisation of the group dramatically

FIGURE VI (12)

PERMEATION OF A COMBINED AQUEOUS SOLUTION 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ ACROSS A WATER SWOLLEN MAA/ACM MEMBRANE AT 37°C



increases the E.W.C. of the preparation.[124] Another complicating factor involves the extent of interchain hydrogen bonding in MAA/ACM preparations which might lead to a gradual increase in E.W.C. with time at 37°C (Chapter III).

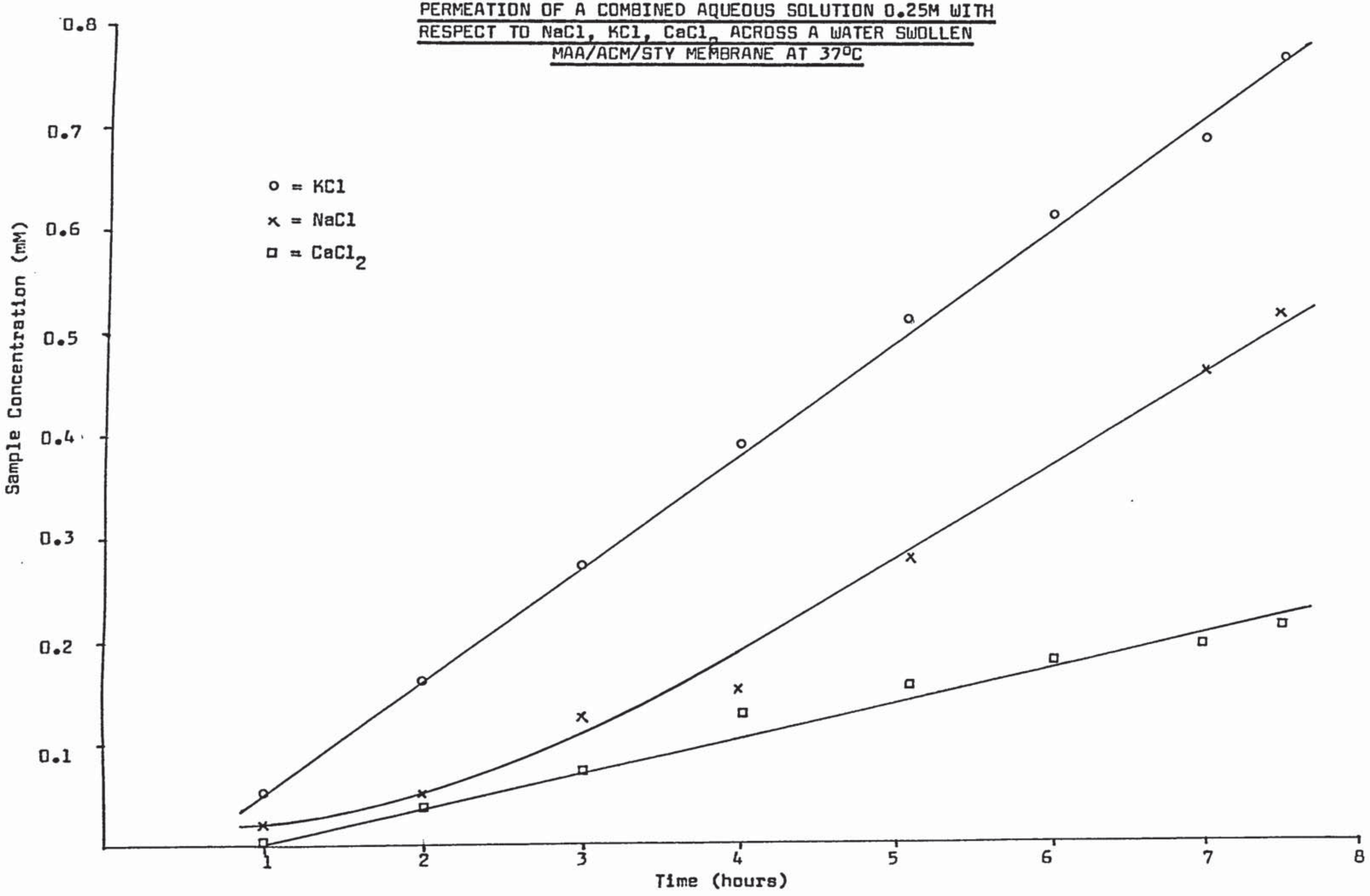
The possible E.W.C. change with time was not considered to be significant for the experimental time and the pH of the system was such as to suggest that increased E.W.C. due to MAA ionisation was also negligible. The preparation's permeability characteristics showed no significant deviations from the properties expected for a hydrogel of this E.W.C. This was exemplified by the linearity of the plots, showing no E.W.C. change with time and also by comparison with results obtained on other hydrogel preparations (see permeation "cut-off" plots later). Overall, short term effects due to the experimental temperature used could be confidently excluded.

VI.2.v Solute Permeation Across Water Swollen MAA/ACM/STY Membranes

Figure VI (13) shows the permeability of a water swollen MAA/ACM/STY membrane (E.W.C. = 27.5) to a combined aqueous solution 0.25M with respect to NaCl, KCl and $CaCl_2$ at 37°C. The low rates of transport correlated with the low E.W.C. of this preparation and the order of rates of transport was again KCl > NaCl > $CaCl_2$. It was also possible to identify a period of transport lag (lag time) from these results, especially in the cases of $CaCl_2$ and KCl transport. This had also been observed for NaCl and KCl transport across HEMA/STY membranes (eg Figure VI (10)). It was also interesting to note that whereas no $CaCl_2$ transport was detected across HEMA/STY membranes of E.W.C. = 23.1%, transport of $CaCl_2$ was found across a MAA/ACM/STY membrane of E.W.C. = 27.5%. Not only did the 5% E.W.C. increase allow $CaCl_2$ transport to occur but it also resulted in a ten-fold increase in NaCl and KCl transport even though the E.W.C's of

FIGURE VI (13)

PERMEATION OF A COMBINED AQUEOUS SOLUTION 0.25M WITH RESPECT TO NaCl, KCl, $CaCl_2$ ACROSS A WATER SWOLLEN MAA/ACM/STY MEMBRANE AT 37°C



both preparations were almost completely composed of non-freezing water.

The predictable permeability properties of this membrane suggested that none of the influences discussed with regard to MAA/ACM membranes were affecting transport across this preparation.

VI.2.vi Effect of Pore Size on Solute Permeation

The effect of a reduction in E.W.C. on pore size and thus on solute permeability of the type described above can be clearly seen from a plot of logP versus ($^1/1$-H) as demonstrated in Chapter V. The data corresponding to all the results discussed so far in this chapter are shown in Table VI.A and log P versus ($^1/1$-H) plots for $CaCl_2$, NaCl and KCl transport are shown in Figures VI (14), VI (15) and VI (16) respectively.

The values of ($^1/1$-H) employed were calculated from the E.W.C's of the preparations at 23°C bearing in mind the negligible influence on E.W.C. of the short term elevated experimental temperature (Chapter III). The results used for pHEMA permeation were those obtained in the repeated experimental run since the variation in permeability coefficients between combined solute solution and separate solute solution experiments was not as great as in the original experimental runs.

Some of the data in Table VI.A can be compared with previously reported values. Yasuda, Lamaze and Ikenberry[119] reported permeability coefficients of 3.12×10^{-7} and 5.64×10^{-7} cm^2 sec^{-1} for NaCl transport across pHEMA membranes cross-linked with 0.97 mole% and 0.37 mole% EDMA. The experimental temperature must be assumed to be room temperature. These values were in agreement with those of 3.40×10^{-7} cm^2 sec^{-1} and 4.25×10^{-7} cm^2 sec^{-1} obtained during studies here on 1 mole% EDMA cross-linked pHEMA membranes at 37°C.

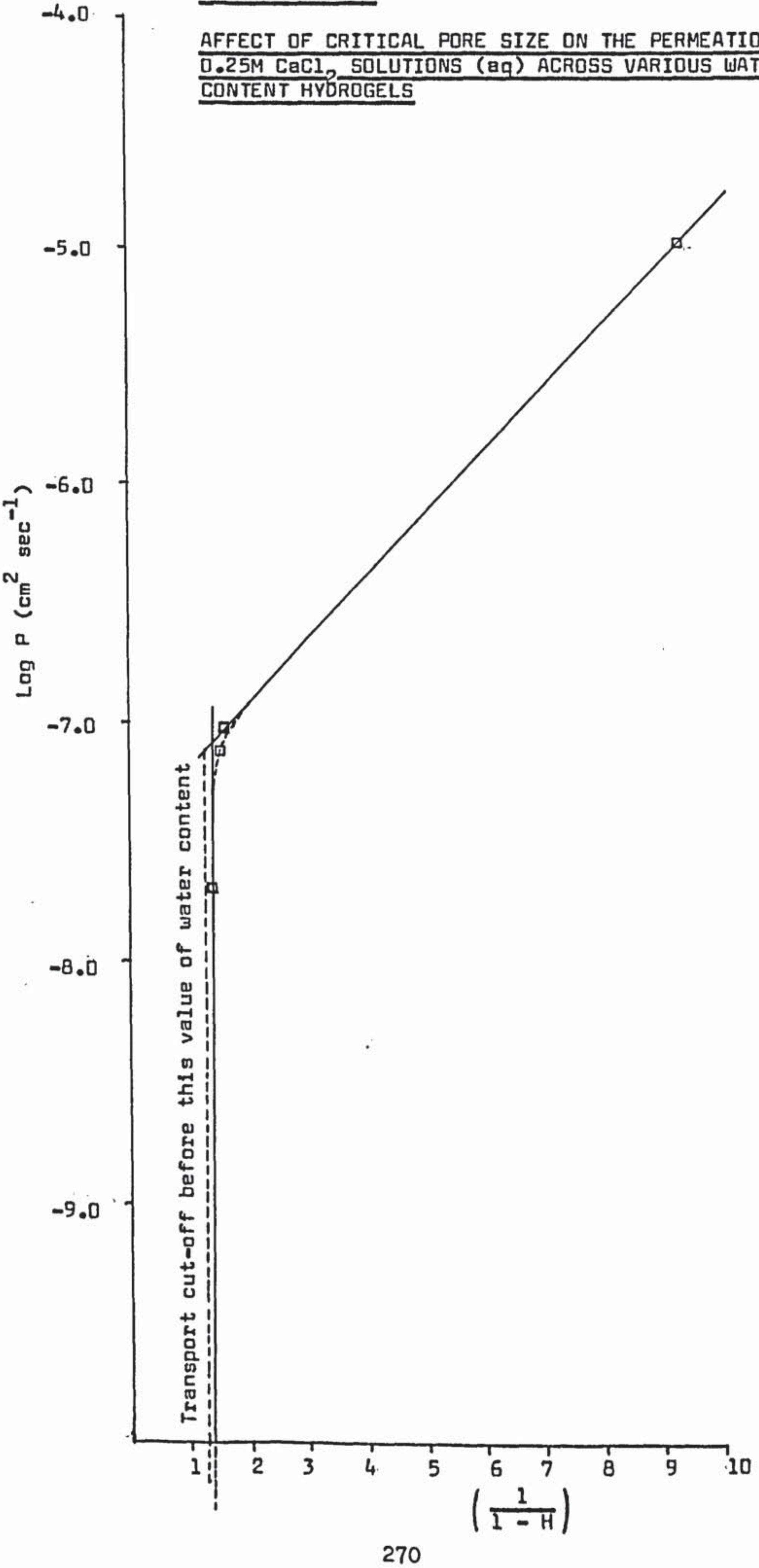
TABLE VI.A

SUMMARY OF PERMEABILITY DATA FOR WATER SWOLLEN HYDROGEL MEMBRANES

Preparation (E.W.C. % at 23°C	Temperature (°C)	Solute(s) Studied	Permeability Coefficient (cm^2 sec^{-1})
pACM (89.3)	37	0.25M w.r.t (NaCl	141.2 × 10^{-7}
		(KCl	148.1 × 10^{-7}
		(CaCl$_2$	98.2 × 10^{-7}
pHEMA (1st run) (35.5)	37	0.25M w.r.t (NaCl	2.8 × 10^{-7}
		(KCl	3.2 × 10^{-7}
		(CaCl$_2$	0.7 × 10^{-7}
pHEMA (1st run) (35.5)	37	0.25M NaCl	3.4 × 10^{-7}
		0.25M KCl	2.2 × 10^{-7}
		0.25M CaCl$_2$	0.6 × 10^{-7}
pHEMA (2nd run) (35.5)	37	0.25M w.r.t (NaCl	2.5 × 10^{-7}
		(KCl	3.7 × 10^{-7}
		(CaCl$_2$	0.7 × 10^{-7}
pHEMA (2nd run) (35.5)	37	0.25M NaCl	4.3 × 10^{-7}
		0.25M KCl	3.8 × 10^{-7}
		0.25M CaCl$_2$	0.8 × 10^{-7}
pHEMA (35.5)	45	0.25M w.r.t (NaCl	2.7 × 10^{-7}
		(KCl	2.9 × 10^{-7}
		(CaCl$_2$	0.6 × 10^{-7}
pHEMA (35.5)	45	0.25M NaCl	3.4 × 10^{-7}
		0.25M KCl	3.6 × 10^{-7}
		0.25M CaCl$_2$	0.8 × 10^{-7}
HEMA/STY (23.1)	37	0.25M w.r.t (NaCl	0.009 × 10^{-7}
		(KCl	0.028 × 10^{-7}
		(CaCl$_2$	Not Detected
HEMA/STY (23.1)	37	0.25M NaCl	0.003 × 10^{-7}
		0.25M KCl	0.050 × 10^{-7}
		0.25M CaCl$_2$	Not Detected
HEMA/STY (23.1)	45	0.25M w.r.t (NaCl	0.056 × 10^{-7}
		(KCl	0.073 × 10^{-7}
		(CaCl$_2$	Not Detected
HEMA/STY (23.1)	45	0.25M NaCl	0.020 × 10^{-7}
		0.25M KCl	0.067 × 10^{-7}
		0.25M CaCl$_2$	Not Detected
MAA/ACM (37.6)	37	0.25M w.r.t (NaCl	4.3 × 10^{-7}
		(KCl	5.7 × 10^{-7}
		(CaCl$_2$	0.9 × 10^{-7}
MAA/ACM/STY (27.5)	37	0.25M w.r.t (NaCl	0.6 × 10^{-7}
		(KCl	0.7 × 10^{-7}
		(CaCl$_2$	0.2 × 10^{-7}

FIGURE VI (14)

AFFECT OF CRITICAL PORE SIZE ON THE PERMEATION OF 0.25M $CaCl_2$ SOLUTIONS (aq) ACROSS VARIOUS WATER CONTENT HYDROGELS



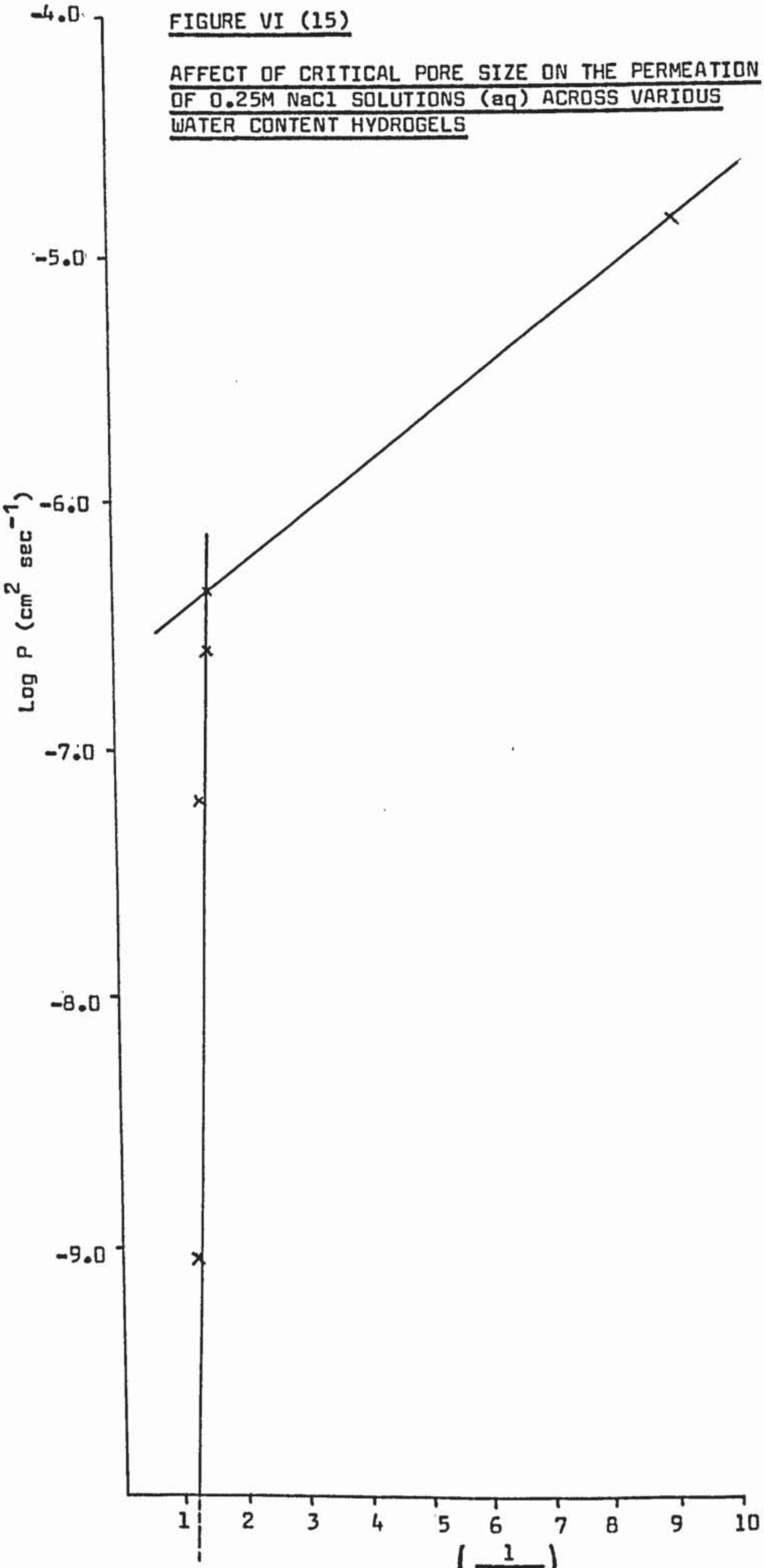
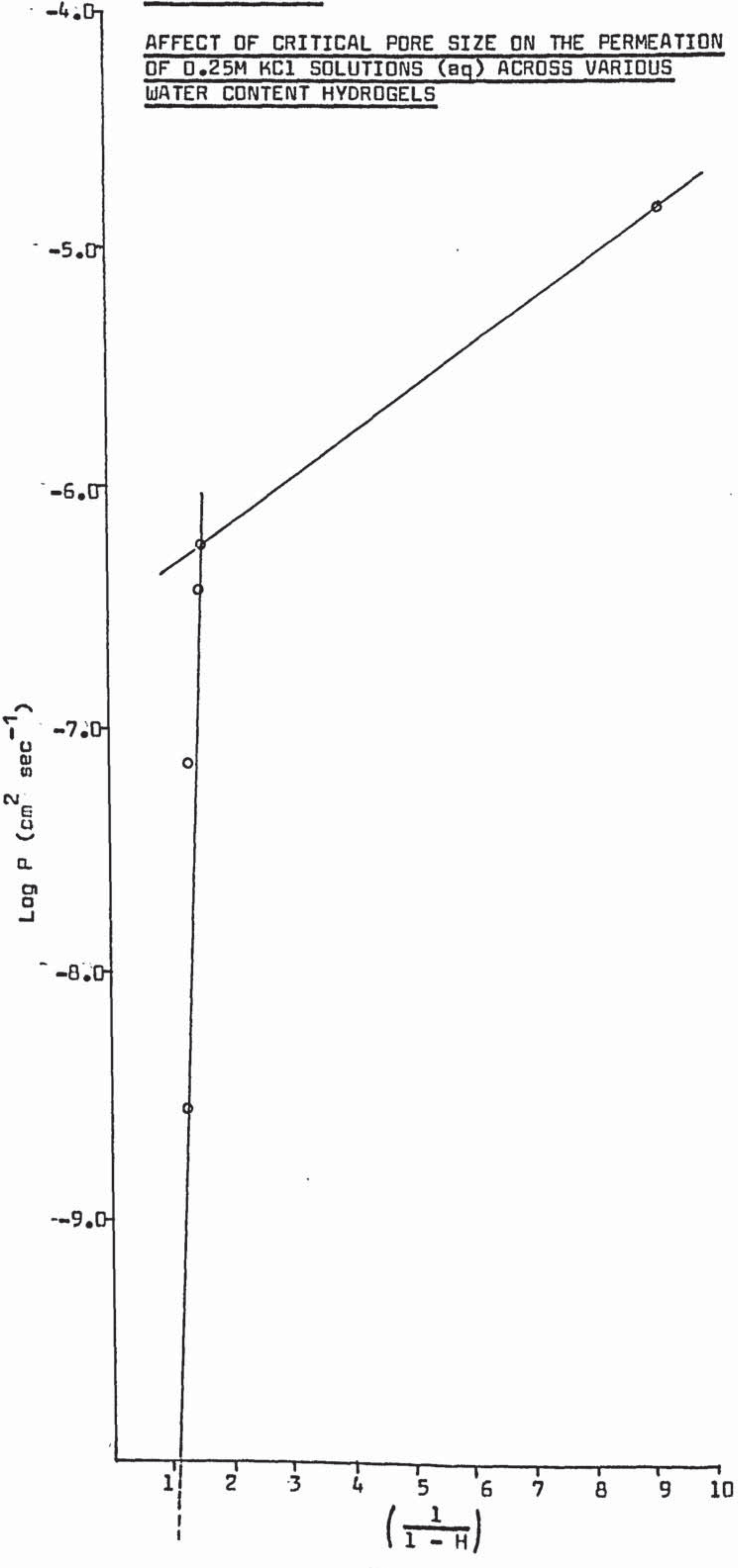


FIGURE VI (15)

AFFECT OF CRITICAL PORE SIZE ON THE PERMEATION OF 0.25M NaCl SOLUTIONS (aq) ACROSS VARIOUS WATER CONTENT HYDROGELS

FIGURE VI (16)

AFFECT OF CRITICAL PORE SIZE ON THE PERMEATION OF 0.25M KCl SOLUTIONS (aq) ACROSS VARIOUS WATER CONTENT HYDROGELS



Figures VI (14), VI (15) and VI (16) clearly show the abrupt "cut-off" points for $CaCl_2$, NaCl and KCl transport. Extrapolation of the plots towards zero permeation yields the point at which the pore size of the matrix prevents any solute transport. The dramatic change of slope which is characteristic of such plots represents the point at which the hydration cospheres of the ions and the water lining the matrix pores begin to interact. The order in which the ions were seen to be affected was Ca^{2+}, Na^+ and then K^+. This followed the order of hydration numbers rather than ionic radii thus illustrating the limiting effects on transport of the hydration cospheres (see Table VI.8).

TABLE VI.8

IONIC RADII AND HYDRATION NUMBERS

	Na^+	K^+	Ca^{2+}	Cl^-
Ionic radius (nm)[179]	0.095	0.133	0.099	0.181
Hydration number for chloride salt[180]	5-6	5-7	6-8	-
Hydration number for Cl^- associated with each cation 180	4-6	2-4	6-8	

The range of water content values over which the transport of all three solutes became severely restricted was small, being between 25% and 35% E.W.C. From Figure III (23) in Chapter III these E.W.C's correspond to pore radii of approximately 2.8 to 3.3 Å although it must be appreciated that the matrices are composed of a distribution of pore sizes at a given E.W.C. which also contributes to the gradual change in slope at the "cut-off" point.

Complete transport "cut-off" was only found for $CaCl_2$ although it must be considered that given sufficiently long periods of experimental time some $CaCl_2$ would be found to permeate even HEMA/STY membranes. Transport of $CaCl_2$ was found to be initially influenced at an E.W.C. corresponding to a pore radius of about 0.33 nm. Given that the radius of Ca^{2+} is 0.099 nm then the addition of one hydration layer of water molecules adds 0.180 nm to the radius, ie total radius of 0.279 nm. This would be slightly smaller than the pore radius at which $CaCl_2$ transport was affected, but the addition of another hydration shell would result in a radius of 0.459 nm. This would be more than sufficient to result in the reduced transport behaviour observed. These calculations agree with the estimated hydration numbers of 6-8 for Ca^{2+} when as $CaCl_2$.

It can also be seen from Table VI.8 that the difference in the hydration numbers of Na^+ and K^+ is small. However, Wiggins presented data (Table VI.8) whereby NaCl transport could be "cut-off" prior to KCl transport "cut-off" due to the hydrated size of the chloride ion associated with each cation. This was clearer in the individual solute solution experiments than in the combined solute solution experiments where the hydrated size of the common anion exerted its influence on permeation to a lesser extent.

Throughout the permeability studies discussed so far a high level of confidence has been placed on the results of any single experiment. This can be justified in two ways. First, a number of repeated experiments on the permeability of water swollen pACM to Lincomycin (0.1M) at 23°C showed a variation better than ± 5% and experiments on KCl transport across water swollen pHEMA showed a variation of less than ± 3.5% from experiment to experiment.

Secondly, in any one experiment the data were in good agreement with each other, often resulting in excellent linearity of the plots or well fitting curves.

Having demonstrated ionic transport selectivity based both on anion hydrated size and associated cation hydrated size the next stage of this research was to study the transport of these solutes across antibiotic incorporated membranes where the water structuring influences of the antibiotics on solute transport could be investigated.

VI.3 Solute Permeation Across Antibiotic Incorporated Membranes

Antibiotic incorporated membranes employed in these studies were soaked in distilled water overnight and then rinsed in fresh distilled water before being fitted to the permeability cell. This procedure eliminated the effects of gradual depletion of antibiotic in the membranes during the experiments in that most of the antibiotic present during experiments was "locked" into the matrix. The procedure also overcame the associated problem of membrane rupture which was observed if a membrane not previously soaked in distilled water overnight was fitted to the cell, due to its swelling characteristics when in contact with the solutions used in permeability runs. The rupture process consisted of a "delamination" of the membrane in a plane perpendicular to solute transport. This process was often accompanied by a very faint and transient blue translucency, as in similar processes reported by Refojo[79] as due to microphase separation of small clusters of water molecules.

VI.3.1 Solute Permeation Across Antibiotic Incorporated pHEMA Membranes

Figure VI (17) shows the permeation of a combined aqueous solution 0.25M with respect to NaCl, KCl and $CaCl_2$ across a 10% 1.0M Lincomycin incorporated pHEMA membrane at 37°C. The corresponding separate solution experiments on the same membrane composition can be seen in Figure VI (18).

FIGURE VI (17)

PERMEATION OF A COMBINED AQUEOUS SOLUTION 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ ACROSS A 10% 1.0M LINCOMYCIN INCORPORATED pHEMA MEMBRANE AT 37°C

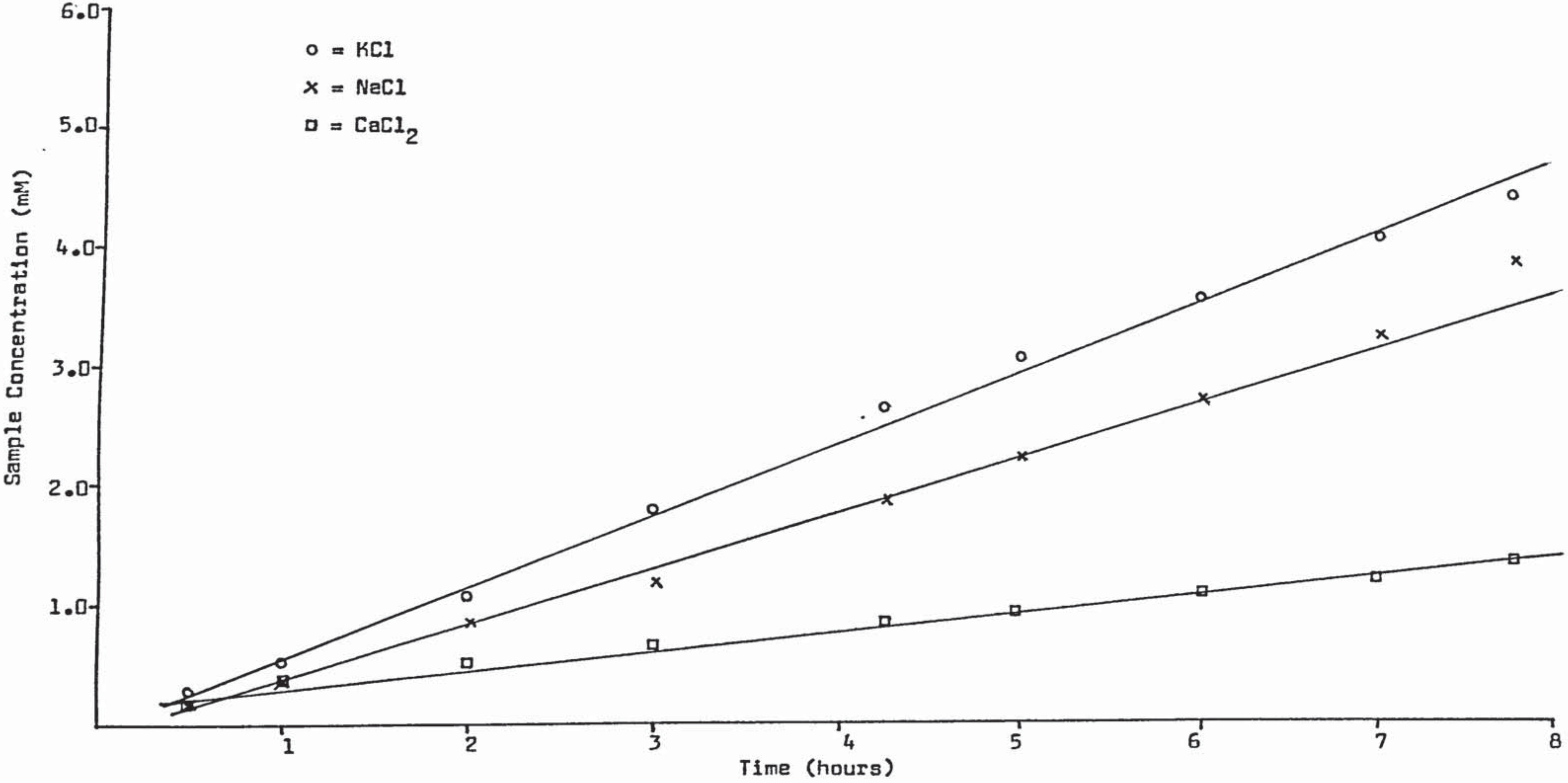
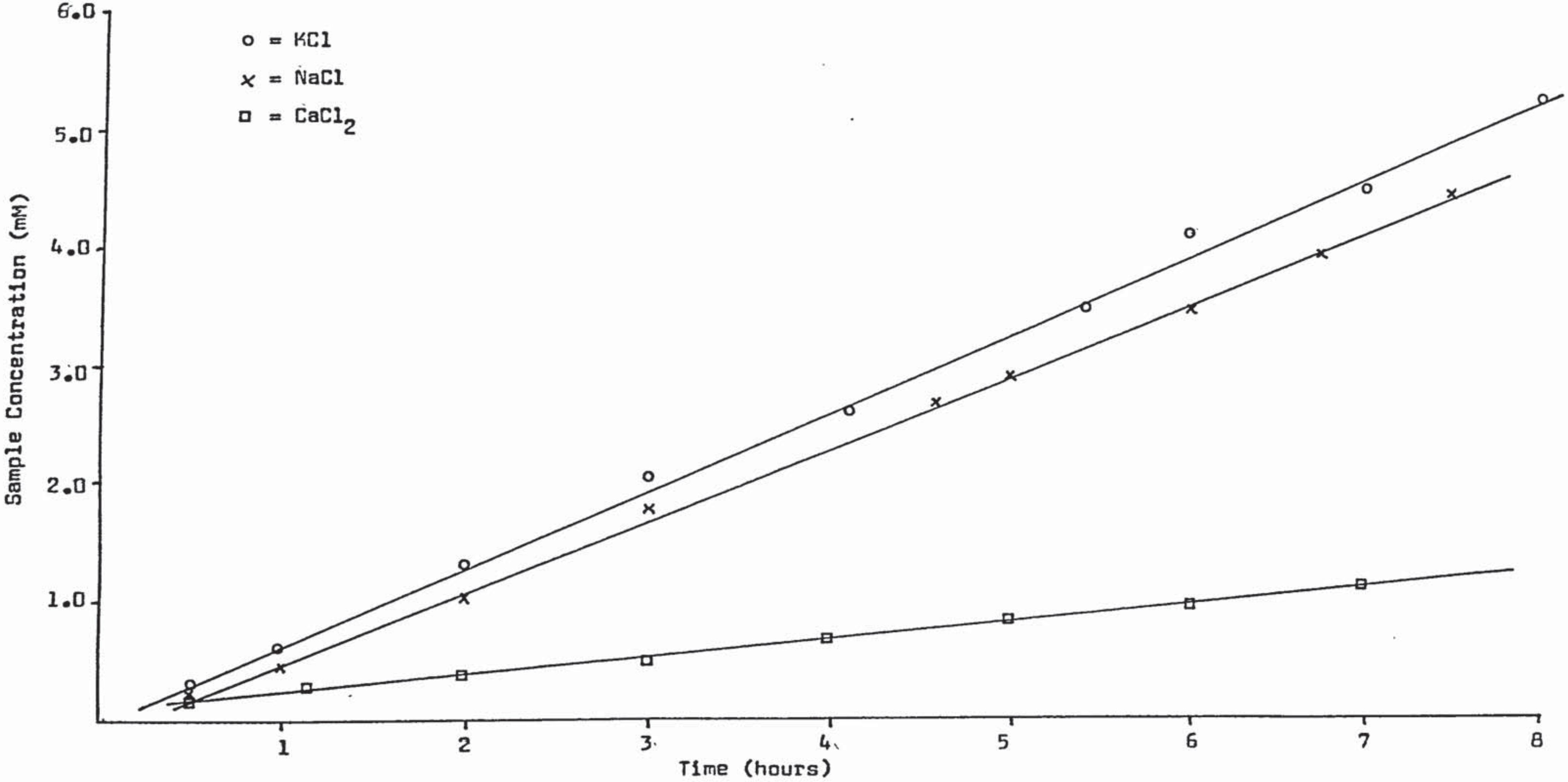


FIGURE VI (1B)

PERMEATION OF SEPARATE 0.25M AQUEOUS SOLUTIONS OF NaCl, KCl and $CaCl_2$ ACROSS A 10% 1.0M LINCOMYCIN INCORPORATED pHEMA MEMBRANE AT 37°C



The order of transport rates in both cases followed the previously observed trend of KCl > NaCl > $CaCl_2$ although the rates of KCl and NaCl transport were lower in the combined solute solution experiments than in the separate solute solution experiments. This indicated the competitive nature of NaCl and KCl transport.

The permeation of a combined aqueous solution 0.25M with respect to NaCl, KCl and $CaCl_2$ across a 10% 1.0M Clindamycin incorporated membrane at 37°C can be seen in Figure VI (19). The corresponding results from separate solute solution experiments can be seen in Figure VI (20). The order rates of transport in both cases was as expected except that NaCl and KCl transport rates were very similar and once again NaCl and KCl transport rates were lower in the competitive combined solute solution experiments than in the separate solute solution experiments.

Comparing the two types of antibiotic incorporated membrane it was found that the transport rate of all three solutes was greatest in the Lincomycin incorporated membrane permeability experiments. The permeability coefficients of all these experiments are presented later in Table VI.C, but Figures VI (21) and VI (22) summarise the data for pHEMA including that for transport across water swollen pHEMA membranes. The E.W.C. of Lincomycin incorporated pHEMA was 39.4% of which 10.1% was freezing water. The E.W.C. of Clindamycin incorporated pHEMA was 40.8% of which 20.2% was freezing water. Water swollen pHEMA had an E.W.C. of 35.5% of which 12.8% was freezing water.

Comparisons of data from water swollen membrane experiments with data from antibiotic incorporated membrane experiments are not strictly valid. However, pHEMA membranes incorporating 10% (by wt) water were not successfully prepared although such preparations would according to

FIGURE VI (19)

PERMEATION OF A COMBINED AQUEOUS SOLUTION 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ ACROSS A 10% 1.0M CLINDAMYCIN INCORPORATED pHEMA MEMBRANE AT 37°C

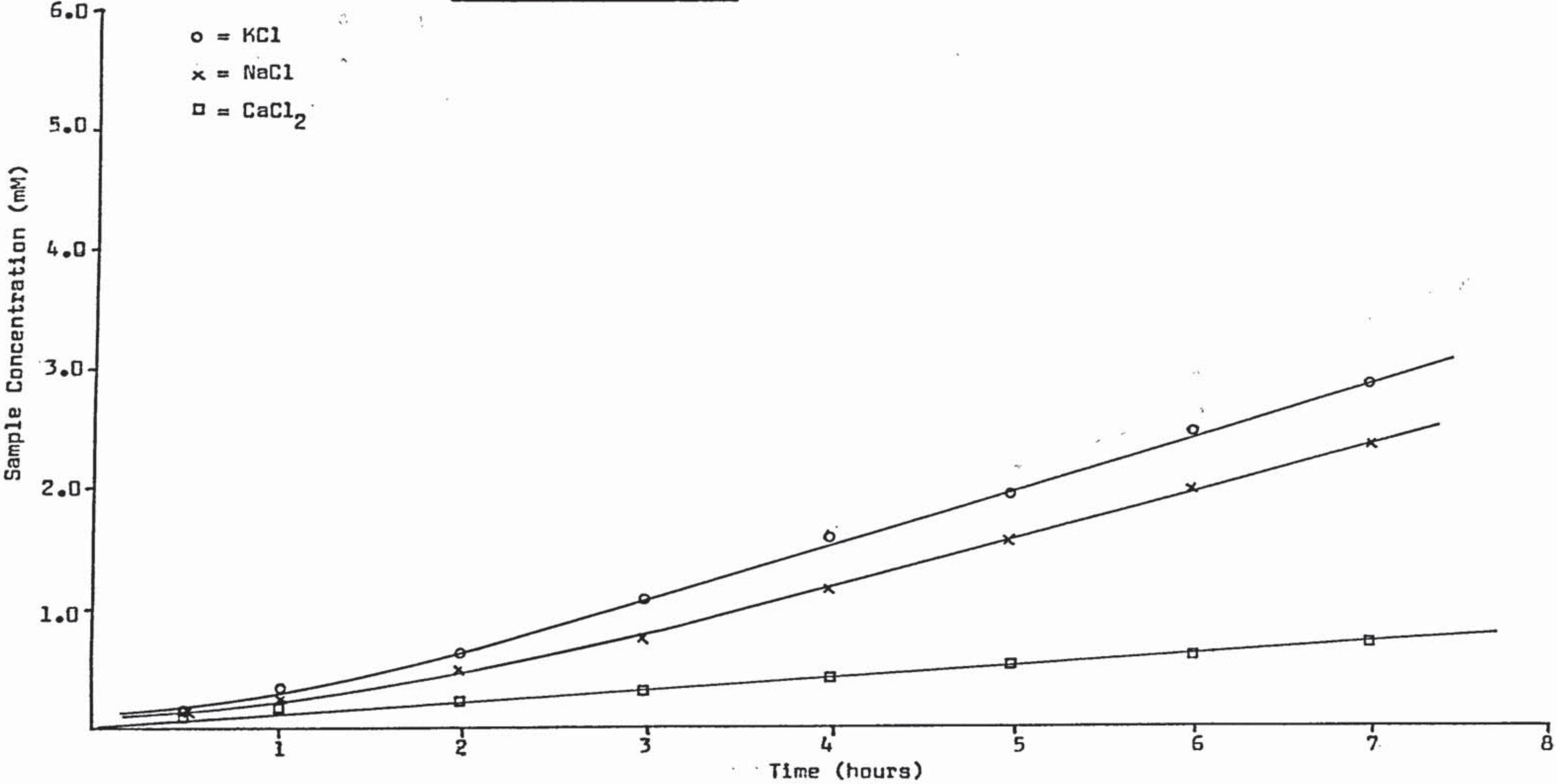


FIGURE VI (20)

PERMEATION OF SEPARATE 0.25M AQUEOUS SOLUTIONS OF NaCl, KCl and $CaCl_2$ ACROSS A 10% 1.0M CLINDAMYCIN INCORPORATED pHEMA MEMBRANE AT 37°C

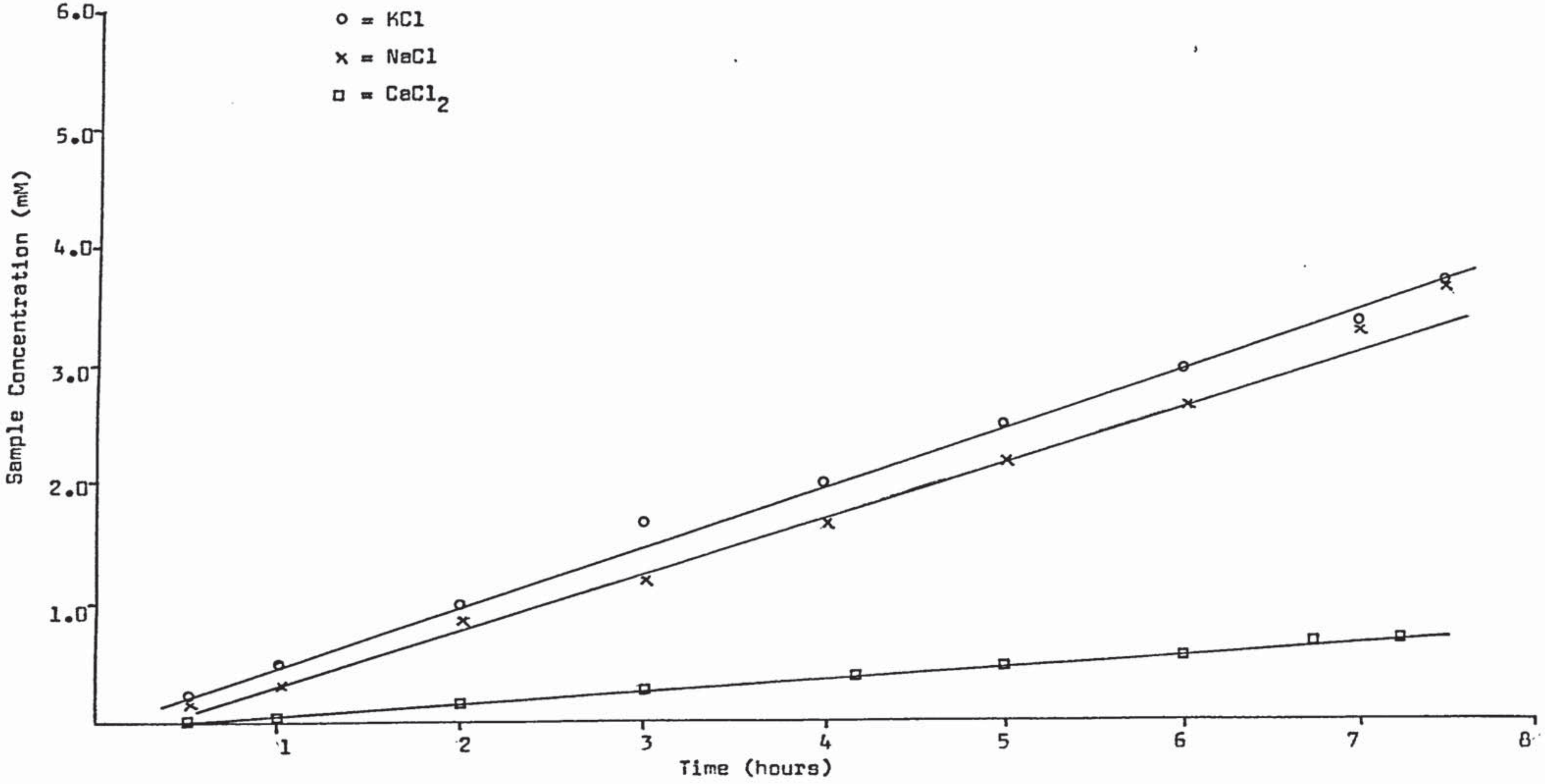


FIGURE VI (21) SUMMARY OF PERMEABILITY COEFFICIENTS OF COMBINED AQUEOUS SOLUTIONS 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ ACROSS VARIOUS pHEMA MEMBRANE PREPARATIONS AT 37°C

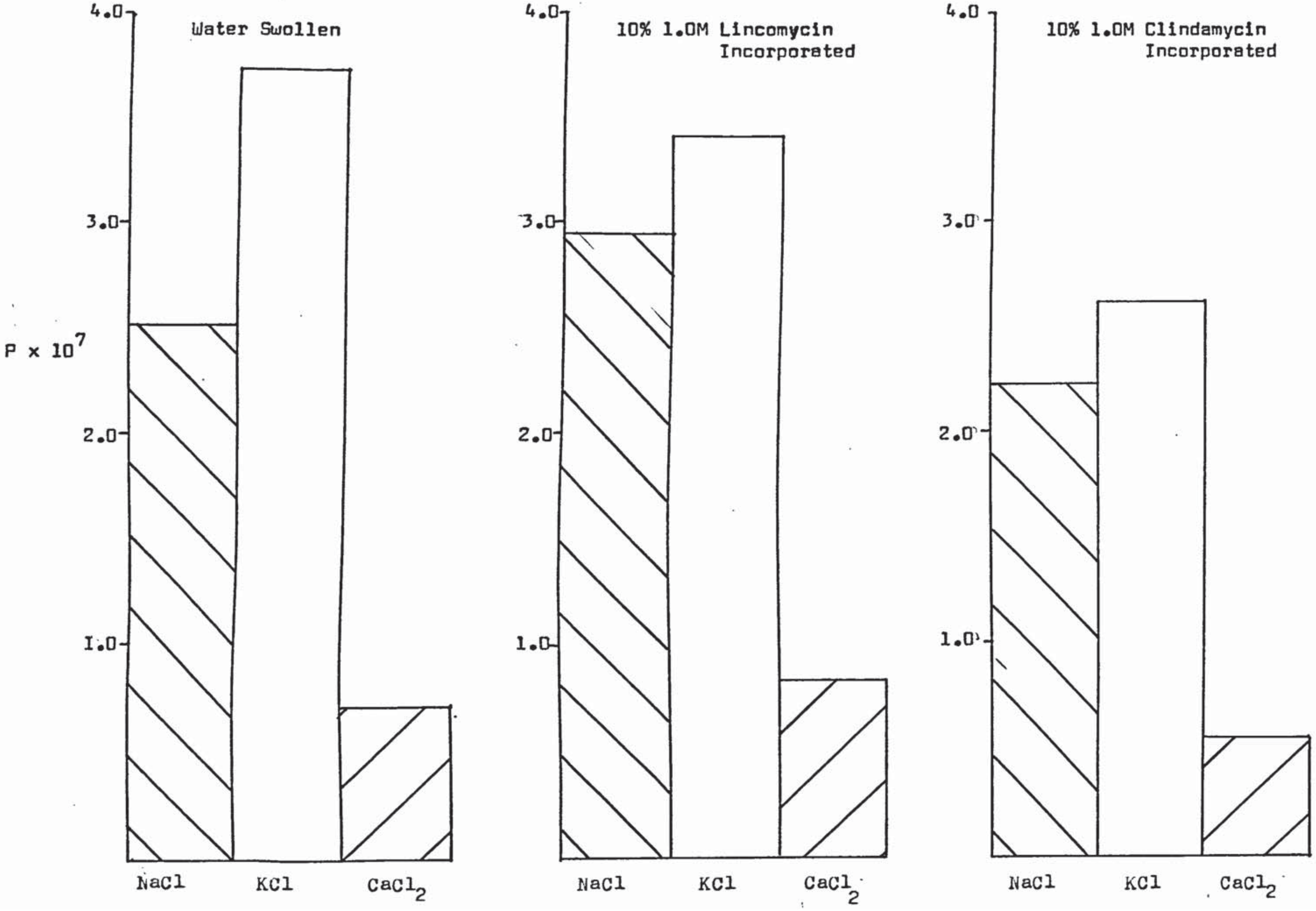
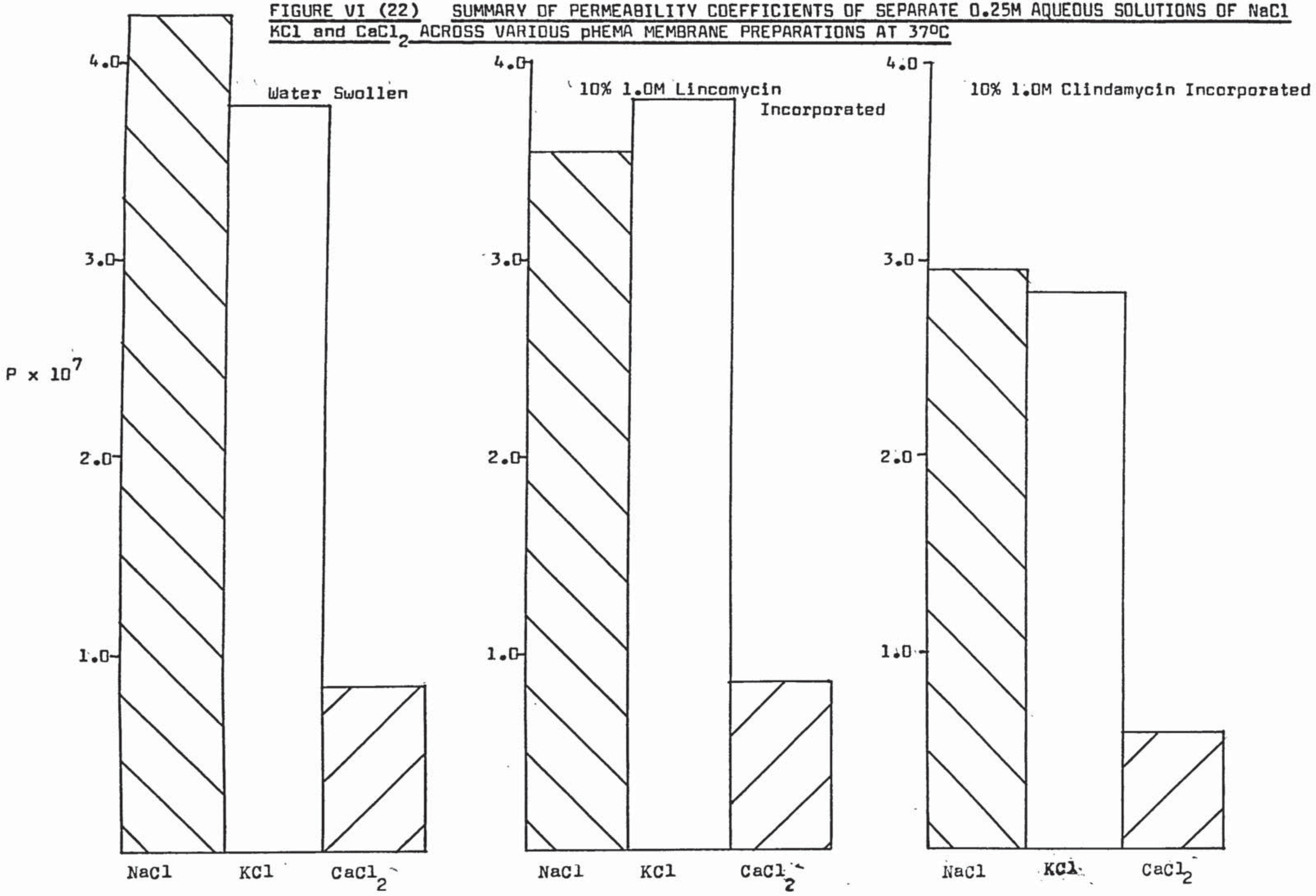


FIGURE VI (22) SUMMARY OF PERMEABILITY COEFFICIENTS OF SEPARATE 0.25M AQUEOUS SOLUTIONS OF NaCl KCl and $CaCl_2$ ACROSS VARIOUS pHEMA MEMBRANE PREPARATIONS AT 37°C



Refojo and Yasuda[78] have the same E.W.C. as water swollen pHEMA.

Figures VI (21) and VI (22) show that with two exceptions the order of rates of transport was $KCl > NaCl > CaCl_2$. This was modified to $NaCl > KCl > CaCl_2$ in combined solute solution experiments on water swollen pHEMA (discussed earlier) and Clindamycin incorporated pHEMA (where NaCl and KCl transport rates were very similar anyway).

It was observed that the higher E.W.C. antibiotic incorporated membranes had lower overall transport rates than the lower E.W.C. water swollen membrane. It was also found that the higher freezing water content Clindamycin incorporated membranes had lower overall transport rates than the lower freezing water content Lincomycin incorporated membranes. These results were contrary to the well-established affects of E.W.C. on permeability.

It was therefore apparent that the antibiotics were capable of influencing solute transport and this was envisaged as being the result of their water structuring properties (Chapter IV). The differences in the water structuring properties of the antibiotics was considered responsible for the lower overall transport rates across Clindamycin incorporated membranes than were found for Lincomycin incorporated membranes.

These observations were extremely important since there was now direct evidence of the differing effects of the two antibiotics on processes relevant to biological systems. The effects of these compounds on transport processes in biological systems could result in the differing extents of irritancy of the antibiotics depending on their in vivo behaviour.

VI.3.ii Solute Permeation Across Antibiotic Incorporated HEMA/Styrene Membranes

The permeation of combined aqueous solutions 0.25M with respect to NaCl,

FIGURE VI (23)

PERMEATION OF A COMBINED AQUEOUS SOLUTION 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ ACROSS A 10% 1.0M LINCOMYCIN INCORPORATED HEMA/STYRENE MEMBRANE AT 37°C

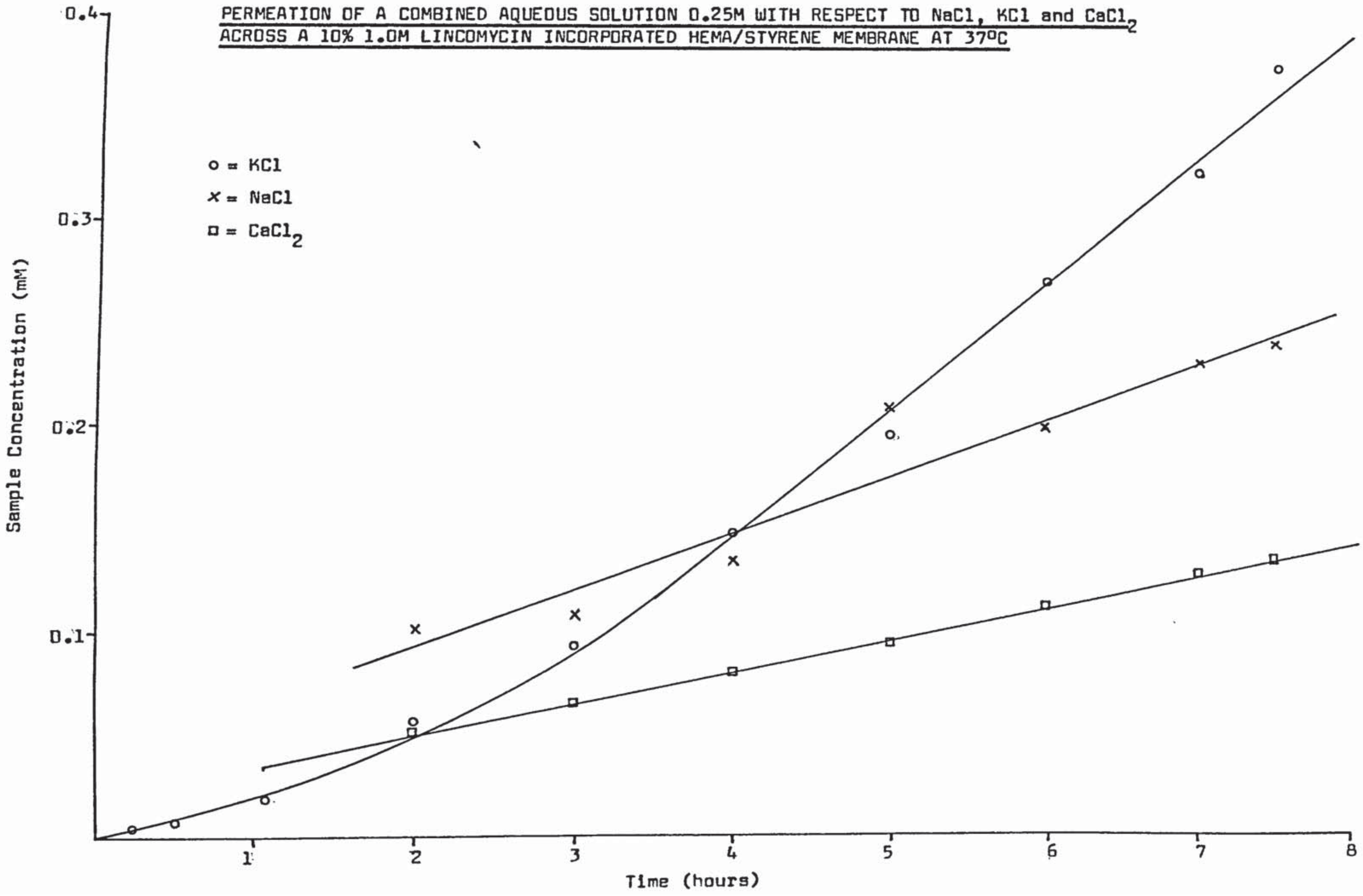


FIGURE VI (24)

PERMEATION OF A COMBINED AQUEOUS SOLUTION 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ ACROSS A 10% 1.0M CLINDAMYCIN INCORPORATED HEMA/STYRENE MEMBRANE AT 37°C

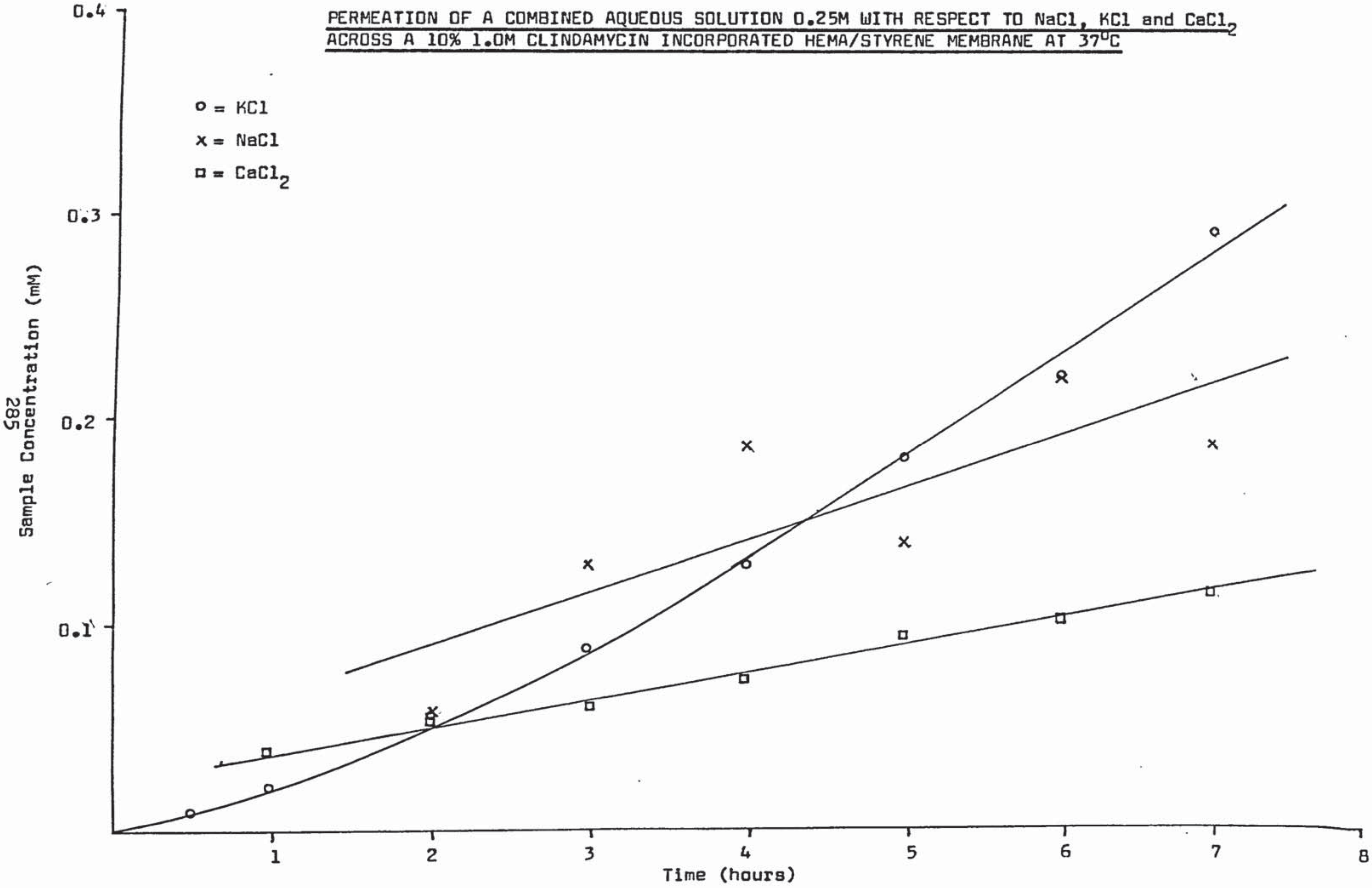
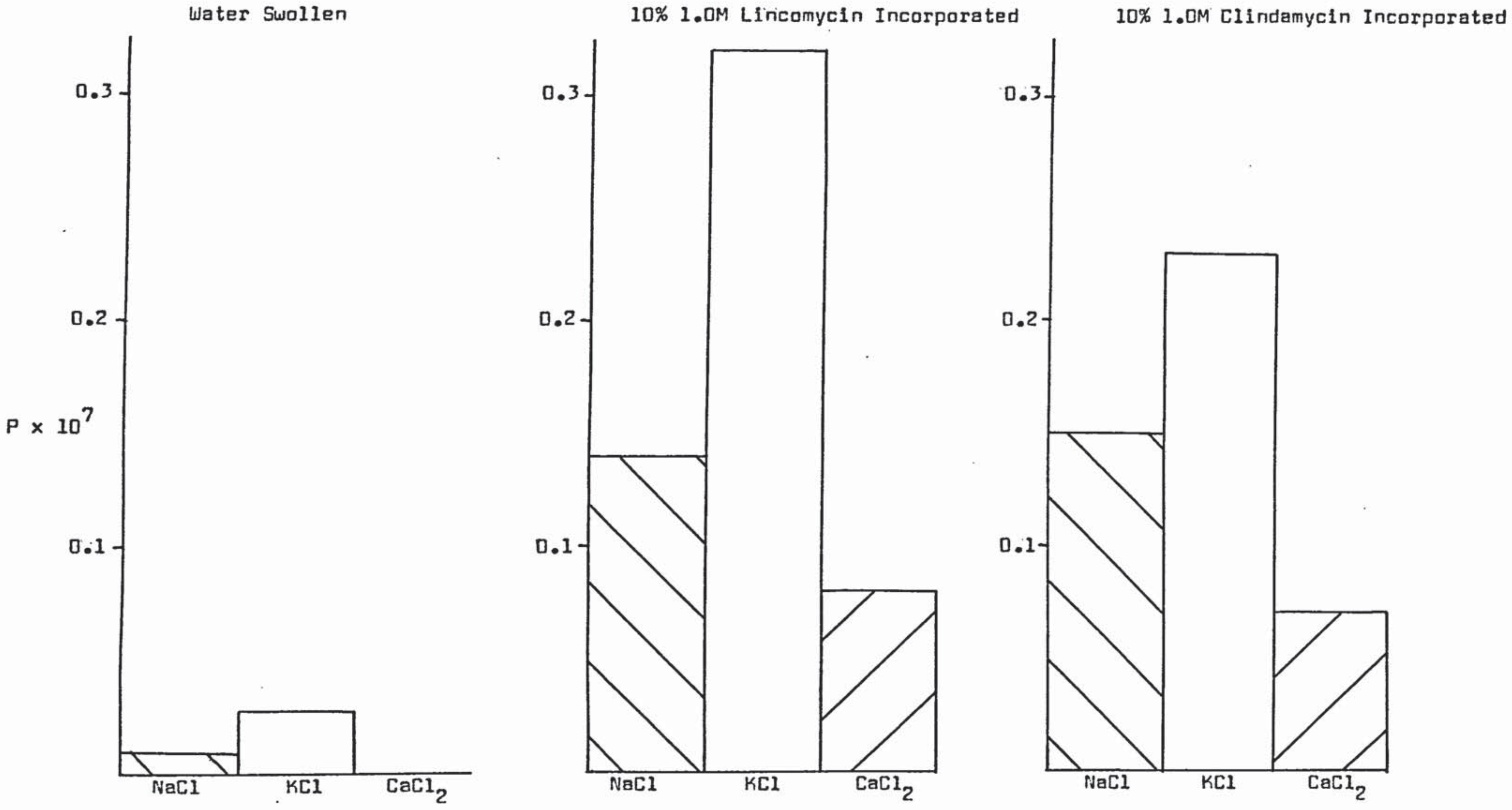


FIGURE VI (25) SUMMARY OF PERMEABILITY COEFFICIENTS OF COMBINED AQUEOUS SOLUTIONS 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ ACROSS VARIOUS HEMA/STYRENE MEMBRANE PREPARATIONS AT 37°C



KCl and $CaCl_2$ across HEMA/Styrene membranes incorporating either 10% 1.0M Lincomycin or 10% 1.0M Clindamycin at 37°C can be seen in Figures VI (23) and VI (24) respectively. The data are to be found in Table VI.C (see later) and are summarised in Figure VI (25).

HEMA/STY incorporating Lincomycin had an E.W.C. of 28.8% of which 1.2% was freezing water whereas HEMA/STY incorporating Clindamycin had an E.W.C. of 34.6% of which 5.4% was freezing water. Water swollen HEMA/STY had an E.W.C. of 23.1% of which only 1.0% was freezing water.

It was immediately apparent that transport rates across both antibiotic incorporated membranes were much higher than for the water swollen membrane. This observation was highlighted by the fact that whereas in the 1.0% freezing water content water swollen membrane no $CaCl_2$ permeation was detected, significant levels of $CaCl_2$ were found to have permeated the 1.2% freezing water content Lincomycin incorporated membrane and 5.4% freezing water content Clindamycin incorporated membrane. It was apparent that $CaCl_2$ transport occurred in the freezing water fraction of the membranes, the small increase in freezing water content of the antibiotic incorporated membranes being sufficient to allow $CaCl_2$ transport.

The rates of NaCl and $CaCl_2$ transport across both antibiotic incorporated membranes were similar but KCl transport was higher for the Lincomycin incorporated membrane than the Clindamycin incorporated membrane. This was again contrary to expectations based on E.W.C. and freezing water content data. The differing water structuring capabilities of the antibiotics could account for such behaviour if the effective pore size and water structures in the two preparations were such so that $CaCl_2$ and NaCl had to exchange their hydration shells with matrix water in order to permeate both membrane types. Such exchange might not be necessary for KCl transport across Lincomycin incorporated HEMA/Styrene membranes but could be at least partially involved in KCl transport across Clindamycin incorporated HEMA/Styrene membranes. The real situation would not be as clear-cut as described above but these types of interactions based on hydration cosphere size could explain the K^+ selectivity observed.

VI.3.iii Solute Transport Across Antibiotic Incorporated MAA/ACM/STY Membranes

The permeation of combined aqueous solutions 0.25M with respect to NaCl, KCl and $CaCl_2$ across either a 10% 1.0M Lincomycin incorporated MAA/ACM/STY membrane or a 10% 1.0M Clindamycin incorporated MAA/ACM/STY membrane can be seen in Figures VI (26) and VI (27) respectively. The data shown in Table VI.C (see later) is summarised in Figure VI (28).

Water swollen MAA/ACM/STY had an E.W.C. of 27.5% of which 0.1% was freezing water. MAA/ACM/STY preparations incorporating Lincomycin or Clindamycin had E.W.C's of 19.4% and 21.1% respectively, in neither case was any freezing water detected. Although the previously noted trend of order of transport rates of KCl > NaCl was observed NaCl and $CaCl_2$ transport rates were similar and it was apparent that solute transport through non-freezing water in the antibiotic incorporated membranes was occurring. This would presumably depend on an exchange of hydration as described earlier and even 0.1% freezing water in the water swollen preparation led to an increase in rate of transport of all three solutes.

It was apparent from the studies on the antibiotic incorporated membranes that NaCl and $CaCl_2$ were restricted to about the same level of transport but the KCl transport was still higher than both NaCl and $CaCl_2$. This again suggested that the antibiotics were capable of exerting a specific influence over KCl transport. The time lags seen for the detection of

FIGURE VI (26)

PERMEATION OF A COMBINED AQUEOUS SOLUTION 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ ACROSS A 10% 1.0M LINCOMYCIN INCORPORATED MAA/ACM/STY MEMBRANE AT 37°C

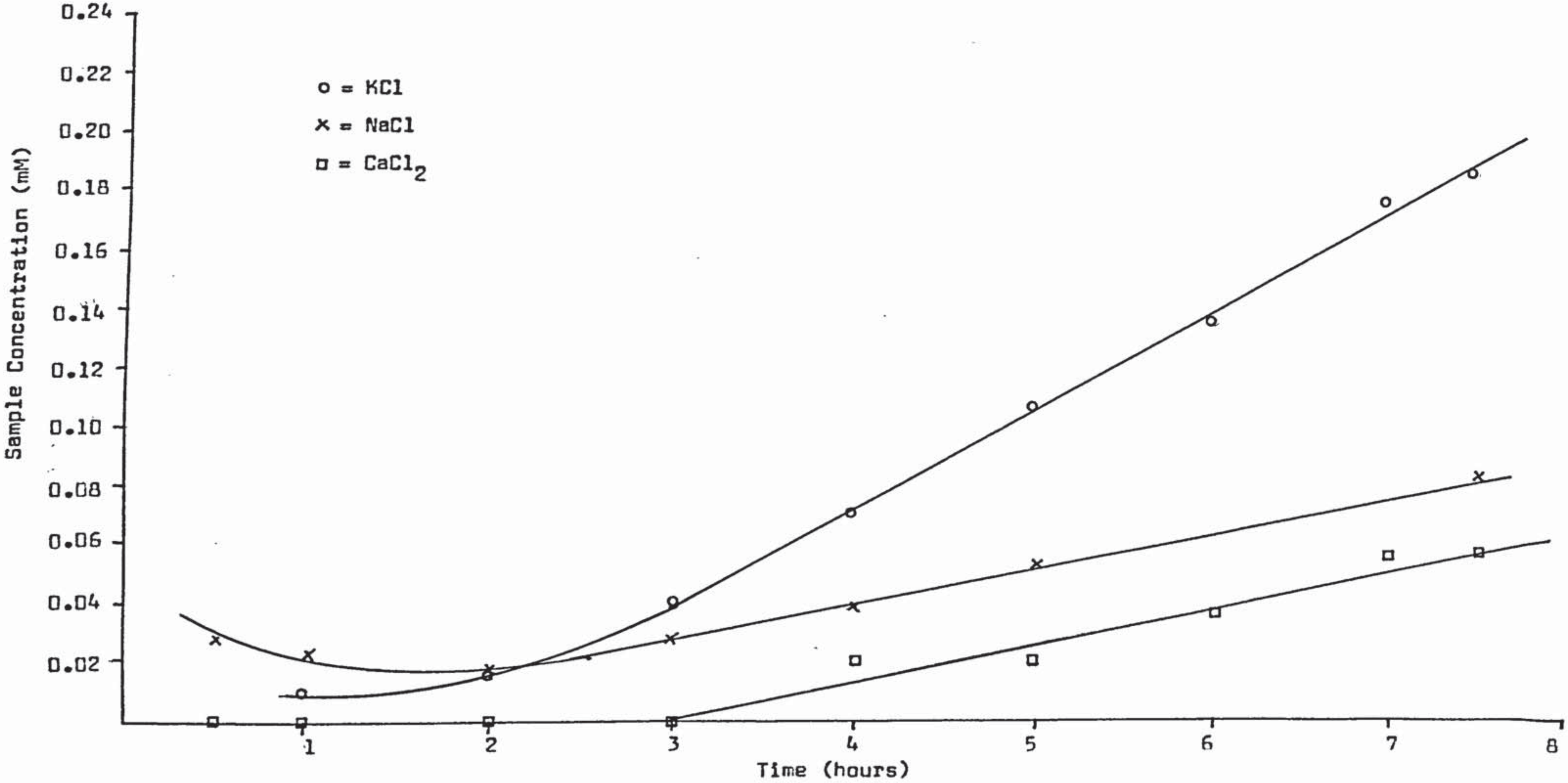


FIGURE VI (27)

PERMEATION OF A COMBINED AQUEOUS SOLUTION 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ ACROSS A 10% 1.0M CLINDAMYCIN INCORPORATED MAA/ACM/STY MEMBRANE AT 37°C

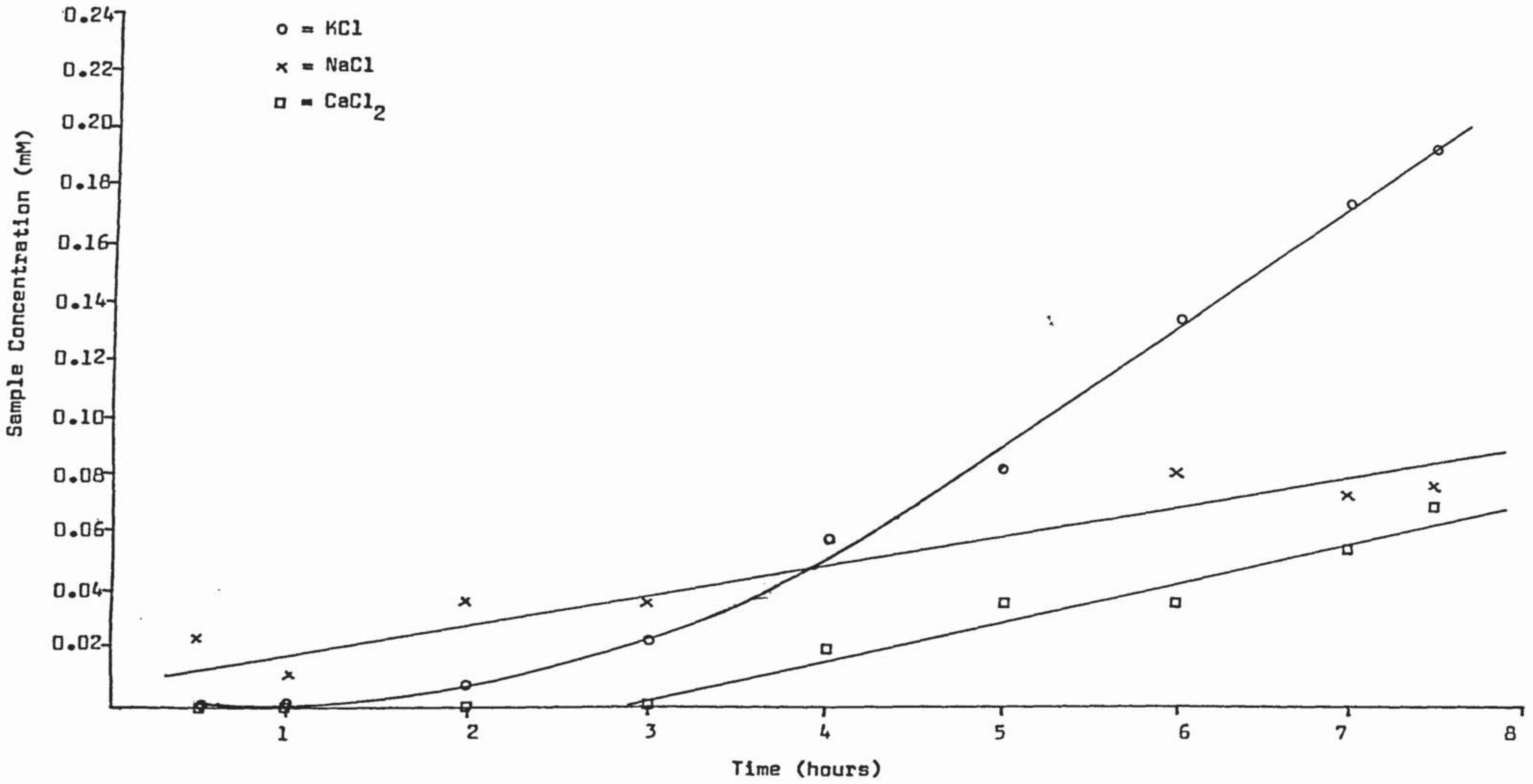
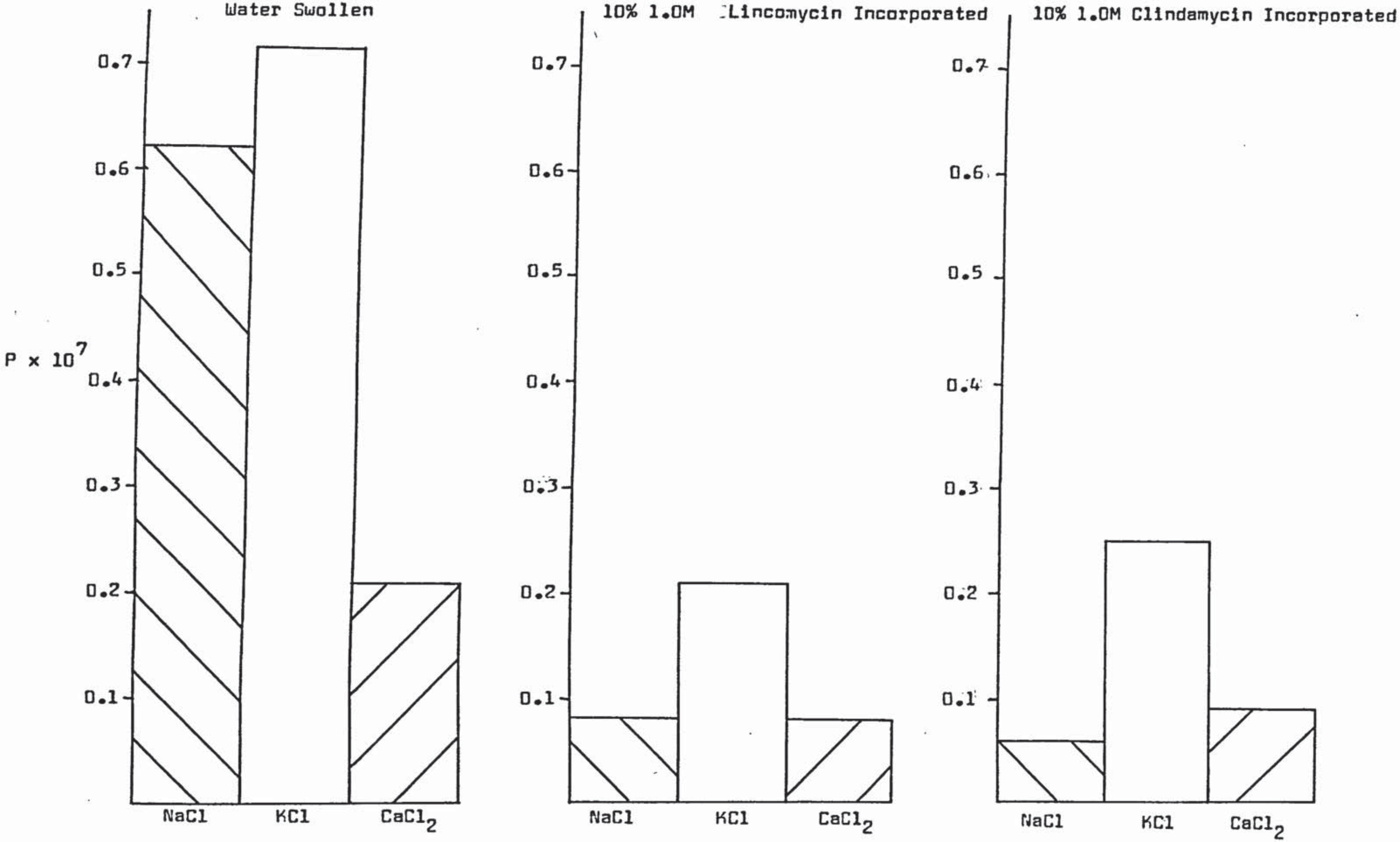


FIGURE VI (28) SUMMARY OF PERMEABILITY COEFFICIENTS OF COMBINED AQUEOUS SOLUTIONS 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ ACROSS VARIOUS MAA/ACM/STY MEMBRANE PREPARATIONS AT 37°C



$CaCl_2$ transport in Figures VI (26) and VI (27) also demonstrate the reduced $CaCl_2$ permeation due to its hydrated size.

VI.3.iv Solute Transport Across Antibiotic Incorporated MAA/ACM Membranes

The permeation of combined aqueous solutions 0.25M with respect to NaCl, KCl and $CaCl_2$ across MAA/ACM membranes incorporating either 10% 1.0M Lincomycin or 10% 1.0M Clindamycin at 37°C can be seen in Figures VI (29) and VI (30) respectively. The data obtained are shown in Table VI.C and summarised in Figure VI (31).

Water swollen MAA/ACM had an E.W.C. of 37.6% of which about 12% would be freezing water (see Chapter III). MAA/ACM incorporating 10% 1.0M Lincomycin had an E.W.C. of 37.7% of which 2.3% was freezing water and MAA/ACM incorporating 10% 1.0M Clindamycin had an E.W.C. of 39.2% of which 7.3% was freezing water.

Higher overall rates of solute transport were observed for both antibiotic incorporated membranes than was found for water swollen MAA/ACM. This was especially significant since all three membrane preparations had about the same values of E.W.C. However, their constituent amounts of freezing water did vary, and the highest overall rates of transport were found for the mainly non-freezing water composition antibiotic incorporated membranes. Once again, of these two membranes highest transport rates were found for the Lincomycin incorporated membrane which also had the lowest freezing water content. It has already been mentioned that such behaviour was contrary to that previously reported[158, 119, 120, 121] as well as being contrary to the behaviour seen for water swollen membranes earlier in this work. In each case the general trend of order of rate of transport being KCl > NaCl > $CaCl_2$ was found so this suggested that the

FIGURE VI (29)

PERMEATION OF A COMBINED AQUEOUS SOLUTION 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ ACROSS A 10% 1.0M LINCOMYCIN INCORPORATED MAA/ACM MEMBRANE AT 37°C

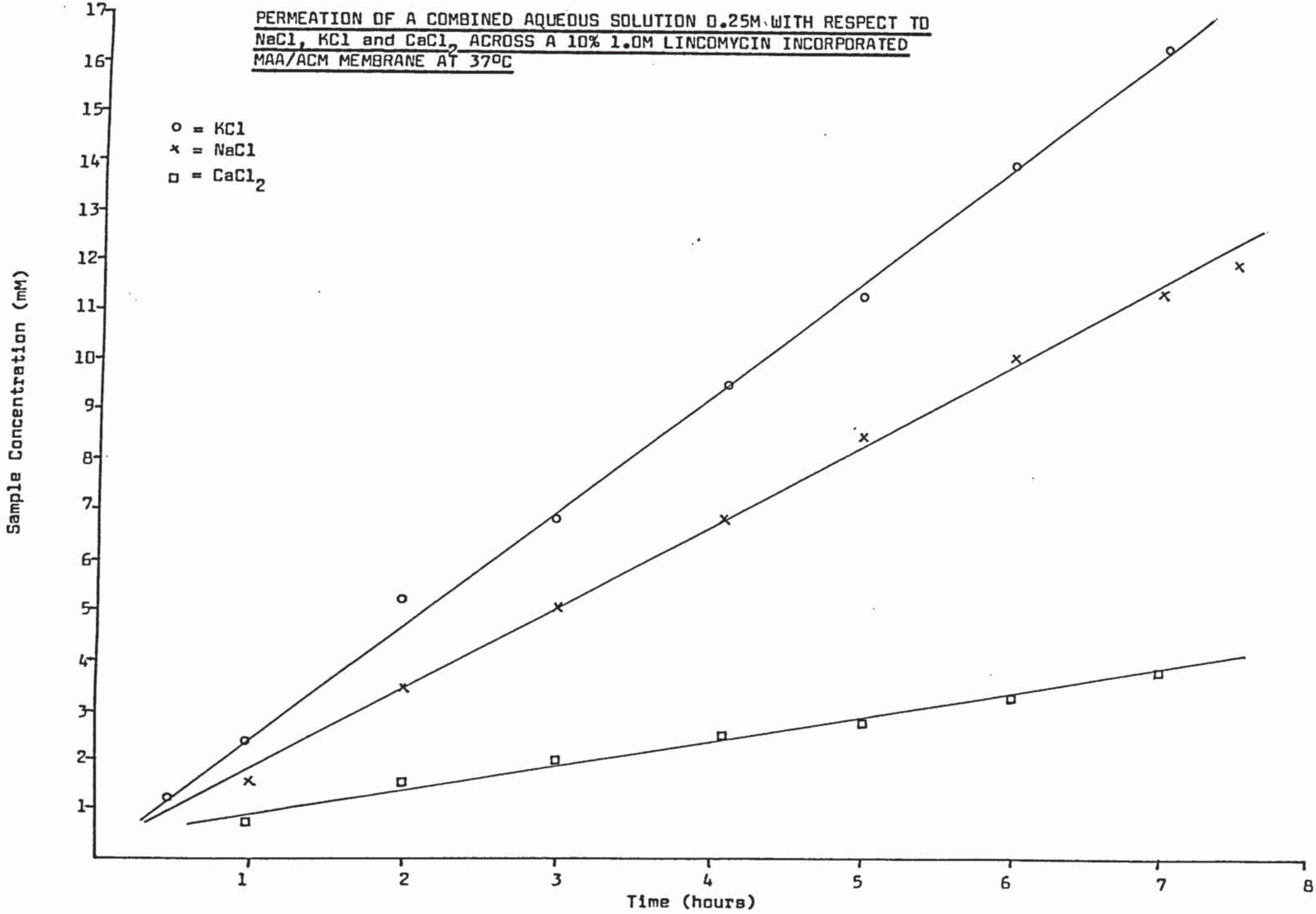


FIGURE VI (30)

PERMEATION OF A COMBINED AQUEOUS SOLUTION 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ ACROSS A 10% 1.0M CLINDAMYCIN INCORPORATED MAA/ACM MEMBRANE AT 37°C

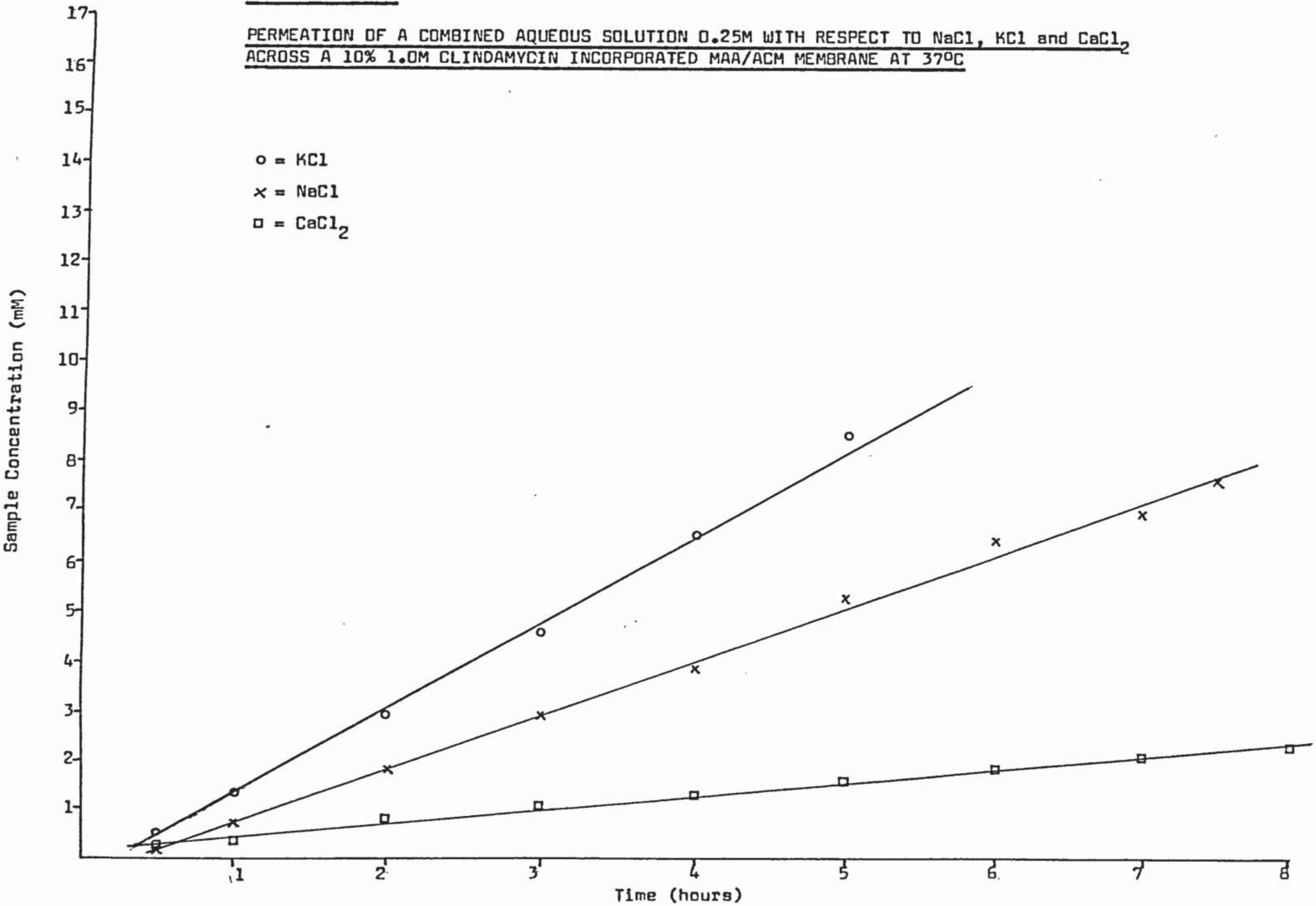
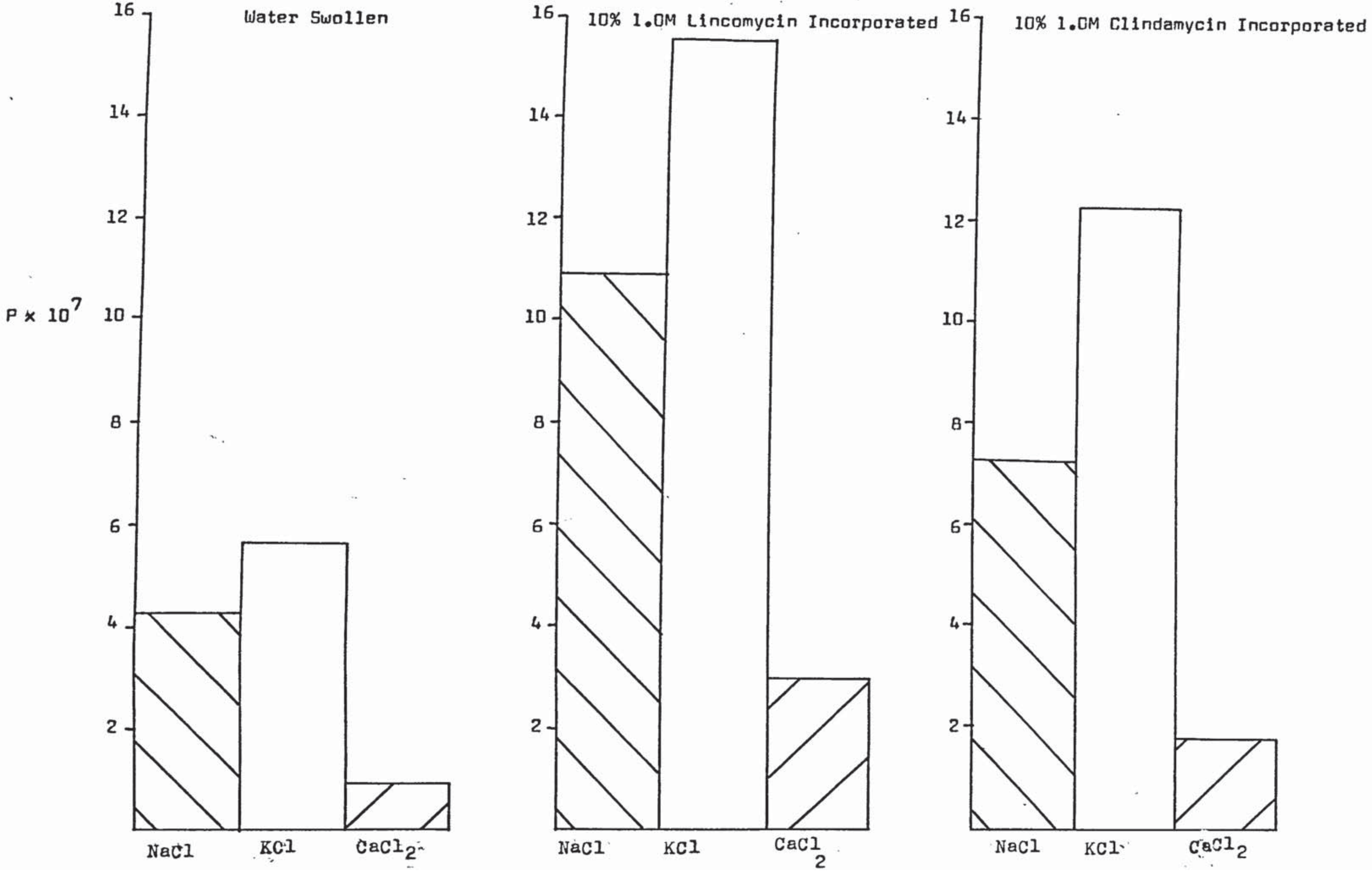


TABLE VI.C

SUMMARY OF PERMEABILITY DATA FOR ANTIBIOTIC INCORPORATED HYDROGEL MEMBRANES AT 37°C

Composition (E.W.C. % at 23°C)	Solute(s) Studied	Permeability Coefficient (cm^2 sec^{-1})
pHEMA inc L/M (39.4)	0.25M w.r.t (NaCl (KCl ($CaCl_2$	2.9 × 10^{-7} 3.4 × 10^{-7} 0.8 × 10^{-7}
pHEMA inc L/M (39.4)	0.25M NaCl 0.25M KCl 0.25M $CaCl_2$	3.6 × 10^{-7} 3.8 × 10^{-7} 0.9 × 10^{-7}
pHEMA inc C/M (40.8)	0.25M w.r.t (NaCl (KCl ($CaCl_2$	2.2 × 10^{-7} 2.6 × 10^{-7} 0.6 × 10^{-7}
pHEMA inc C/M (40.8)	0.25M NaCl 0.25M KCl 0.25M $CaCl_2$	2.9 × 10^{-7} 2.8 × 10^{-7} 0.6 × 10^{-7}
HEMA/STY inc L/M (28.8)	0.25M w.r.t (NaCl (KCl ($CaCl_2$	0.14 × 10^{-7} 0.32 × 10^{-7} 0.08 × 10^{-7}
HEMA/STY inc C/M (34.6)	0.25M w.r.t (NaCl (KCl ($CaCl_2$	0.15 × 10^{-7} 0.23 × 10^{-7} 0.07 × 10^{-7}
MAA/ACM/STY inc L/M (19.4)	0.25M w.r.t (NaCl (KCl ($CaCl_2$	0.08 × 10^{-7} 0.21 × 10^{-7} 0.08 × 10^{-7}
MAA/ACM/STY inc C/M (21.1)	0.25M w.r.t (NaCl (KCl ($CaCl_2$	0.06 × 10^{-7} 0.25 × 10^{-7} 0.09 × 10^{-7}
MAA/ACM inc L/M (37.7)	0.25M w.r.t (NaCl (KCl ($CaCl_2$	10.9 × 10^{-7} 15.5 × 10^{-7} 3.0 × 10^{-7}
MAA/ACM inc C/M (39.2)	0.25M w.r.t (NaCl (KCl ($CaCl_2$	7.3 × 10^{-7} 12.2 × 10^{-7} 1.8 × 10^{-7}

FIGURE VI (31) SUMMARY OF PERMEABILITY COEFFICIENTS OF COMBINED AQUEOUS SOLUTIONS 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ ACROSS VARIOUS MAA/ACM MEMBRANE PREPARATIONS AT 37°C



same fundamental factors controlled permeation throughout all of these studies but that their effectiveness was modified to different extents by the presence of either Lincomycin or Clindamycin. The MAA/ACM systems stand out in particular because of the large overall increase of transport of both antibiotic incorporated preparations over the rates of transport found in water swollen MAA/ACM despite the simultaneous reduction in freezing water content. Such behaviour could be explained in terms of water channels organised by the antibiotics (or at least favourable transport routes) which would allow faster solute permeation. Some degree of selectivity based on hydrated solute size must remain since the characteristic order of transport rates of $KCl > NaCl > CaCl_2$ was maintained.

This type of behaviour in similar water environments in biological systems could result in a breakdown of certain cellular processes possibly leading to cell lysis. This could in theory provide a basis for an explanation of the different extents of irritancy of these two antibiotics based on their different water structuring capabilities.

VI.4 Solute Absorption Studies

As part of this research an investigation of the weight of NaCl, KCl and $CaCl_2$ absorbed per gram of hydrogel for a range of preparations was carried out. The membrane preparations were cut into discs and their surfaces wiped dry in the manner described for the determination of E.W.C. (Chapter II). A known weight of hydrated discs was added to 10 ml of a combined aqueous solution which was 5 mM with respect to NaCl, KCl and $CaCl_2$. The samples were then left to soak at 37^oC for about 4 weeks. At the end of this period the solution concentration of each solute was determined and the amount of each solute absorbed per gram of sample was calculated.

TABLE VI.D

SOLUTE ABSORPTION STUDY RESULTS (37°C)

Sample Composition	Amount of Solute Absorbed (mg/g)	
pACM (H_2O)	NaCl	0.19
	KCl	0.20
	$CaCl_2$	0.64
pHEMA (H_2O)	NaCl	0.20
	KCl	0.18
	$CaCl_2$	0.72
HEMA/STY (H_2O)	NaCl	0.01
	KCl	0.03
	$CaCl_2$	Not detected
MAA/ACM (H_2O)	NaCl	0.57
	KCl	0.55
	$CaCl_2$	1.21
MAA/ACM/STY (H_2O)	NaCl	0.65
	KCl	0.64
	$CaCl_2$	1.43
pHEMA inc L/M	NaCl	0.26
	KCl	0.31
	$CaCl_2$	0.41
HEMA/STY inc L/M	NaCl	0.21
	KCl	0.21
	$CaCl_2$	0.34
MAA/ACM inc L/M	NaCl	0.49
	KCl	0.51
	$CaCl_2$	1.63
MAA/ACM/STY inc L/M	NaCl	0.66
	KCl	0.67
	$CaCl_2$	2.09
pHEMA inc C/M	NaCl	0.18
	KCl	0.25
	$CaCl_2$	0.24
HEMA/STY inc C/M	NaCl	0.28
	KCl	0.37
	$CaCl_2$	0.13
MAA/ACM inc C/M	NaCl	0.23
	KCl	0.30
	$CaCl_2$	0.92
MAA/ACM/STY inc C/M	NaCl	0.71
	KCl	0.71
	$CaCl_2$	2.50

The results obtained from these studies can be seen in Table VI.D. The results obtained for water swollen preparations are also shown in Figure VI (32). From Figure VI (32) it can be seen that for each water swollen preparation equal amounts of Na^+ and K^+ were absorbed. However, in each case there was a markedly higher level of Ca^{2+} absorption and there was no apparent trend according to water content. Such findings were comparable to those of Yasuda[119] who reported that the distribution of NaCl in hydrogel/NaCl systems responded with great sensitivity to change in the type of polymer involved. From the results obtained here it was also noted that the presence of MAA in a hydrogel led to increased absorption of all three solutes in an unspecific manner presumably due to the partly ionic nature of the hydrogel.

These results can also be compared with studies reported by Wiggins[6,181] in which silica gel and rat kidney cortex slices were found to selectively absorb K^+ over Na^+. Ca^{2+} was effectively excluded. The model developed to explain these findings was based on the free energies of hydration of the ions as shown in Table VI.E.

TABLE VI.E

FREE ENERGIES OF HYDRATION (ΔG_{HYD}) OF IONS[7]

	Ca^{2+}	Na^+	Cl^-	K^+
$-G_{HYD}$ (k J mol $^{-1}$)	1593	388	317-340	314

Wiggins suggested that highly hydrated ions such as Ca^{2+} had to use more energy to acquire primary hydration from networks of water molecules of higher hydrogen bond energy. As its activity in such surroundings would increase its tendency would be to diffuse spontaneously into the bulk

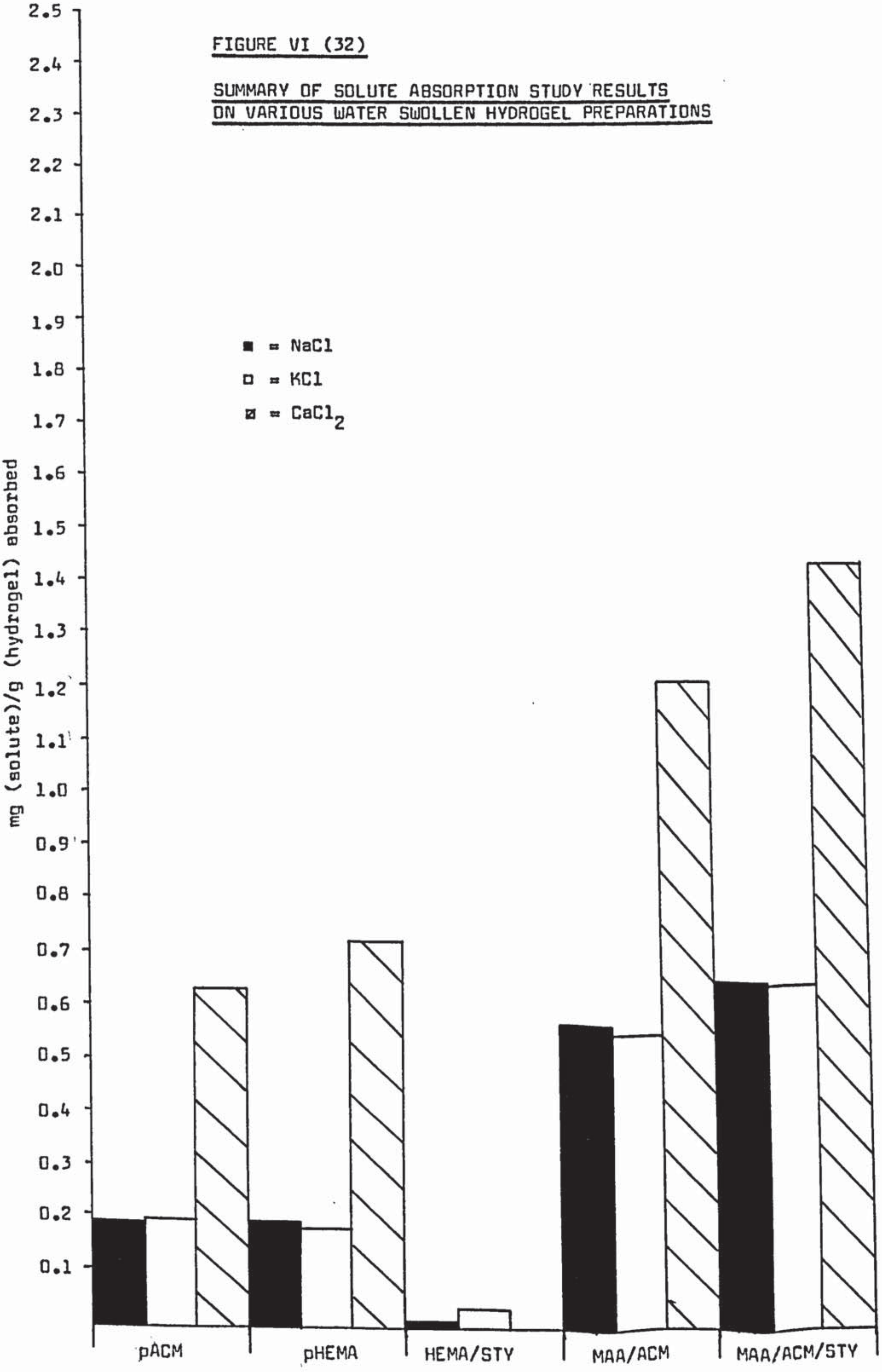
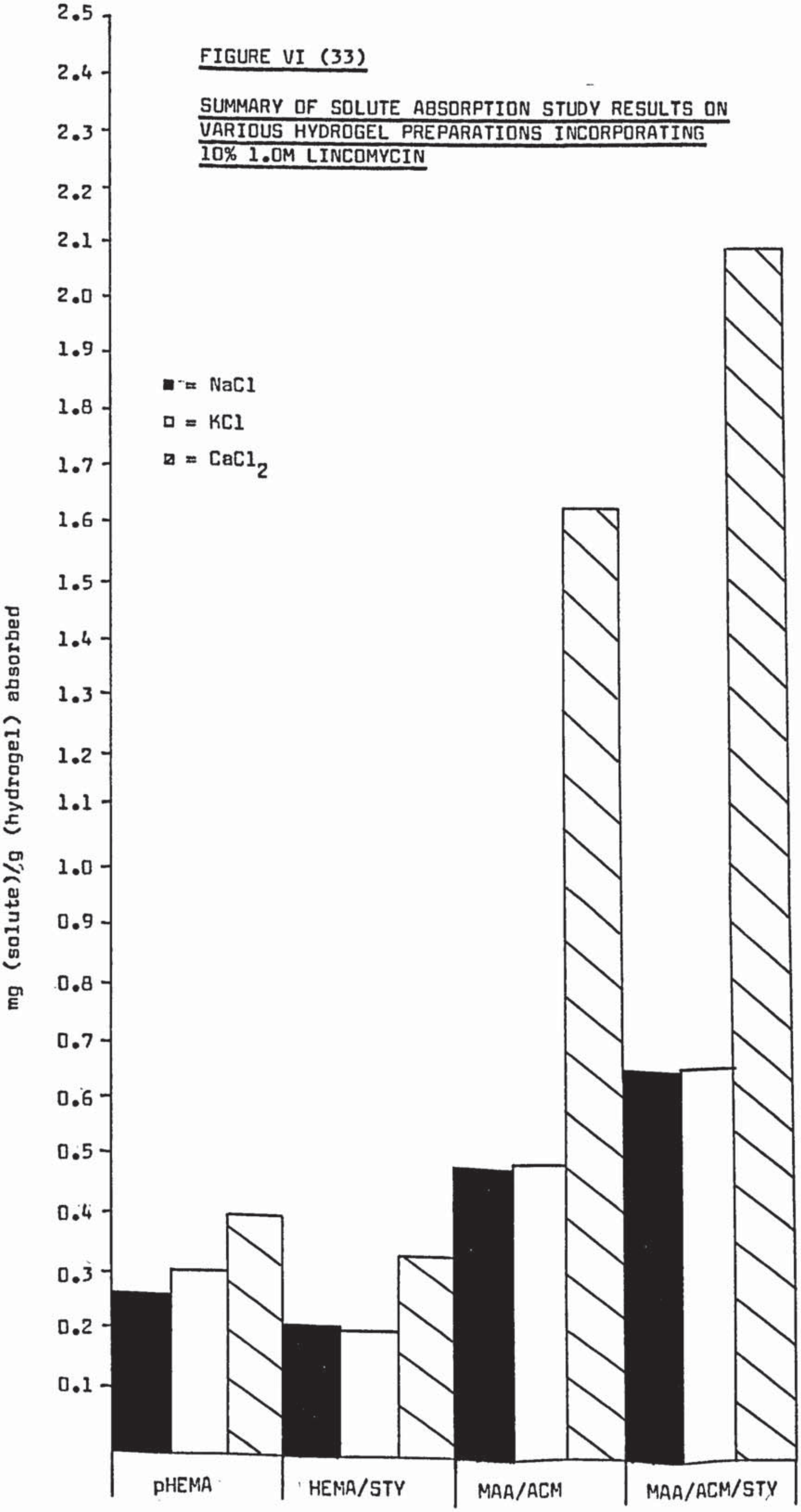


FIGURE VI (32)

SUMMARY OF SOLUTE ABSORPTION STUDY RESULTS ON VARIOUS WATER SWOLLEN HYDROGEL PREPARATIONS

FIGURE VI (33)

SUMMARY OF SOLUTE ABSORPTION STUDY RESULTS ON VARIOUS HYDROGEL PREPARATIONS INCORPORATING 10% 1.0M LINCOMYCIN



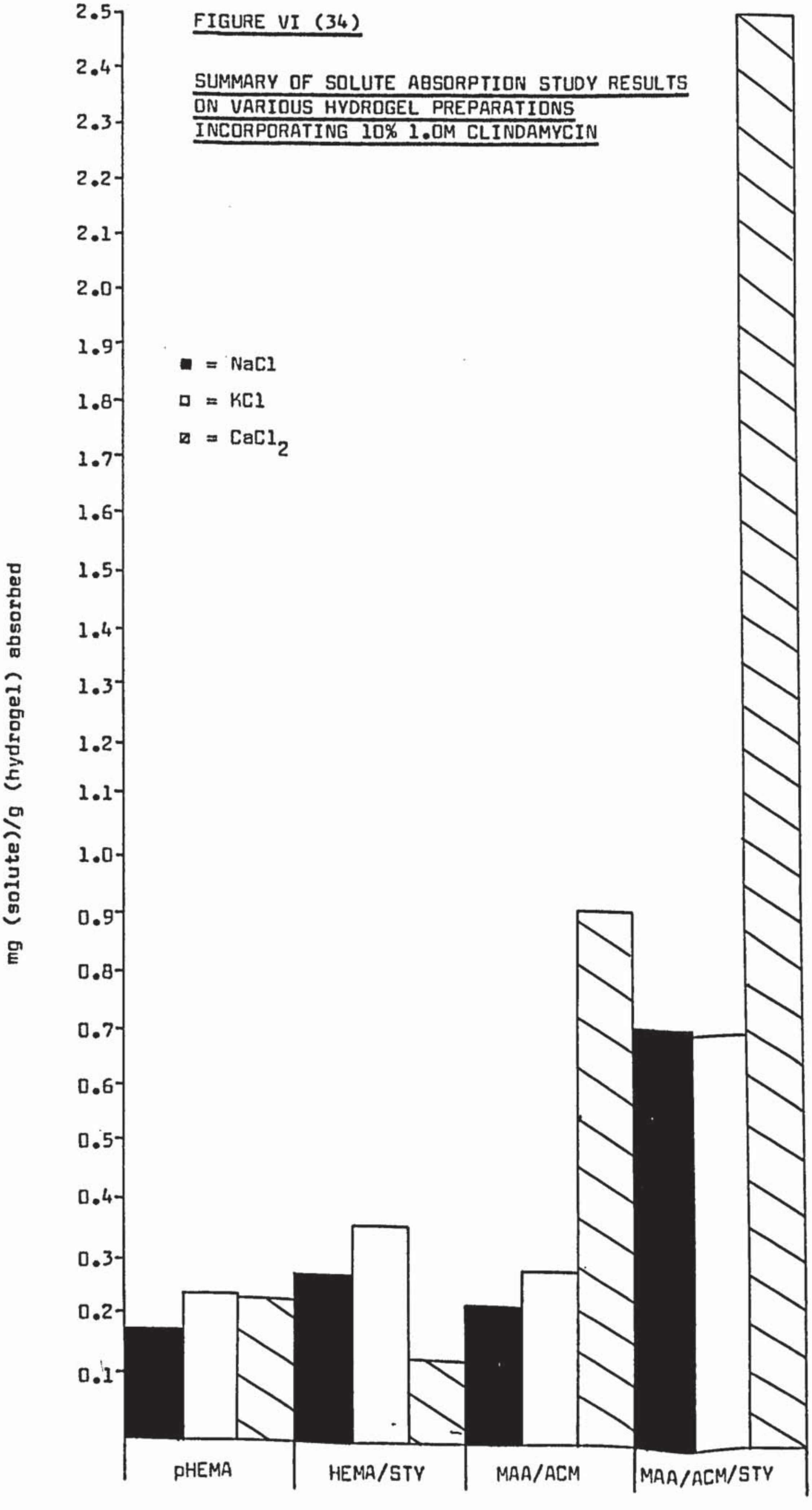


FIGURE VI (34)

SUMMARY OF SOLUTE ABSORPTION STUDY RESULTS ON VARIOUS HYDROGEL PREPARATIONS INCORPORATING 10% 1.0M CLINDAMYCIN aqueous phase. However, such theory did not correlate with the preliminary findings reported here.

The solute absorption study results for Lincomycin and Clindamycin incorporated membranes are summarised in Figures VI (33) and VI (34) respectively. These figures once again showed that overall solute absorption was highest for hydrogels containing MAA. Such hydrogels also appeared to have more highly elevated Ca^{2+} levels than Na^+ and K^+ levels. The Lincomycin incorporated membranes in general showed no significant difference in the amounts of Na^+ and K^+ absorbed whereas a small but consistent increased absorption of K^+ over Na^+ was found in all but the lowest freezing water content (MAA/ACM/STY) Clindamycin membranes.

Direct comparison of the data for the antibiotic incorporated membranes showed that solute absorption by low E.W.C. membranes was greatest for the Clindamycin incorporated membranes but that solute absorption by high E.W.C. hydrogels was greatest for Lincomycin incorporated membranes.

It has already been mentioned that the affinity of the antibiotics themselves for the hydrogels could not be carried out without the development of a more sensitive assay although this area was well worth further study. However, on the basis of the preliminary studies reported here the incorporation of Clindamycin into a hydrogel seemed to endow it with a measure of K^+/Na^+ selectivity not seen for water swollen or Lincomycin incorporated membranes.

The results in general suggested that the higher rates of solute transport across Lincomycin incorporated hydrogel membranes than were found across Clindamycin incorporated hydrogel membranes, could not be accounted for on a basis of differing solute solubility in the various hydrogels. Such behaviour seemed to be the result of the influence of the antibiotics on the diffusive contribution to the permeability coefficients.

VI.5 Extended Solute Permeation Studies

A few permeability studies were carried out on solutes other than NaCl, KCl and $CaCl_2$ across antibiotic incorporated membranes and the assay of all solutes described in this section other than NaCl, KCl and $CaCl_2$ was carried out by P A Caeser using a Technicon Autoanalyser (Chapter II).

VI.5.1 Phenylalanine Permeation Across a Water Swollen HEMA/STY Membrane

The experiment described here was to investigate the possibility of ionic transport being restricted whilst amino acid transport was maintained on a hydrated solute size basis.

Figure VI (35) shows the results of the permeability study at 37°C of a water swollen HEMA/Styrene membrane to a combined aqueous solution 0.25M with respect to NaCl, KCl and $CaCl_2$ + 15 mg/l (nitrogen) phenylalanine. However, no phenylalanine transport was detected but $CaCl_2$ transport was detected whereas experiments on solutions not containing phenylalanine showed no $CaCl_2$ transport across water swollen HEMA/STY membranes. These results are summarised in Table VI.F.

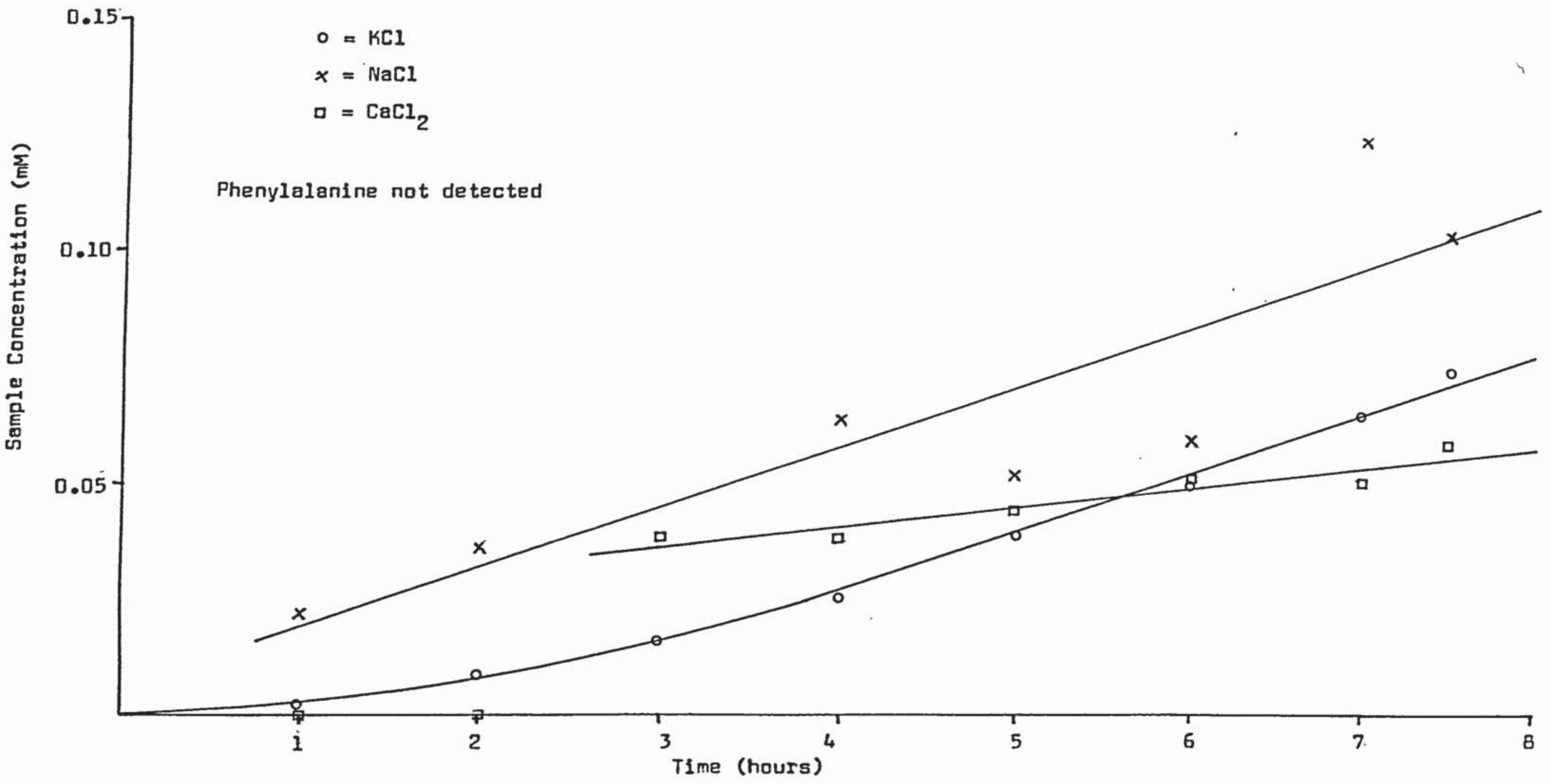
TABLE VI.F

PERMEABILITY DATA FOR HEMA/STYRENE (H_2O) AT 37°C

Solute System	Permeability Coefficients ($cm^2\ sec^{-1}$)		
	NaCl	KCl	$CaCl_2$
0.25M w.r.t NaCl, KCl, $CaCl_2$	0.009×10^{-7}	0.028×10^{-7}	Not detected
0.25M w.r.t NaCl, KCl, $CaCl_2$ + 15 mg/l (nitrogen) phenylalanine	0.067×10^{-7}	0.066×10^{-7}	0.023×10^{-7}

FIGURE VI (35)

PERMEATION OF A COMBINED AQUEOUS SOLUTION 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ + 15 mg/l (NITROGEN) PHENYLALANINE ACROSS A WATER SWOLLEN HEMA/STYRENE MEMBRANE AT 37°C



It was apparent that phenylalanine had interfered with the process of selectivity of solute transport on the basis of hydrated solute size.

VI.5.ii Phenylalanine Permeation Across Water Swollen MAA/ACM Membranes

The above experiment was repeated on a higher E.W.C. MAA/ACM water swollen membrane. The permeability of this membrane to a combined aqueous solution 0.25M with respect to NaCl, KCl and $CaCl_2$ + 15 mg/l (nitrogen) phenylalanine at 37°C can be seen in Figure VI (36).

Once again, no phenylalanine transport was detected but the transport characteristics were different to those found for the permeation of the same solution in the absence of phenylalanine. NaCl transport tailed off whilst KCl transport rose. It appeared that phenylalanine was once more disrupting the process of solute hydrated size selectivity.

VI.5.iii Glycine Permeation Across MAA/ACM Membranes

The lack of any detectable phenylalanine transport over the periods studied in the above experiments led to the extension of such studies to glycine transport.

The results of these studies using various concentrations of glycine in conjunction with water swollen and antibiotic incorporated MAA/ACM membranes at various temperatures are shown in Figure VI (37). The preliminary run of 15 mg/l (nitrogen) glycine at 23°C was successful, and so the experiment was repeated on other MAA/ACM membranes at 37°C using 60 mg/l (nitrogen) glycine solutions.

Figure VI (37) clearly shows that higher glycine transport was found using a Lincomycin incorporated MAA/ACM membrane than was found for either Clindamycin incorporated or water swollen MAA/ACM membranes. The permeability coefficients were ; water swollen MAA/ACM = 0.212×10^{-7} cm^2 sec^{-1},

FIGURE VI (36)

PERMEATION OF A COMBINED AQUEOUS SOLUTION 0.25M WITH RESPECT TO NaCl, KCl and $CaCl_2$ + 15 mg/l (NITROGEN) PHENYLALANINE ACROSS A WATER SWOLLEN MAA/ACM MEMBRANE AT 37°C

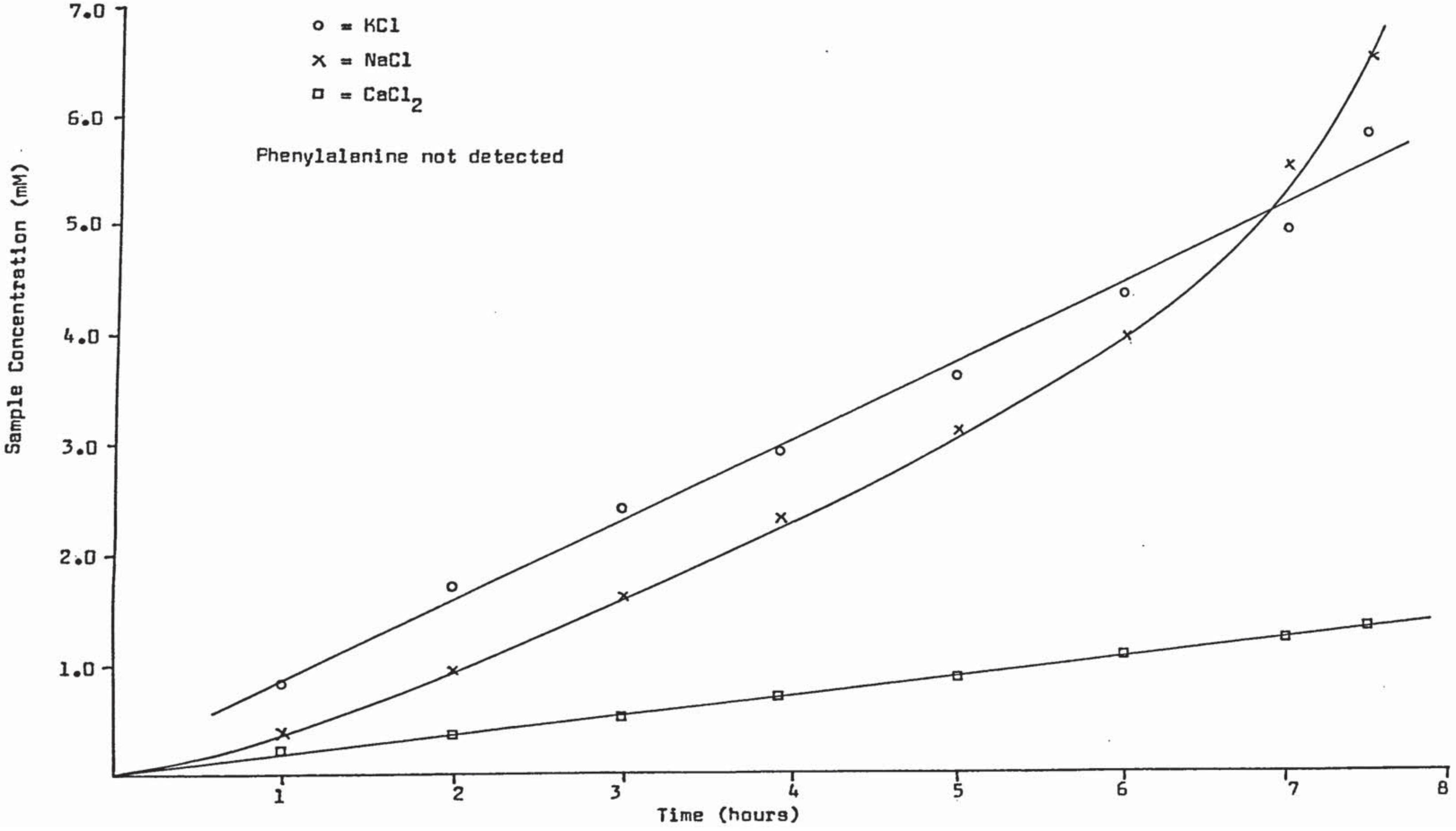
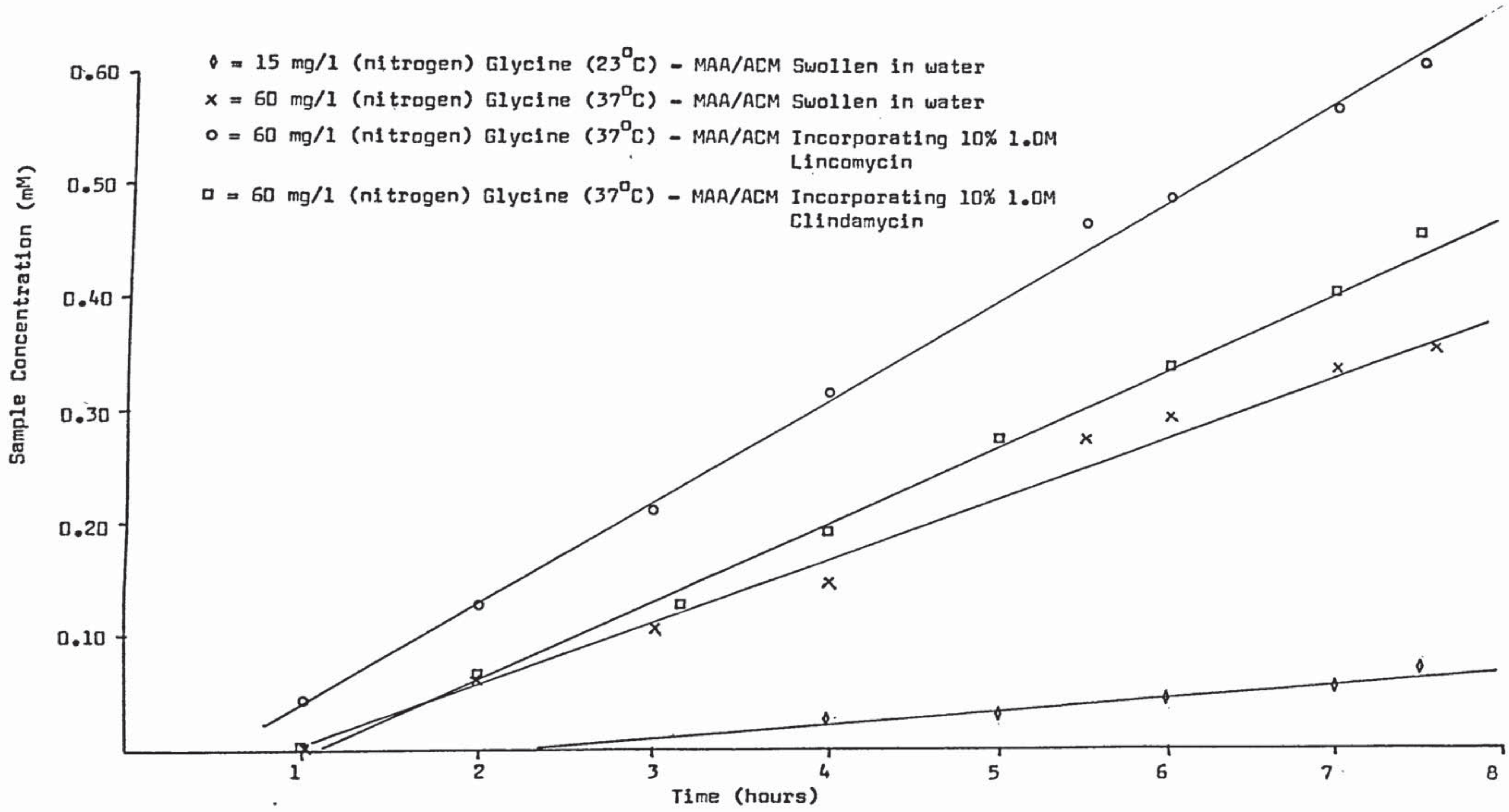


FIGURE VI (37)

PERMEATION OF VARIOUS CONCENTRATION AQUEOUS GLYCINE SOLUTIONS ACROSS VARIOUS MAA/ACM MEMBRANE PREPARATIONS



Lincomycin incorporated MAA/ACM = 0.303×10^{-7} cm^2 sec^{-1} and Clindamycin incorporated MAA/ACM 0.280×10^{-7} cm^2 sec^{-1}.

The observation that glycine transport was greater through Lincomycin incorporated MAA/ACM than Clindamycin incorporated MAA/ACM, despite the lower freezing water content of the preparation, was in agreement with the observations on NaCl, KCl and $CaCl_2$ transport reported earlier.

VI.5.iv Phosphocreatine Permeation Studies Across MAA/ACM Membranes

The promising results of the above experiments led to permeation studies on substances that in some way might be implicated in the irritation process and release of creatine kinase described in Chapter I. One such substance was phosphocreatine but very low levels of this substance were found to permeate MAA/ACM water swollen membranes at 23°C over the time of study (8 hours). It was obvious that a slightly different membrane composition would have to be developed before further permeability studies in this direction could be carried out.

The results presented in this chapter were subject to even more complex competitive interactions than those presented in Chapter V. The balance of interaction could be expected to be sensitive to changes in temperature and solute concentrations. The overall results suggested that for the solutes studied here the order of membrane permeability was Lincomycin incorporated > Clindamycin incorporated > water swollen. The precise nature of the controlling forces could not be rationalised during these studies but changes in the nature of water structure due to the antibiotics were implicated in the processes.

CHAPTER VII

CONCLUDING DISCUSSION AND SUGGESTIONS FOR FURTHER WORK

VII.1 Concluding Discussion

The original aim of this research was the study of the possible role of water structuring effects of the antibiotics concerned as the basis of an explanation of the widely different irritant properties of apparently very similar compounds.

It must be appreciated that the investigation did not concern the actual mechanism of irritation itself but the way in which two compounds which are chemically very similar can show such widely different physico-chemical behaviour. Models for the possible explanation of the differing extents of irritancy of these antibiotics were therefore based on the physico-chemical characteristics of the compounds as determined during this research.

Although water structure in bulk water form and aqueous solution has not been precisely identified, many of the unusual properties of water have been known for a long time. The main feature of water structure of concern during these studies was its ability to exist in free and bound water states. The amounts and definition of water types in a given sample vary according to the technique used and since differential calorimetry was used extensively here free water was described as "freezing" water and bound water was described as "non-freezing" water. A continuum of water states between these extremes is also envisaged.[10]

Water and water structure play important roles in biological processes such as stabilising intramolecular bonds that maintain proteins and enzymes in their native conformation as well as stabilising the double helix of D.N.A. Water structure has also been implicated in models of cellular control such as those of Wiggins[6,7] and Etzler and Drost-Hansen.[52] Employing the concept of water structure and its modifications it was possible that water structuring effects of the Lincomycins in some way modified or disrupted established water structures in the cellular environment with resultant irritation.

Although early studies were conducted in aqueous solution the majority of the work presented here concerned the effects of the antibiotics on water structure in model systems. The model systems used were hydrogels or water swollen polymer networks. Through selection of appropriate hydrophilic and hydrophobic monomers, these could be synthesised, in membrane form, so as to result in desired water content and freezing/non-freezing water composition preparations.

Hydrogels were also impregnated with antibiotics for some studies and a number of methods of achieving this were tested. Of these, the incorporation of aqueous antibiotic solution into the matrix during the polymerisation stage was considered the best method of achieving high levels of matrix antibiotic.

Although the extent and nature of the differences in irritancy of the antibiotics studied are difficult to quantify,[9] Clindamycin was regarded as "irritant" and Lincomycin as "non-irritant". Clindamycin is also more active than Lincomycin in vivo and in vitro.[63]

The initial characterisation studies on aqueous antibiotic solutions revealed a number of differences in the properties of Lincomycin and Clindamycin. Not only were aqueous solutions of Clindamycin increasingly more acidic than aqueous Lincomycin solutions with increasing solution concentration, but Clindamycin was also found to be more soluble than Lincomycin in water. The solubility of Clindamycin in water also increased with temperature (over the temperature range studied) far more than was found for Lincomycin. Vapour pressure osmometry studies on aqueous solutions of the antibiotics also provided evidence of intermolecular association of Clindamycin at high aqueous solution concentrations of a type not seen for Lincomycin.

It was suggested that the intermolecular association of Clindamycin and its high solubility might in some way result from a hydrophobic interaction which would allow both association and high solubility. Such an interaction would be expected to break down at higher temperatures and the properties of the antibiotics seemed to be to some extent dependant on solution concentration and temperature throughout all the studies reported here.

The effects of the antibiotics on the water content and freezing/non-freezing water composition of hydrogels was also studied. The effect of solutes in general has often been reported to be to deswell hydrogels[11,79] but incorporation of antibiotics into hydrogel matrices resulted in higher water content preparations in most cases. There was also a significant trend of higher freezing water contents in Clindamycin incorporated preparations than was found in Lincomycin incorporated preparations.

The MAA/ACM preparations incorporating antibiotics seemed to display additional properties in that almost all the water present in these compositions was non-freezing and in fact no freezing water at all was detected in the antibiotic incorporated MAA/ACM/STY preparations. Various models to explain such behaviour were proposed whereby antibiotic/matrix interactions could lead to a stabilised water structure that was propagated throughout the preparation.

It was also found that MAA/ACM and MAA/ACM/STY preparations synthesised using MBACM as a cross-linking agent had equilibrium water contents (E.W.C's) that were stable with time of hydration at room temperature. Previous studies of EDMA cross-linked MAA/ACM preparations had suggested that gradual solvation of interchain hydrogen bonds with time lead to increasing E.W.C's.[15,111] However, any stabilisation due to the use of MBACM here was overcome at elevated hydration temperatures where increased E.W.C's were recorded.

Differential scanning calorimetry (D.S.C.) studies showed that substantially larger amounts of non-freezing water were associated with high concentration aqueous solutions of Clindamycin than were found for Lincomycin. The number of non-freezing water molecules associated with Clindamycin were also found to increase with increasing solution concentration, again in a manner not found for Lincomycin.

All of these results showed a series of small but significant differences in the aqueous solution behaviour of Lincomycin and Clindamycin. Further evidence was obtained from low concentration aqueous antibiotic solution melting endotherms using D.S.C. These revealed an additional melting transition at a lower temperature (269K) than the melting of pure water for Lincomycin solutions but not Clindamycin solutions. The area of the transition increased with increasing Lincomycin concentration but its temperature position hardly varied. At higher solution concentrations the separation of the melting of pure water and depressed water components was observed for both antibiotics but at the highest solution concentrations an additional endothermic transition at 259K was found for Clindamycin and an exothermic transition at the same temperature was found for Lincomycin. The possibility of the low temperature endothermic transitions being due to the melting of eutectic compositions was investigated, phase diagrams constructed and an eutectic explanation all but eliminated.

The additional peak transitions were attributed to the melting of "restricted" water fractions, these being intermediate to the bound and free states. Studies on water swollen hydrogels, antibiotic solution swollen hydrogels and antibiotic incorporated hydrogel preparations revealed more complex multiple transition melting behaviour. Throughout the studies a number of characteristic transitional temperatures were noted, all occurring well before the melting of the "pure" water components of the systems, and these were attributed to further "restricted" water fractions. These represented particular types of stabilised water fractions that melted at characteristic temperatures. These observations agreed well with the postulated "continuum" of water states between bound and free water forms.[10] Further evidence of such a continuum was provided by the occasional superimposition of other transient melting peaks on the usual melting behaviour of some preparations. These were considered as "semi-stable" water states which form spontaneously and disappear after only one temperature cycle although a few samples showed similar peaks which persisted for about two or three temperature cycles.

A limited number of D.S.C. cooling studies were carried out which seemed to suggest that the "perfection of crystallisation" process[10], whereby the pure water melting peak of a sample increases on temperature cycling at the expense of imperfect crystalline structures, was more complex than originally envisaged in systems including the antibiotics.

It appeared that the stabilisation of water structures in the systems by antibiotics could lead to the freezing peak of pure water in the system moving to progressively lower temperatures on temperature cycling, eventually approaching the homogeneous nucleation temperature of water at the same time as the size of the "pure" water transition decreased.

The overall conclusion of the D.S.C. studies was that both antibiotics were capable of structuring water in the same manner but that the type and extent of water structuring was dependant on the nature of the water environment in the system in which the antibiotics were present. As has been mentioned already the Clindamycin incorporated preparations had higher freezing water contents than the Lincomycin incorporated preparations. This might suggest that the bulk of the water structuring effects of the antibiotics result from the hydroxyl groups of the sugar ring but that the process is influenced by the Cl/OH group change between Clindamycin and Lincomycin. This region of the molecules could have its own individual water structuring effects, or could be affecting the conformation of the molecules (via water structure) so controlling the water structuring effects of the molecules as a whole. This situation would be more complex in concentrated aqueous solution since the intermolecular association of Clindamycin could alter its water structuring properties.

Such explanations were highly speculative and so the experimental work progressed to the study of antibiotic permeation across water-swollen hydrogel membranes. Of the preparations studied only water swollen pACM (high E.W.C. high freezing water content) membranes were permeable to the antibiotics and a series of solution concentration and temperature variation studies were conducted. There was little overall difference in the permeation of Lincomycin and Clindamycin across such high E.W.C. membranes but anomolous transport behaviour of Clindamycin was found at low temperature and high solution concentration. This was considered to reflect the intermolecular association of Clindamycin in high concentration solution which may be disrupted at higher temperatures. The permeation of aqueous solutions of NaCl, KCl and $CaCl_2$ across a range of E.W.C. and freezing/non-freezing water content preparations were studied for the purpose of comparison with the transport behaviour of the same solutes across a range of antibiotic incorporated preparations. The order of rates of transport was generally KCl > NaCl > $CaCl_2$ which corresponded with the order of increasing hydrated solute size. Pore size "cut-off" plots for the transport of these solutes were constructed which also showed the influence of hydrated solute size on transport. In fact $CaCl_2$ transport across water swollen HEMA/Styrene membranes was eliminated and KCl and NaCl transport rates were well distinguished. Such observations have important implications in fields such as dialysis.

The permeation studies of NaCl, KCl and $CaCl_2$ across antibiotic incorporated membranes revealed small but significantly higher solute transport rates across Lincomycin incorporated preparations than across Clindamycin incorporated preparations. This was despite the fact that the E.W.C's and freezing water contents of Lincomycin incorporated preparations were generally lower than for Clindamycin incorporated preparations.

Studies of Glycine transport demonstrated the same permeability trends as those described above for NaCl, KCl and $CaCl_2$. It was concluded that the effects of the antibiotics was to alter water structure to varying extents so that selectivity of solute transport (based on hydrated solute size) was modified and that this effect was different for each antibiotic. The selectivity of solute transport was found to be reduced by the presence of phenylalanine in the permeating solution either by it physically obstructing solute permeation or by interacting with the particular water structuring forces present in the matrix.

Such alterations of membrane transport behaviour based on water structuring effects would have severe implications for biological systems. Disruption or alteration of membrane transport processes could lead to loss of cellular control and possibly to the type of irritation differences recorded with use of these antibiotics.

Water structuring models for the control of cellular processes have been developed by Wiggins.[67] Based on observations that ions can be selectively accumulated or excluded by cells depending on the hydration requirements of the ions and the ordered (structured) water nature of the systems a number of transport systems were described. These are best illustrated by the ($Na^+ + K^+$) ATPase model in which a membrane undergoes a conformational change, resulting in an "ordered" state (upon hydrolysis of ATP) but spontaneously collapses to a "disordered" state in response to thermal fluctuations. During the "ordered" condition it was postulated that the inner surface of the ATPase has hydrogen bonding donor and acceptor groups orientated in such a way as to allow them to participate in water structuring. The structured water regions could be stabilised and propagated throughout intracellular water adjacent to the membrane. Structured water would almost totally exclude small cations whilst accumulating large cations, most anions, amino acids and sugars to varying degrees. Permeability changes of these membrane regions, resulting in highly specific solute transport, were also postulated.

In the disordered period a membrane would have aqueous channels large enough to allow free passage of hydrated K^+ and there would be a large driving force for Na^+ influx which would be restricted since the pore size would be too small to allow free passage of hydrated Na^+ ions. However, during an ordered period the water zone just inside the membrane has a greatly reinforced hydrogen bonded structure and so would not be readily available to provide primary hydration for the ions. A Na^+-specific ionophoric channel at each (Na^+ + K^+) ATPase would allow dehydrated Na^+ ions to have their water of hydration replaced by a protective tunnel of oxygen atoms so that Na^+ can move rapidly in either direction. However, the driving force for Na^+ transport has reversed because the water structure inside the membrane is incompatible with the stable existence of Na^+ ions with their absolute requirement of primary hydration. Net efflux of Na^+ thus occurs whilst K^+ finds the intracellular environment a more favourable environment (a water structure breaker)[41] and so tends to be accumulated.

The model as outlined above has been greatly simplified but was developed by Wiggins to explain a number of transport system processes.[6,7] It is easy to see how disruption of the water structure of such a process could lead to uncontrolled K^+ and Na^+ transport. The levels of extracellular K^+ would thus rise and this process would agree with the observed K^+ loss of red blood cells (before cell lysis) in the presence of Clindamycin which was found to a far smaller extent in the presence of Lincomycin.[9]

The differences in the water structuring capabilities of the two antibiotics in certain water environments could lead to different extents of disruption or modification of water structure and as cellular metabolism becomes uncontrolled lead to the different extents of irritancy of the two compounds. Clindamycin would presumably have the greatest effect in this way since the higher levels of K^+ release from red blood cells incubated with Clindamycin rather than Lincomycin could be correlated with its greater irritant effects.

The differing extents of irritancy of the two compounds might also be the result of water structuring effects at a slightly different level to that described above. It might well be that certain cellular transport processes are modified rather than completely disrupted and that certain substrates and compounds essential for the maintenance of cell metabolism are selectively or even unselectively transported out of the intracellular environment via modified water structure based transport systems that eventually lead to cell and tissue degradation and hence the irritation reported. Such a modification might lead to the release of compounds such as creatine kinase (as reported)[9] into the extracellular environment as a cause of cellular degradation rather than as a result of cell lysis.

Either way, the water structuring effects of the Lincomycin antibiotics could result in the different extents of irritancy of the various compounds due to the occurrence of interactions of water structures or water structure disruptions in biological systems.

In the broader context of drug induced irritation the results of this research are particularly interesting. It is unlikely that the water structuring abilities seen here are limited to the Lincomycin group of antibiotics alone and in future years it may well prove that a large number of diverse compounds demonstrate hitherto unreported complexity of thermal behaviour in aqueous systems.

That withstanding, the differences in the physical-chemical behaviour of Lincomycin and Clindamycin are interesting in that the association of

Clindamycin and not Lincomycin and the large differences in the solubility characteristics of the two compounds were largely unexpected. These studies, combined with the investigation of the thermal behaviour of the compounds when in aqueous solution suggested that the interactions responsible for these observations were sensitive to concentration and temperature changes.

The large differences in the water structures of these compounds also has wider implications in other fields where the influence of water structure has already gained credibility. One such area is that of biocompatibility where the nature of the water associated with an implant governs its successful use to a large extent. The studies described here may eventually lead to a better understanding of interactions in which water structure is involved.

It is impossible to say at this stage what feature of the water structuring capabilities of Clindamycin results in its more marked irritancy than Lincomycin. However, given that high concentrations of some antibiotics are readily accumulated at certain sites, it may well prove that the intermolecular association of Clindamycin but not Lincomycin in high concentration aqueous solutions is the main factor controlling the differences in water structuring and extents of irritancy of these compounds.

VII.2 Suggestions for Further Work

Several suggestions for the extension of particular aspects of the research described in this thesis have been made as follows:

1) The study of the intermolecular association of Clindamycin in aqueous solution as a function of temperature bearing in mind the apparent concentration and temperature dependance of processes studied during

this research. Investigations of Lincomycin and Clindamycin in non-aqueous solution (eg D.M.S.O.) by vapour pressure osmometry would aid the assessment of the importance of association of the antibiotics to their water structuring differences.

2) Studies of the amount of each antibiotic absorbed by the various hydrogel preparations employed would allow a more detailed consideration of the permeation of the antibiotics across such membranes although this in itself requires the development of a more sensitive assay for the antibiotics.

3) The amount of each drug associated with the hydrogel preparations employed during this research could also be determined from C.H.N. analysis and would clarify some of the results of the water contents of the various antibiotic impregnated hydrogels as regards the amounts of non-freezing water rather than absorbed antibiotic present.

4) The MAA/ACM and MAA/ACM/STY preparations used during this research need further characterisation of their E.W.C. stability with time and hydration temperature. Any stabilisation of the network due to antibiotic incorporation might be reflected in the E.W.C. versus hydration temperature profiles of such preparations. The variation of the E.W.C's of such preparations might also be studied with regard to the pH of the hydrating medium.

5) Further D.S.C. studies need to be carried out on the antibiotic incorporated hydrogel preparations that resulted in multiple peak melting behaviour to investigate the relationships between the amounts of antibiotics present and the size of the water component under each thermal transition.

Extended D.S.C. studies, in order to resolve the problems of possible eutectic formation and their contribution to the thermograms, are also required as well as further studies on the thermal transitions of aqueous monomer solutions. The multiple transition behaviour of the samples studied should be repeated using a technique such as n.m.r. or dielectric in order to substantiate the assignment of these transitions as the melting of "restricted" water fractions.

6) D.S.C. studies evaluating the effects of the antibiotics on the gradual process of incorporation of non-freezing water into the freezing water fraction during temperature cycling are also required together with the investigation of the behaviour of water associated with the exothermic transitions seen during the heating of some samples.

7) Permeability studies on the effects of antibiotic incorporation into hydrogel membranes on the transport of solutes other than those studied here that might be implicated in the irritation process (such as phosphocreatine and creatine kinase) would be of considerable interest. Such studies would require the selection of hydrogel compositions with water environments and pore sizes suited to the study of the solutes under investigation.

The suggestions outlined above are all based within the general area of research already carried out, however, new areas of study must involve the investigation of the water structuring effects of many other compounds which need not necessarily be limited to pharmaceuticals or biologically significant species.

Such investigations could then be extrapolated to permeability and other studies as a general improvement of our knowledge of the nature of water structures and their importance in physical-chemical and biological systems.

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