NOVEL POLYMERIC CONTROLLED RELEASE SYSTEMS

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A Thesis Submitted for the Degree of

Doctor of Philosophy

THE UNIVERSITY OF ASTON IN BIRMINGHAM

September 1986

The University of Aston in Birmingham

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SUMMARY

The primary object of this research has been to evaluate the potential of biodegradable polymers as matrices for macromolecular controlled drug release systems. Initial work was carried out on the *in vitro* breakdown of members of a novel poly ester copolymer series - polyhydroxybutyrate-co-hydroxyvalerate, in various aqueous buffers by bulk and surface measurements of the varying physical forms of the copolymer matrices.

In line with the reported hydrolytic degradation of other polyesters the degradation was found to be by a predominantly homogeneous (bulk) mechanism. The initial molecular weight, copolymer composition and physical form of the polymer matrix determining the rate of hydrolysis. Comparison with established biodegradable suture materials showed a relatively slow rate of hydrolysis for the butyrate / valerate copolymers. However, differences in matrix molecular weights and crystallinities did make equivalent comparisons difficult. A link between matrix surface physical properties and the extent of bulk degradation was found, with the surface techniques providing a more detailed reflection of the extent of bulk matrix hydrolysis than weight loss measurements during the initial degradation stages.

Drug release in the form of a series of macromolecular FITC dextran dyes was followed from two types of polyhydroxybutyrate-co-hydroxyvalerate matrices (i.e. solvent cast films and cold compressed tablets). Release was found to occur predominantly by diffusion with matrix degradation playing an insignificant part in dye dissolution. Contrary to expectation, the rate of dye release increased with increasing dye molecular weight. This was due to changes in matrix porosity rather than dye diffusivity. Tablet dye release was found to be effected by the addition of various excipients, with matrix porosity governing the overall release rate. It is suggested that the tabletted form could be used as sustained release non-disintegrating oral dosage forms.

KEY PHRASES

BIOPOLYMERS
HYDROXYBUTYRATE co HYDROXYVALERATE COPOLYMERS
ESTER HYDROLYSIS
MACROMOLECULAR DYE RELEASE

THIS THESIS IS DEDICATED TO THE MEMORY OF MY GRANDPARENTS

ACKNOWLEDGEMENTS

I sincerely thank my supervisor Dr B.J. Tighe for his helpful advice and useful discussions throughout the last three years.

Thanks are also extended to my associate supervisor Dr A.J. Amass and my industrial supervisor Dr P.L. Gould, together with all in 248 and 358 for their constructive advice and criticisms.

My thanks also go to Pfizer Central Research, Sandwich, Kent. For the use of the tabletting and dissolution facilities, and to everyone in the Pharmaceutical Research and Development department there. Special thanks are extended to Dr G. Henderson for assistance with the tablet dissolution runs.

I should also like to thank Mrs E. Ince for the 'Blood letting' and to all in the computing department at Aston University for advice on the wordprocessor and on the construction of the graphs.

Finally, I would like to thank my wife Julie, for her encouragement and never-ending support.

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LIST OF ABBREVIATIONS

FITC-Dextran	=	O-(Fluoresceinylthiocarbamoyl)-dextran
GA	=	Glycolic Acid
GF	=	Gloss Factor
GPC	=	Gel Permeation Chromatography
HET	=	7-Hydroxyethyltheophylline
HPMC	=	Hydroxypropylmethylcellulose
I_d	=	Diffuse Reflectance
I_s	=	Specular Reflectance
IM	=	Injection Moulded
LA	=	Lactic Acid
MCC	=	Microcrystalline Cellulose
MEL	=	Minimum Effective Level
$\overline{\overline{Mn}}$	=	Number average molecular weight
$\overline{\overline{Mw}}$	=	Weight average molecular weight
MWD	=	Molecular Weight Distribution
Mwt	=	Molecular Weight
PCA	=	Poly Caproamide
PCL	=	Poly Caprolactone
PDS	=	Poly para Dioxanone
PGA	=	Poly Glycolic Acid
PHB	=	Poly hydroxybutyrate

LIST OF ABBREVIATIONS (Cont)

PHV	=	Poly hydroxyvalerate
PLA	=	Poly Lactic Acid
SEM	=	Scanning Electron Microscopy
SGN	=	Simulated Gastric Fluid
Tg	=	Glass transition temperature
THF	=	Tetrahydrofuran
TMC	=	Trimethylene Carbonate
TS	=	Tensile Strength
USP	=	United States Parmacopoeia
$W_{1/2}$	=	Peak Width at Half Height
γd	=	Dispersive Component of the Surface Energy
γp	=	Polar Component of the Surface Energy
γt	=	Total Surface Energy
δ	=	Solvent Solubility Parameter
θ	=	Contact Angle

CHAPTER ONE

INTRODUCTION

1.1 PHILOSOPHY OF CONTROLLED RELEASE

At the present time in the pharmaceutical industry there is a considerable effort to develop extended release drug delivery systems. This stems from the increasing difficulty in obtaining official (c.f. in USA the Food and Drug Administration (FDA)) approval of new drug entities along with the long term, high risk investment associated with full scale development of a new 'active' molecule. This has led to a focus of attention on developing new methods for delivering existing drugs for a prolonged period which can increase drug patent life and reduce the scale of official testing.[1,2] This prolonged release is often termed sustained or controlled release. In this thesis sustained release is considered as being relatively short term (less than 2 days) and controlled release as being extended release.

Conventional oral tablet and intravenous drug delivery systems frequently give poor control of drug plasma concentrations, exhibiting initially high (and possibly toxic) drug concentrations within the body which then tails off due to metabolisation of the drug, with the consequence that additional dosage is needed when the drug concentration has fallen below its minimum effective level (MEL). Many drugs have a small difference

between the toxic and MEL levels and particular attention must be paid when evaluating the residuum of active drug in the blood plasma and other tissues in order to avoid overdosing. Other disadvantages of these types of systems are the possible side effects in surrounding tissues induced by the loss of drug into the environment of the target organ. This leads to an expensive treatment due to the inherent inefficiency. An ideal controlled release system would avoid these problems by maintaining the drug plasma concentration between the toxic and the minimum effective levels for prolonged time periods at a constant concentration (i.e. zero order release of drug from the matrix), followed by rapid tail off when the continuous release device is depleted. This system and other conventional systems release profiles are illustrated in Figure 1.1.

Obvious advantages of controlled release systems are:

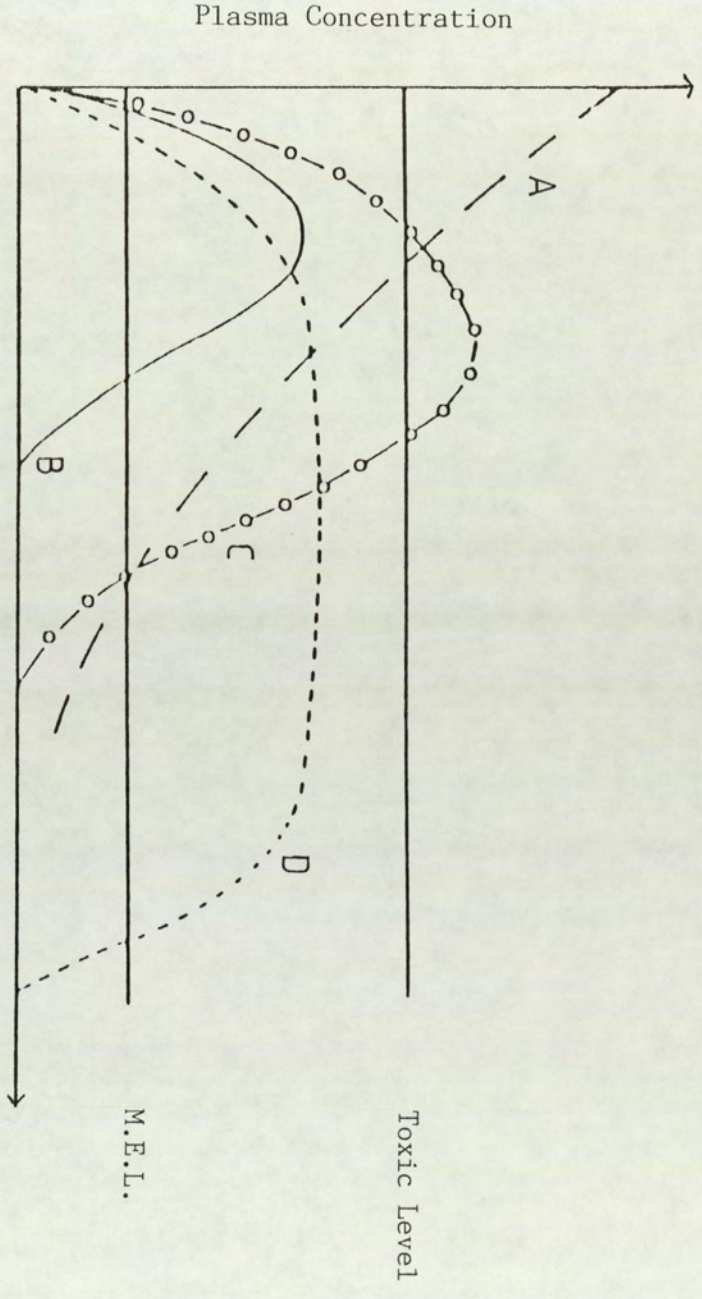
(i) A reduction in the frequency of administration.

(ii) Reproducible extended constant delivery rates.

(iii) Reduced side effects provided the dose remains below the toxic level.

(iv) For a biodegradable implant matrix the additional advantage that the drug vehicle would degrade into non toxic products and hence obviate surgical removal.

Figure 1.1 Plasma drug concentration profiles for various methods of administration



(A) = I.V. Injection, (B) = Oral tablet dose, (C) = Oral overdose, (D) = Ideal Controlled Release dose.

Many other important advantages of controlled release systems from the commercial point of view have been considered by Kydonieus.[3]

As with all processes there are some potential disadvantages associated with controlled release systems, for example, for a polymer/drug matrix, important factors to be considered include the mechanism of matrix degradation, the nature and toxicity of its metabolites, the biocompatibility of the matrix and the effect of any additives in the polymer on the body. Other possible disadvantages are the financial aspects corresponding to the cost of preparation of controlled release forms and as already mentioned the difficulty in obtaining full registration of a controlled release product.

Some of these problems can be circumvented by using a biodegradable polymer with natural metabolites as the matrix, such as the lower members of the poly alpha ester series e.g. poly lactic acid, which degrades to the free acid.

1.2 AREA OF RESEARCH

Prolonged controlled release of drugs from polymeric matrices is now well established. In contrast, however, release of macromolecules presents more difficulties and in consequence is less well studied and relatively rarely discussed in the literature.

From Ficks law of diffusion macromolecular drug release from within a polymer/drug matrix cannot be diffusion controlled, with true diffusion playing a very small if not insignificant part in the overall release rate. However controlled bioerosion of a polymeric matrix, releasing the drug as it is eroded, appears to be an appropriate method for accomplishing controlled release of these high molecular weight compounds.

This is the area of controlled release technology that my work is based, with the main objective being to design a system to release macromolecular drugs at a constant rate by erosion of a matrix of drug and biodegradable polymer. The primary step was to evaluate the published work in this field with a view to selecting a suitable biodegradable polymer which would provide an erodible matrix from which macromolecular drug entities could be released.

1.3 POTENTIAL POLYESTERS FOR CONTROLLED MACROMOLECULAR RELEASE SYSTEMS

In considering the potential of biodegradable polymers for use in controlled macromolecular release systems existing systems are first considered, followed by a more precise definition of the terms biodegradation and bioerosion. A description of the four main controlled release systems is proceeded by a detailed individual evaluation of chemically erodible polymers with a summary of possible polymers for macromolecular release studies.

Early work on drug release from polymeric matrices was concentrated on the release of relatively low molecular weight species, with the preparation of a Silastic (silicone rubber) / isoproterenol matrix reported by Folkman and Long[4] as early as 1964. The first clinically tested controlled release system was the Ocusert ethylene vinyl acetate copolymer system[5] designed to release Pilocarpine into the eye to combat glaucoma. The initial use of biodegradable polymers for extended release focussed on poly lactic acid, with a drug delivery system reported by Yolles et al[6] in 1970. There has more recently however, been a growing interest in extending the principles involved in low molecular weight drug release to the release of macromolecular compounds such as growth hormones. For the controlled release of macromolecules, the release rates obtained by predominantly diffusional processes are frequently too slow to be practical and much research activity currently centres on the achievement of enhanced release by erosional breakdown of the polymer matrix. Thus present interest in the utilisation of biodegradable polymers for controlled release of macromolecules is illustrated by references to this area in more general reviews[7-10] and papers[11-16] dealing with controlled release. Many of the aspects of the polymer behaviour involved are also important in the design of polymers for absorbable surgical sutures,[17,18] for seedling protection[19,20] and for pesticide release[7].

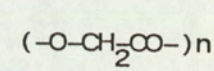
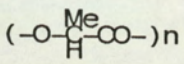
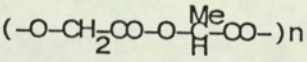
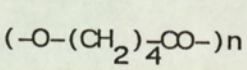
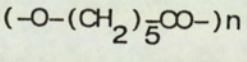
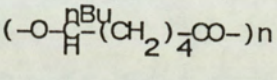
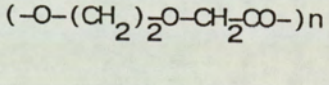
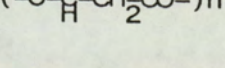
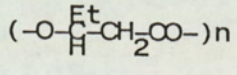
The release of macromolecular species (taken here to include molecular weights (Mwt) in excess of 1500 Daltons) from polymeric vehicles has been mainly achieved by four processes. These are: diffusion controlled release from ethylene vinyl ester copolymers (especially ethylene vinyl acetate[7,9,13-15,21-29]) in which a porous structure develops as a result of macromolecular release, and diffusional release from other polymers;[16,30] diffusional release by controlled swelling of poly vinyl alcohol and related materials;[31] release by erosion of cross-linked polyester hydrogels[12,32] and, finally, erosion of polylactide and polyglycolide[33] (the so called poly alpha hydroxy acids or poly alpha esters). None of these processes is without problems in its application to macromolecular release and none can be applied with equal facility to the whole range of molecular sizes and stabilities of the potentially important macromolecules for which release processes are sought. It is this very aspect of controlled macromolecular release that demands versatility and control of the release system. Thus the range of species involved (extending for example from relatively simple peptides to complex proteins), the range of physical properties and stabilities that they possess, and the very wide range of delivery profiles required, present the need for a range of release systems that may be adapted to individual cases. In this review of the literature attention is concentrated on the last release process described, namely the poly glycolide and poly lactide based systems, and the extent to which they may be

adaptable to meet the particular demands of macromolecular controlled release systems. Polyesters of interest in this general field are listed in Table 1.1.

At the present time there is comparatively little literature dealing with the successful design of controlled macromolecular release systems, as distinct from those concerned with relatively low molecular weight species. Amongst the examples that do exist, however, are to be found the apparently succesful uses of poly glycolide - lactide systems which encourage the belief that other so called 'biodegradable' polyesters might be harnessed in the same way. One particular advantage of this type of polymer in the present context is the fact that the hydrolytic lability of the ester group has been exploited in the design of other types of biodegradable medical device. As a result there is a great deal of literature that could provide a useful base of systemised knowledge which would assist in the design or selection of polymers for controlled macromolecular release systems in which biodegradation or bioerosion is the important controlling feature. On this basis an attempt has been made to bring together literature from the various fields where these processes are important in order to illustrate and assess the potential of such processes as a basis for the controlled release of macromolecules.

Many problems are encountered in incorporating large molecules into polymeric matrices. These derive mainly from the interactions between the polymer matrix and the incorporated

TABLE 1.1 Commercial bioerodible polymers

POLYMER NAME	COMMERCIAL NAME	STRUCTURE
POLY GLYCOLIC ACID	DEXON®(sutures)	
POLY d,l LACTIC ACID	---	
POLY GLYCOLIC–co– –LACTIC ACID	POLYGLACTIN 910® or VICRYL®(sutures)	
POLY VALEROLACTONE	---	
POLY ε CAPROLACTONE	---	
POLY ε DECALACTONE	---	
POLY DIOXANONE	P.D.S®(sutures)	
POLY HYDROXY– –BUTYRATE	BIOPOL®	
POLY HYDROXY– –VALERATE	(used as a comonomer with the butyrate)	

macromolecule, that is 'polymer-polymer' compatibility phenomena. The mutual incompatibility which is normally encountered with two polymers means that homogeneous distribution is difficult to achieve. In addition greater solubility problems arise with high molecular weight than with low molecular weight compounds. Thus incorporation of macromolecular drugs or markers into a polymer matrix frequently requires the difficult combination of an aqueous solvent system and an organic solvent system, with further complications arising from drug instability at raised temperatures and in acidic and alkaline pH's.[32] There are therefore many problems associated with drug loading, device fabrication and drug stability that are much greater than those encountered with the release of low molecular weight species.

An additional problem associated with controlled release is the aim of delivering the 'active' at a constant non time dependent rate i.e. zero order release; as opposed to the more usual simple diffusional release of a drug which gives essentially a square root time dependence. In this connection alteration of the drug matrix geometry and drug loading to give pseudo zero order release for swellable hydrogel systems has been discussed by Lee,[34-36] and additionally by Langer and co-workers,[37] Colombo et al,[38] Heuvelsland et al[39] and de Haan and Lerk.[40] However the application of these principles to general matrix controlled release systems is as yet unreported.

One of the first problems to become apparent in overviewing the literature in this area is the fact that there are varying definitions of biodegradation and bioerosion. Williams[41] defines Biodegradation as biological breakdown of the polymeric material, as opposed to simple hydrolytic breakdown[42], a definition also held by Gilbert[43]. Gilding[44] on the other hand reports that the phrase 'biodegradable polymer' is widely used for any polymer that undergoes in vivo degradation. Similarly, Graham[45] defines biodegradable systems as those which degrade after a period of time to soluble products easily removed from the implant site and excreted from the body. A much more complex picture emerges with the various definitions listed according to their degradative pathway by Griffin[46]. Thus direct biodegradation is taken as being the enzymatic scission and metabolisation of macromolecules, indirect biodegradation as being the oxidative cleavage of a polymer followed by metabolisation and macrobiological biodegradation as involving mechanical degradation by animals.

Although Zaikov[47] gives no definition of biodegradation he considers the in vivo breakdown of a polymer in terms of three degradative components, these being water (diffusivity), salt content / pH of the physiological environment, and lastly enzymatic attack.

Bioerosion is much less widely defined in the literature. Heller[11] defines the term as the conversion of an initially water insoluble material to a water soluble material which may or may

not involve major chemical degradation. Langer on the other hand initially made no distinction between bioerodible and biodegradable[8], but later[9] discusses the term 'erodible' in the context of the ability of polymer matrix degradation to control the rate of drug release. In this thesis the term biodegradation is taken to refer to hydrolytic, enzymatic or bacteriological degradation processes occurring in a polymer, which do not necessarily proceed to a stage where the physical form of the polymer is altered. Bioerosion on the other hand necessitates physical loss from the polymer matrix and this maybe brought about by a variety of physical or chemical processes which are not always specified.

Macromolecular release by bioerosion occurs when the release of an active ingredient from a polymer matrix follows erosion of the polymer surface and/or bulk matrix, rather than occurring by simple diffusion. The use of the term bioerosion does not necessarily define the chemical processes involved as they are invariably complex. The various steps that must be taken into account, for example, in considering the degradation of a polymer matrix include diffusion of water into the matrix and subsequent hydrolytic chain scission, the removal of low molecular weight polymer degradation products (which may be in the form of a 'gel') by diffusion out of the matrix, hydrolytic and enzymatic erosion to form pores, inward diffusion of enzymes (in the latter stages of matrix degradation) and ultimately breakdown of the polymer to

fragments followed by enzymatic degradation and tissue assimilation. It is generally impossible from the limited data in the various papers reviewed here to make sharp distinctions between the processes involved.

Biodegradable systems are often referred to as undergoing chemical or enzymatic erosion, a subdivision that is retained in this discussion. In practice the distinction is more correctly regarded as simple hydrolytic and enzymatically induced hydrolytic degradation.

1.3.1 CONTROLLED RELEASE SYSTEMS

Controlled release systems can generally be divided into four sections depending on their general release mechanisms[9], i.e. diffusion, chemical erosion, solvent activated, magnetic and more recently ultrasonic[48,49] control.

CHEMICAL EROSION: Heller[50] has suggested three types of chemical erosion; firstly dissolution by hydrolytic degradation of cross-links of water soluble polymers rendered insoluble by the cross-linking; secondly aqueous dissolution of water insoluble polymers by hydrolysis, ionisation or protonation of a pendant side chain; and finally random cleavage of an insoluble polymer backbone producing aqueous oligomers. Williams in a similar fashion has listed classes of chemical erosion in terms of polymer

hydrophobicity / hydrophilicity, solubilisation and physical degradability.[41]

The two main mechanisms of chemical erosion can be considered as being heterogeneous and homogeneous. Heterogeneous erosion occurs at the polymer surface only and is often referred to as surface erosion.[11] Homogeneous erosion, often but misleadingly called bulk erosion, causes degradation (at a constant rate) throughout the polymer matrix. It should be noted that a combination of them usually occurs depending on the physical properties of the specific polymer (for example, polymer crystallinity, molecular weight, hydrophobicity, and glass transition temperature (Tg) and in the case of pharmaceutical tabletting, the nature of the additives). Diffusion[2,51-53] frequently plays a significant part in the rate of drug release from bioerodible systems. As drug molecules are released, pores and channels are formed in the polymer matrix which then allows more drug to diffuse. Alternatively, it has been suggested that the voids formed by dissolution contain a viscous oligomeric solution which reduces the rate of diffusion of the drug from the matrix through the channels. High loading levels of drugs can induce major contributions of diffusion to the overall release rate.[8]

An important subsection of chemical erosion (normally simple hydrolysis) is ENZYMATIC EROSION. Apart from the proven enzymatic degradation of naturally occurring polymers, (for example, cat

gut, collagen and cellulose[43,44,54]) there has been much debate about enzymatic involvement in the degradation of synthetic polymers. Many papers declare no enzymatic involvement on the basis that their *in vivo* and *in vitro* data are well correlated[55,56]; whilst others propose enzymatic involvement due to poor *in vitro*/*in vivo* correlation.[44,57] However as drug release and polymer degradation product removal occurs through an outer boundary layer around the polymer matrix, tissue encapsulation of the polymer matrix *in vivo* may inhibit removal of drug and the polymer degradation products from the boundary layer. On this basis, in the degradation process, a poor correlation does not necessarily prove an enzymatic involvement.

Enzymatic involvement in the degradation of biodegradable polymers has been discussed by Williams[58], who found that out of a total of fourteen enzymes tested *in vitro*, four enzymes affect the rate of degradation of poly glycolic acid (PGA); namely , Ficin, Carboxypeptidase A, alpha Chymotrypsin and Clostridiopeptidase A. A further three enzymes (Bromelain, Esterase and Leucine aminopeptidase) also had some affect on PGA. However, they were tested in an ammonium sulphate medium, which can perturb enzyme activity[59]. Hydrolytic degradation could also have played a part in the influence of the first four enzymes on PGA, as these enzymes were contained in Tris and phosphate buffers which can themselves degrade PGA hydrolytically. The other seven ineffective enzymes were Acid phosphatase, Papain, Peptidase, Pepsin, Pronase,

Proteinase K and Trypsin. The studies of Salthouse[60] revealed the presence of Leucine aminopeptidase and Acid phosphatase at the site of PGA implants and in addition Herrmann[61] suggests that tissue esterases play an important part in PGA degradation.

Recent work by Williams[62] has found a sharp difference between the *in vitro* and the *in vivo* degradation rates (as measured by tensile strength (TS)) over the first two days. *In vivo* (rat) studies showed an appreciably higher reduction in TS over the first two days, after which loss followed *in vitro* TS-time profile results. When the samples were exchanged after two days the same enhanced reduction in TS occurred *in vivo*, whilst the *in vitro* sample showed the expected reduction in TS. This suggested that specific conditions around the implant site caused enhanced initial degradation *in vivo*. Further *in vitro/in vivo* comparisons have indicated that lipids (e.g. butyric, caproic, heptanoic and stearic acids) can bring about a similarly rapid initial degradation.

Williams[54] studies have also included work on poly lactic acid (PLA), and show that Proteinase K brings about PLA hydrolysis. Reed[63] indicates that there could be a considerable enzymatic involvement in PLA degradation, having reported and observed a substantial difference between *in vitro* and *in vivo* molecular weight changes (18% more loss *in vivo*). Later work by Williams[64] however, has shown that Lactate dehydrogenase gave ambiguous results with PLA, but Proteinase K, Pronase and Bromelain had

significant effects, as shown by the detection of lactic acid in solution. The effects of Esterase, Ficin and Trypsin were found to be minor. Williams suggests that since Pronase and Bromelain caused physical breakdown of the polymer, and Proteinase K although inducing a reduction in molecular weight caused no physical damage, these enzymes showed exo- and endo-kinase behaviour respectively. However, the *in vivo* presence of all these enzymes has to be questioned.

It has been reported[65] that there is no diffusion of enzymes into nylon 6 (poly caproamide (PCA)), and that attack occurs on the surface only, although work with enzymes in the vicinity of PCA implants (rat) by Salthouse[66] has shown no effect. This is supported by work[67] on Vicryl sutures (Ethicon Inc.), which contain 90 PGA / 10 PLA, where no enzymatic contribution during the bulk of the degradation process was found. It was concluded however, that cellular oxidative enzymes had a role in the degradation of primary hydrolysis products. This secondary role of enzymes in biodegradation is also supported by Gilbert and co-workers[43], who consider that enzymatic involvement becomes important after the polymer has been considerably degraded by hydrolytic chain scission processes, that is after onset of weight loss. This is due to an increase in the available surface area which increases the enzyme flux into the polymer matrix.

Poly ε caprolactone (PCL) features widely in studies that are designed to isolate the various contributory factors involved in

in vivo hydrolysis. Woodward and co-workers collaborating with Schindler and Pitt[68] have attempted to identify the relative importance of enzymatic surface erosion and phagocytosis in the intracellular degradation of PCL. Interest in this arose from earlier work by Pitt and Schindler[69,70] in which elastomeric copolyesters of PCL and poly valerolactone cross-linked with bis caprolactone, were shown to undergo surface erosion by enzymatic attack at a rate 600 times faster than normal hydrolytic attack. This was inferred to be due to the fact that polymer chains were in the rubbery state, with the result that the chains had enough free movement to form the correct chain conformation for enzymatic attack. They also noted that for glassy biodegradable polymers this configuration cannot be easily obtained and hence small (if any) enzymatic degradation occurs. Other enzyme degradation studies which either demonstrate[71-73] or infer[57] involvement have been reported, and the effects of lipids on PGA sutures have been discussed by Sharma and Williams.[74]

On the basis of the literature, it is therefore concluded that: a) For polymers in the glassy state, little enzyme involvement is expected in the early stages. This can become more pronounced in the later stages, however, as erosion and physical fragmentation of the polymer occurs.

b) For polymers in the rubbery state, enzymes can play a significant role in their degradation.

Very few studies involving micro-organisms have been carried out and for convenience these are included here. PCL degradation using a yeast (Cryptococcus laurentii) and a fungus (Fusarium) by Jarret et al[75], has shown that the amorphous regions of the polymer are preferentially degraded; growth of fungi into polyester copolyurethane elastomers and starch-ethylene vinyl acetate blends with a theoretical treatment of possible 'tunnel growth' of bacteria have been considered by Griffin[46] and the effect of bacteria on PGA sutures studied by Williams[76] has shown that a decrease in degradation occurs when compared to hydrolytic degradation processes. In addition sterilisation by gamma irradation produces a further type of degradation often overlooked with implants and sutures. Under these conditions a decrease in molecular weight has been reported by many workers,[17,56,69,77,78] with a possible mechanism for PGA degradation being discussed by Chu[79] and Gilding.[80]

1.3.2 ERODIBLE POLYMERS FOR CONTROLLED RELEASE OF MACROMOLECULES

Individual polymers which have been, or could be, used for controlled release by 'erosion' of the drug loaded matrix are now discussed. Also included in the discussion are the mechanisms of biodegradation and release where information is available, and the suitability of the individual polymers for macromolecular controlled drug release.

1.3.2.1 Poly Glycolic Acid (PGA)

The most widely studied biodegradable polymer has been PGA. It is most often used in the form of a highly crystalline, high molecular weight absorbable surgical suture; Dexon®(American Cyanamid co). A great deal of work has been conducted evaluating its potential as the basis of a controlled release delivery system.

The degradation of PGA occurs essentially by bulk erosion. Chu[59,78,79,81-85] describes the *in vitro* degradation as a two stage process. The first stage consists of diffusion of water into the amorphous regions of the polymer with random hydrolytic chain scission of the ester groups occurring. The second stage starts when most of the amorphous regions are degraded, hydrolytic attack then focuses on the crystalline domains. An apparent increase in crystallinity with (degradation) time is explained by the chain fragments of the hydrolysed amorphous regions having a lesser degree of entanglement; and their ability to realign themselves into a more ordered crystalline state.

In vitro tests[82] on Dexon sutures show that degradation occurs in two stages; the first from Day 0 to Day 21, and the other from approximately Days 21 to 49. Maximum crystallinity occurs around the transition between the two stages. Tensile strength was lost completely by Day 49, at which point 58% of the suture by weight remained. It is thought that diffusion of oligomers formed during

the degradation is probably a significant component of the erosion process during the latter stage of the degradation.

Strongly alkaline environments[59,81,83,84] increase the rate of hydrolysis with respect to normal physiological (pH=7.4) or acidic conditions. Gel permeation chromatography (GPC) by Reed[86] indicated a growing oligomeric fraction of the molecular weight distribution (MWD) as the degradation proceeded. Gilding[87] reports that degradation products of Dexon sutures are eventually eliminated from the body as carbon dioxide through the respiratory system.

Zaikov[47] reports that buffer salt concentration affects PGA degradation. The rank order of degradation rates being: 1 mol / litre (buffer) concentration > <u>in vivo</u> (rabbit) > 0.5 mol/litre > 0.1 mol/litre >> water.

Miller et al[88] have found that the 'curing time' (which the authors relate to the polymerisation time) of PGA affects its degradation rate, with fast curing polymers degrading more quickly. This is presumably due to differences in molecular weight and MWD between fast and slow cured polymers.

The form of PGA encountered in sutures is too crystalline to be used for drug release. Since PGA is the most hydrophilic member of the poly alpha ester series currently used, polymers with low molecular weights and crystallinities could be suitable. No significant work has been reported using the homopolymer as a matrix for drug release, but there are a considerable number of reports on the copolymer of PGA with PLA (see below). Another

copolymer system of PGA with unhindered glycols has been described by Casey et al.[83] Possible copolymers for drug release are described and the authors claim that surface erosion is the main degradation process. Their claim for continuous release suitability, however, is purely speculative.

1.3.2.2 Poly Lactic Acid (PLA)

Due to the presence of a chiral centre, there are three forms of PLA; those deriving from D(-), L(+), and the (racemic) D,L forms of the parent acid. D and L terms derive from the configurations of optically active (+) and (-) forms of glyceraldehyde, respectively; the (+) and (-) specifying the direction of rotation of the sodium D line. However, on dimerisation the direction of the sodium D line is reversed, hence the D(-) lactic acid gives the D(+) dilactide and the L(+) lactic acid gives the L(-) dilactide. Since ring opening of the lactides is commonly used in poly lactide synthesis, an apparent complexity arises because poly (L+) lactic acid and poly (L-) dilactide are structurally equivalent. For this reason the abbreviations P(D)LA, P(L)LA and P(D,L)LA are sufficient unless it is specifically intended to show the synthetic origin of the polymer. Thus, poly L(-) lactide and poly D(+) lactide are sometimes used.

The polymers derived from the optically active D and L monomers are semi-crystalline and at similar levels of crystallinity degradation would be expected to be similar to, or

slightly slower than that of PGA due to the extra methyl group increasing hydrophobicity and restricting water uptake to about 2%.[80,86] Reed[86] has shown this similarity with work on P(L)LA which showed a 50% loss in molecular weight, but only a 10–15% loss in weight, during a sixteen week in vitro degradation study. (c.f. 58% weight loss in 49 days for PGA). Since it is the L(+) form of lactic acid that is metabolised in the body, P(L)LA is much more commonly employed than P(D)LA. The work of Vert et al[90] however, throws considerable doubt on the results of Reed and of that of several previous workers. In tabulating and comparing absorption rates of various forms of poly lactic acid they emphasise the wide variation in published results. Their analysis leads to the expectation that the time period required for complete absorption is substantially longer than had been previously supposed; with the work of Hyon et al[91] showing very little change in TS and weight loss for a high molecular weight fibre immersed in phosphate buffered saline at 37°C for six months.

P(D,L)LA is amorphous and since it has only one morphological phase it can be considered as being a preferable candidate for a drug release matrix. Although this material does not have the high crystalline melting point (180°C) of P(L)LA, it does undergo a solid-melt transition in the region of 130–135°C and therefore represents a processable alternative to the polymers of its optically active counterparts.

Pitt and Schindler et al[42,92] have shown that the *in vivo* (rabbit) degradation of P(D,L)LA occurs by essentially homogeneous erosion over two stages. The first occurs by random hydrolytic chain scission of the ester groups accompanied with a linear loss in molecular weight. The second stage begins at a number average molecular weight ($\bar{M}n$) of 15,000, at which onset of weight loss and total loss of TS occurs, along with an increase in chain breakdown unique in the poly alpha ester series. The duration of the first stage depends on the initial $\bar{M}n$. *In vitro* work[92] gave a similar (slightly faster) degradation rate than the *in vivo* results suggesting non-enzymatic involvement. The degradation[93] is generally assumed to be autocatalytic in nature due to the carboxylic acid end groups formed during degradation. P(L)LA behaves as PGA in that it is eliminated from the body through the respiratory system.[94]

Drug release from P(D,L)LA has generally been concerned with relatively low molecular weight drugs such as steroids for contraceptive use and narcotic antagonists. These systems are discussed by Heller.[50] Eenink et al[95] describe release of a low molecular weight dye from a series of P(L)LA's and poly amino acids. Pitt and Schindler et al[96] have shown that steroid release occurs by a mechanism which departs from simple diffusion and may involve polymer erosion. PLA copolymers derived from mixtures of L and D,L lactic acids having a $\bar{M}n$ = 180,000 have been used for hormone release by Wise et al.[97] An average release rate of 25μg per

day was observed for 100 weeks in vivo (rat), but a tissue encapsulation effect of the implant decreased the rate near the end of the observation period.

Other low molecular weight drug release from P(D,L)LA films[98], membranes[99,100] and microcapsules[101] has been discussed by Spilizewski et al[98], Benita et al[99], Nakano et al[100] and Suzuki and Price[101].

Recent work on the evaluation of poly L(-) lactide for bone fixation devices has been published by Tunc et al.[102,103]

There has been no reported work on macromolecular release from any of the PLA forms. It has been suggested[33] that the copolymers of PLA with PGA discussed in the following section are more suitable for this type of release.

1.3.2.3 Copolymers of PGA with PLA

It is here that the confusion in the literature referring to definitions of the various forms of PLA homopolymers and copolymers is at its greatest. Langer[9] correctly defines the cyclic dimers of lactic acid, the 'lactides', as being the L(-) and D(+) forms, and the polymers of lactic acid as being poly (L+) lactic acid, poly (D-) lactic acid and the racemate poly (D,L) lactic acid. Rosensaft[104] however describes his poly lactide as being poly L(-) lactide i.e. using the dimeric form. On the other hand Kiebner and Simpson[105] together with Ludwig and Boisvenue[106] do not define which form of PLA is employed in their work. The confusion in the literature extends as far as Beck and Tice's[56] discussion of

the work of Miller and co-workers[88], whose experiments were based on P(L)LA which is mistakenly taken by Beck and Tice take as being P(D,L)LA. These points emphasise the need for correct use of nomenclature and careful definition of the forms of lactic acid, lactide and poly lactic acid used in experiments.

The copolymers of lactic acid (LA) and glycolic acid (GA) have been extensively studied over their complete composition ranges. The two major copolymer series are those of (L) LA/GA and (D,L) LA/GA.

Gilding[80] reports that most of the copolymer composition range is taken up by amorphous polymers. For the (L) LA/GA system 25 to 75% GA compositions are amorphous, whilst for the (D,L) LA/GA series compositions of 0 to 70% GA copolymers are amorphous.

Most of the literature reported has been for the (L) LA/GA system. The whole compositional range of (0 to 100%) (L) LA has been studied by Miller et al[88] and Gilding et al.[80,86] Copolymer ratios rich in P(L)LA or PGA are very much more stable to hydrolytic attack than the intermediate compositions. Elegant photographic evidence of this is provided by Vert et al.[90] Gilding[80] on considering water uptake by the copolymer series, predicted that as the 70% GA copolymer was the most hydrated, it would be the most likely to degrade preferentially. Indeed <u>in vitro</u> tests showed total weight loss after four weeks. Alternatively Miller[88] considered the equimolar copolymer as being the most unstable, since it had an <u>in vivo</u> (rat) lifetime of about five weeks (5-6% of original mass

remaining after four weeks compared with Gilding's work which reported 30% remaining (*in vitro*) after the same time period). In both cases the major weight loss occurred over the two to four week period. Notwithstanding differences in *in vivo* absorption data and techniques employed in their evaluation, a striking difference in absorption rates does occur between either of the PGA/PLA homopolymers and the copolymers containing 25% or more of either component.[88,90] The figures of Miller[88], which can be taken as broadly representative, indicate a ten fold decrease in the absorption half life on moving from the homopolymers to the copolymers.

Preferable alternatives to surgical sutures based on P(L)LA, first described two decades ago[107] are made from the 92 molar % GA copolymer with LA known as Vicryl®or Polyglactin 910®(Ethicon Inc.). Fredericks and co-workers[108] have found that *in vitro* the degradation of Vicryl is, as one would predict, very similar to PGA, with two stages of degradation; the first involving hydrolytic degradation of the amorphous regions over the first 28 days, after which the crystalline domains are degraded. The degradation is accompanied by an increase in crystallinity which has a maximum value between the two stages. As with PGA, degradation is most marked in alkaline environments[109], with complete loss of TS at the beginning of the latter stage at which time considerable weight loss begins. Fredericks and co-workers[108] note that there was no preferential attack on either the lactide or

glycolide units of the polymer backbone despite their difference in hydrophilicity.

The copolymer compositions of the (D,L) LA/GA system have been studied by Pitt et al,[96,110] Hutchinson,[33] Beck and Tice,[56] Gresser and Sanderson[111] and to a lesser extent by Reed.[86] *in vivo* work (in rabbit) by Pitt et al over a sixteen week period showed that for a glycolide content of approximately 19% a reasonably high molecular weight was needed (corresponding to an intrinsic viscosity >1.36 dl/g), to avoid complete degradation. Hutchinson considered the degradation process as homogeneous erosion of the polymer matrix. Beck and Tice report that complete *in vivo* (rat) degradation of copolymers from 0 to 26% GA varies from approximately 20 weeks for the 26% GA, to very much greater than 45 weeks for the parent P(D,L)LA.

Drug release from (L) and (DL) LA/GA copolymers has been studied by Wise et al,[112-116] but this was restricted to relatively low molecular weight drugs such as steroids. (see also Langer[9] and Heller[50])

Controlled release of antibiotics, growth factors and endoparasitic protection formulations from materials desribed as 'lactide'/GA copolymers have been reported by workers[105,106,117] at Eli Lilly. They describe a copolymer composition of 80% lactic acid possessing a weight average molecular weight ($\bar{M}\bar{w}$) of approximately 30,000. Release of a growth factor (50% w/w) in this particular copolymer from a large steel bolus at a rate of 170mg

per day into ruminants was demonstrated for three months. Release of Fenbendazole (50% w/w) from a similar copolymer of $\bar{M}w$ 25,000 was shown over a thirty day period. However, the rates of release varied considerably around the average value.

In vitro studies of progesterone release from (D,L) LA/GA copolymer films have been made by Pitt et al;[96,110] Diffusion controlled kinetics (release rate $\propto \sqrt{\text{time}}$ i.e. $t^{1/2}$) were found for the higher glycolide copolymers studied; (19 and 21% w/w) over a period of twenty and thirty days respectively. A secondary release phase, very much greater in magnitude than the first was then observed, which also showed $t^{1/2}$ dependence. However total TS loss occurred at this time, together with fragmentation of the film, and so the authors considered this secondary phase to have a purely coincidental $t^{1/2}$ correlation. The 21% GA copolymer also exhibited this $t^{1/2}$ dependence for some time after thirty days. Copolymers with low levels of glycolide show very little release over fifty days and can be approximated to the P(D,L)LA homopolymer. Beck and Tice and co-workers[56,77,118-122] have shown that steroid release from microspheres of (D,L) LA/GA copolymers is in two stages, with release depending on diffusion and biodegradation.

In vivo (rat) release studies of a decapeptide hormone analogue from a wide range of (D,L) LA co GA copolymers has been described by Sanders et al.[123-124]

Macromolecular controlled release studies have been reported by Hutchinson.[33,125] He investigated *in vitro* and *in vivo* (rat) release of polypeptides with a molecular weight range of 633 to 22,000 Daltons from (D,L) LA/GA copolymers containing from 25 to 100% P(D,L)LA. The 'lactides' had differing mean molecular weights, MWD's and matrix thicknesses. He found that release of active ingredient was biphasic with an initial surface release (or for high molecular weight polymers no initial release) followed by a period of very little or zero release termed by the author as the 'lag period' and then continuous release until exhaustion. He considered the release as matrix diffusion proceeded by an erosion mechanism, with diffusion of active ingredient through aqueous channels formed by matrix erosion, and noted that matrix diffusion was at a minimum for high molecular weight polymer / high molecular weight polypeptide formulations.

The 'time lag' period was reduced or eliminated by increasing the glycolide content; widening the MWD by controlling the polymerisation; or by blending two or more 'lactide' copolymers of differing molecular weights and copolymer compositions. The properties of a wide MWD are such that the low molecular weight portion provides immediate release of polypeptide whilst the high molecular weight fraction gives an extended release period and slows down the overall rate of release. Blending high and low molecular weight components alters the water uptake characteristic of the copolymer in the same way as having a wide MWD because the

author found that water uptake was proportional to the amount of polypeptide released.

The rate of polypeptide release could be increased by increasing the D,L PLA content, decreasing the molecular weight or decreasing the thickness of the test implant.

Hutchinson found that the most suitable copolymers for release of polypeptides with a range of molecular weights were novel low molecular weight polymers, prepared from the cyclic dimers of LA and GA (namely lactide and glycolide) in the presence of transfer agents such as lactic acid to give the low molecular weight / wide MWD characteristics that are required. The polymers formed are heterogeneous and as the GA blocks erode preferentially the LA/GA ratio increases with degradation time.

Later work[125] on a polypeptide releasing system (Zoladex from P(D,L)LA co glycolide) exploited this diffusion / erosion mechanism of release. The time lag mechanism described by Hutchinson can possibly be applied to the work of Pitt et al[96] discussed above, if the initial $t^{1/2}$ release is compared with surface diffusion and the secondary $t^{1/2}$ relationship is correlated with the matrix erosion / diffusion mechanism.

Sanders et al[124] found that a similar time lag mechanism applied to a system of (D,L)LA / GA copolymer microspheres containing a low molecular weight pro-fertility agent. They found a three phase release pattern consisting of an initial diffusional release from the outer layers of the microspheres followed by a

period of low release until erosion of the polymer system leads to an increase in release of the entrapped active.

From the work of Hutchinson, Pitt et al, Beck and Tice, and Sanders; it can be concluded that copolymers of D,L LA/GA over the composition ranges 25 to 75% are good candidates for macromolecular release, even though zero order release rates may prove difficult to achieve due to the homogeneous erosion / diffusional nature of the polymer degradation.

1.3.2.4 Poly Dioxanones and Poly Oxalates

These series of polymers can be prepared from dioxane-diones and oxalates, some of which are lactide / glycolide hybrids. They will be considered in four sections (i) The unsymmetrically substituted poly-1,4-dioxane-2,5-diones,[126,127] (ii) Copolyoxalates,[44,128] (iii) Poly 1,3-dioxane-2-one and poly 1,4-dioxane-2,3-dione[104,129,130] and (iv) Poly para-dioxanone and relatives.[17,55,131] Figure 1.2 illustrates their preparations and structures.

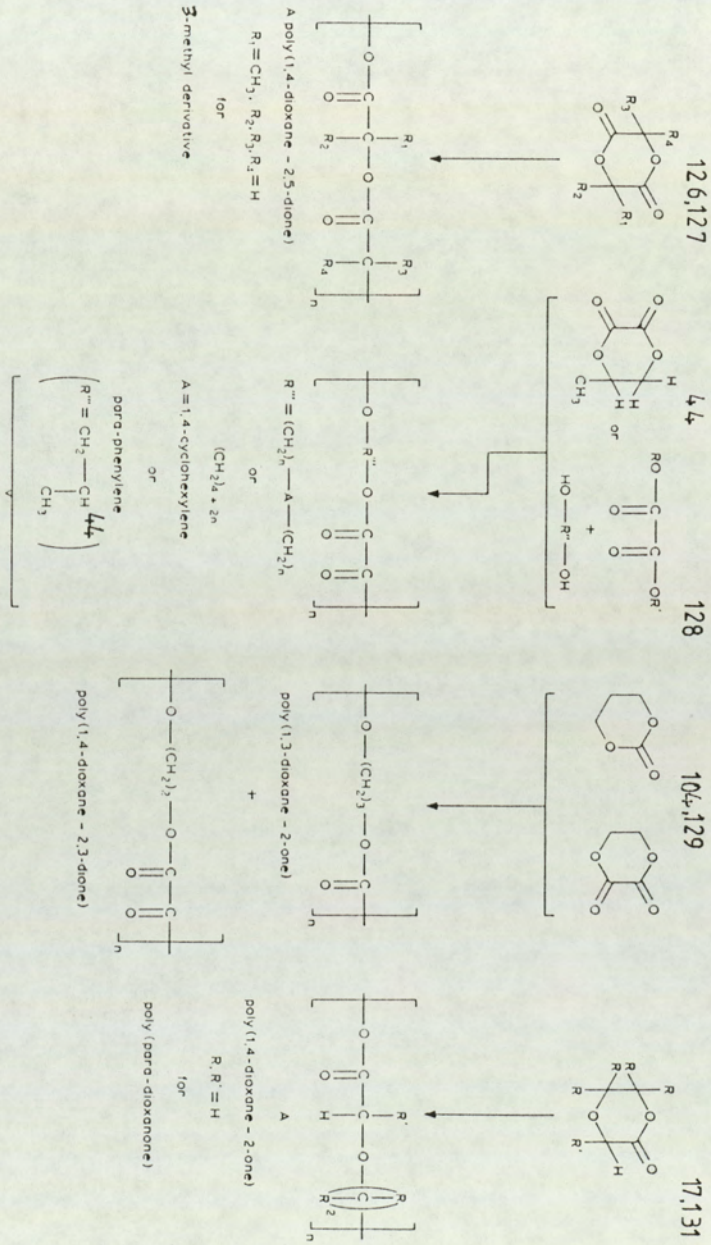
(i) The unsymmetrically substituted 1,4-dioxane-2,5-diones have been prepared by Augurt and co-workers.[126,127] The 3-methyl member of the series is a hybrid of half a lactide and half a glycolide molecule fused together. The 'dimer' is then polymerised to give a polymer with a very similar GA,LA,GA,LA.... sequence. The authors claim it is suitable for use as an absorbable surgical suture or as a bone pin. It is suggested that copolymers of this

family with lactide or glycolide produce a family of copolymers with varying LA/GA compositions. The high GA containing copolymers could find use in controlled drug delivery systems.

(ii) Copolyoxalate polymers have been fabricated by Shalaby and Jamiolkowski[128] and a polyoxalate suture made by Ethicon has been discussed by Gilding.[44] The Ethicon suture undergoes ester hydrolysis to give oxalic acid and propylene glycol as by-products. The author states that the hydrophobicity is between that of PGA and PLA, and as it is less crystalline than PGA, he predicts absorption of a monofilament suture would be quicker than PGA sutures (Dexon). The copolyoxalate polymers listed by Shalaby and Jamiolkowski are prepared by ester interchange reactions between a mixture of diols and an ester of oxalic acid, preferably diethyl oxalate.

The poly (trans 1,4-cyclohexylenedicarbinyl-co-hexamethylene oxalate) copolymer series[128] show an increasingly fast <u>in vivo</u> (rat) weight loss as the 1,4-cyclohexylenedicarbinyl content decreases, except for the 30% 1,4-cyclohexylenedicarbinyl copolymer which regains some <u>in vivo</u> lifetime above the trend. The poly (1,4-phenylenedicarbinyl-co-hexamethylene oxalate) copolymers on the other hand show a decrease in <u>in vivo</u> (rat) weight loss as the 1,4-phenylenedicarbinyl content decreases. The authors suggest that these copolymers could be used for controlled release of steroids and narcotic antagonists (but there is no indication of any actual release work). The 1,4-cyclohexyleneoxalate series show

Figure 1.2 Various poly dioxanones and poly oxalates



'slight' and 'mild' tissue reactions during the first five days of *in vivo* (rat) implantation. Their suitability as polymeric drug delivery matrices has to be questioned however with long term *in vivo* toxicity studies being needed.

(iii) Rosensaft and Webb[104,129] have prepared triblock polymer structures by copolymerising L(-) LA (= L(+) LA ?) or 1,3-dioxane-2-one (trimethylene carbonate (TMC)) with GA to form TMC/GA/TMC (or LA/GA/LA) structures, with polymer chains rich in TMC for monomer compositions of less than 15 mole percent TMC, or greater than 85% GA. Higher TMC ratios up to 57.5 mole percent can be made. These higher TMC/GA copolymers showed a decrease in *in vivo* (rat) TS as the TMC ratio was increased from 48.5 to 57.4% and it is reported that TMC/GA copolymers have a greater *in vivo* (rat) strength than PGA when in suture form. Poly 1,4-dioxane-2,3-dione can be used instead of TMC.

Rosensaft and Webb claim[104,129] that the major use of these dioxanones is for making absorbable surgical sutures, and Katz et al[130] describe a new commercial suture material Maxon®. It is a copolymer of TMC and glycolide (32.5% TMC), and is reported to have better handling properties than the equivalent PGA suture Dexon. However, there is no mention of use as a polymeric matrix for drug release.

It seems that TMC or 1,4-dioxane-2,3-dione homopolymers or copolymers with LA/GA should be suitable for bioerodible drug

release, again providing they show no long term toxicological reactions.

(iv) Poly para-dioxanone is primarily used as an absorbable suture material PDS[55] (manufactured by Ethicon Inc.), because it has superior TS properties to PGA, and it has the ability to form monofilaments. Doddi et al[17] describe the polymerisation of para-dioxanone as well as the methyl and dimethyl homologues. *In vivo* (rat) degradation work on PDS sutures[55,131] shows a slow linear cross-sectional area profile loss for five months followed by a complete loss during the sixth month. ^{14}C studies on weight loss show that it is slow for the first twelve weeks, with major loss occurring between 12 to 18 weeks and complete degradation after 26 weeks. Since there is correlation between *in vitro* and *in vivo* degradation, this suggests that the degradation mechanism involves non-enzymatic hydrolysis of the ester bonds, i.e. homogeneous erosion. Doddi[17] reports that PDS suture material has a crystallinity of 37%, thus the degradation mechanism could be similar to PGA with the amorphous regions preferentially eroded. However, ^{14}C studies[55] have shown that *in vivo* degradation products, unlike those of PGA and PLA, are removed in the urine (93%). This may suggest a different enzymatic degradation of oligomer fragments from that occurring with PGA. When in the form of a ligating clip total absorption of PDS is extended to greater than 180 days and Schaefer[132] reports that the main degradation product is 2-hydroxyacetic acid.

It seems that PDS in suture form is too crystalline to be considered for drug release, but as suggested by Doddi[17] the absorption rate of PDS can be increased by forming copolymers with GA over the 5 to 25% GA composition range, and these polymers are claimed[131] (without experimental backup) to be suitable for drug release.

Controlled release of the anthelmintics Mebendazole and Levamisole for up to seven weeks duration, from an undisclosed form of PDS has been reported by Guerrero et al[133] There have been no reports of macromolecular drug release, but the most suitable form of PDS would appear to be the 25% GA copolymer with a wide MWD.

Most work on dioxanones and oxalates has been done with a view to making absorbable surgical sutures. However as dicussed, the copolyoxalate homopolymers have been recommended for drug release, and this family, and copolymers of the dioxanone series with hydrophilic bioerodible polymers such as P(D,L)LA or possibly PGA, could provide suitable matrices for macromolecular drug release. However further work is needed to evaluate detailed biodegradation biocompatabilities of these polymers.

1.3.2.5 Poly Caprolactone and other Lactones

Pitt, Schindler and co-workers[92,96,134] have shown that poly caprolactone (PCL) degrades similarly to P(D,L)LA, with random chain scission by ester hydrolysis, with the process being

autocatalysed by the generation of carboxylic acid end groups. The rate of degradation however is almost three times slower. The critical $\bar{M}n$ at which linear molecular weight loss with time is disrupted with an accompaning loss of TS, is about 5,000. Subsequent studies by Woodward et al[68] support the proposition that intracellular degradation of PCL is the principal in vivo degradation pathway once the molecular weight ($\bar{M}n$) of the polymer falls to 3,000, with a microbial degradation (P.Pullulans) study by Fields et al[135] showing the necessity of low polymer molecular weight for enhanced degradation.

PCL behaves similarly to PGA in that residual crystallinity increases with time. Scanning electron microscopy (SEM) work by Jarrett et al[75] shows that the amorphous regions of the polymer are eroded first; hence bulk erosion is predominant.

Release of steroids and other relatively low molecular weight molecules using a yeast and a fungus (see above in the enzymatic erosion section) occurs essentially by diffusion. The progress of a commercial PCL system for contraceptive control - Capronor, developed by Pitt and Schindler[136] is discussed by Gabelnick.[137] Once again diffusion was the main release mechanism.

A disadvantage of PCL for in vivo work is that from an initial $\bar{M}n$ of 50,000, three years is required for total removal from the body and only two years of this could be used for controlled drug release,[137] since the $\bar{M}n$ is less than 5,000 after

100 weeks. No studies of macromolecular drug release from a PCL matrix have been reported.

Copolymers of PCL with other lactones and with PGA and PLA degrade far more quickly than the parent homopolymer. PCL-co-decalactone[105] copolymers with 8 and 13% of decalactone erode faster than PCL, as do PCL-co-P(D,L)LA or PGA. PCL-co-valerolactone copolymers with high $\bar{M}\bar{w}$'s (~150,000) exhibited different behaviours depending upon the copolymer composition.[92] The 9% valerolactone copolymer was similar to PCL, but the 23% valerolactone copolymer had less crystallinity, a lower melting point than PCL and a Tg less than body temperature and therefore degraded quickly. An order of degradability is given by Pitt et al[92] with respect to loss of intrinsic viscosity, the order being: PCL/P(D,L)LA (11 and 27%) > PCL/ Valerolactone (23%) > P(D,L)LA > PCL/ Valerolactone (9%) ~ PCL/ Decalactone (8%) > PCL. However, steroid release from PCL (60%)-co-P(D,L)LA and PCL (90%)-co-PGA is approximately the same as from PCL homopolymer[96] with a report[138] of continuous release for 80 days after an initial 'burst' period from PCL-co-P(D,L)LA.

Blends of PCL with D,L PLA prepared by Gupta from 20 to 80% P(D,L)LA have been mentioned by Thies,[139] and <u>in vivo</u> (unspecified species) absorption of fibres of PCL-co-glycolide have been described by Shalaby et al,[140] with drug loaded P(L)LA and P(D,L)LA--co-PCL fibre systems elaborated on by Dunn et al.[141]

Elastomeric copolylactones of ε caprolactone and valerolactone, cross-linked with Bis caprolactone, have been described by Schindler and Pitt.[69,70] They degrade by classical random hydrolytic chain scission in vitro and additionally in vivo (rabbit and rat) with enzymatic surface erosion.[142] Varying the cross-link density alters the degradation rate in an inverse fashion. The suitability of these polymers for continuous release of 'medical agents' has been claimed.[70]

The diffusive nature of PCL drug release, and its long in vivo lifetime, would seem to suggest that it is not a suitable macromolecular release agent. However sustained release of steroids from PCL-co-P(D,L)LA or PGA has been demonstrated. This suggests that macromolecular release is possible, but zero order release improbable.

1.3.2.6 Poly D(-) 3-hydroxybutyric Acid (PHB)

This novel polymer is obtained from strains of bacteria. Gilding[143] reports extraction from Azobacter Beijeinckii and Korsatko[144] claims that PHB can be readily manufactured, with various molecular weight fractions being obtainable depending on the extraction method. Other bacteria - Bacillus Megatenium, Rhodosririllum Rubrum and Bacillus Cereus can also produce PHB.[145] Grassie and Murray[146] describe how low molecular weight PHB may be prepared synthetically. ICI manufacture PHB from Azobacter Beijerwekii cultures and copolymers of PHB with poly 3-hydroxy

valerate (PHV) from 0 to 30% PHV are available as the 'Biopol' range.

Work by Gilding[44] on a PHB with a molecular weight greater than $2x10^6$ showed that thermal processing caused a decrease in molecular weight and *in vitro* hydrolysis showed no apparent degradation after six months. However early work by Kronenthal[147] showed an onset of degradation, *in vivo* (in an unspecified species), after eight weeks. The thermal degradative properties have been extensively reported by Grassie and Murray.[146,148,149]

Stinson and Merrick[150] report *in vitro* degradation of PHB by Pseudomonas Lemoignei, and Korsatko[57,144,151] found that the *in-vitro* release of 7-Hydroxyethyltheophylline (HET) was three times faster than that *in vivo* (mice). He concluded that the *in vivo* rate was due to enzyme degradation. In addition, Korsatko found that HET decreases hydrolase activity, the enzyme causing non-specific degradation, because of its ability to inhibit Phosphodiesterase. However *in vitro* results showed that the polymer can be degraded non-enzymatically, probably by hydrolysis. The authors claim that *in vitro* hydrodynamics may also have affected release and since the hydrodynamic boundary and less than perfect sink conditions exist to a greater extent *in vivo*, this may explain the poor *in vitro*/*in vivo* correlation.

Thus, the degradation mechanism of PHB seems to be complex with the literature[152] giving broad indication only about the extent and nature of enzymatic involvement.

The release of HET from PHB in the form of a tablet matrix has been reported to be diffusion controlled,[144] the release rate depending on the drug loading. Release from 60 to 80% drug loadings required 8 to 24 hours; from 40 to 60% loading 1 to 8 days and 5 to 30% loadings extended the release up to 50 days. The authors found that there was no contribution to release from polymer degradation with the release rate depending on the surface area of the tablet. Later studies[57] with 10% HET loadings showed that surface release occured for two weeks followed by twenty weeks of linear release. These studies were carried out with PHB of a relatively high molecular weight (260,000 Daltons) and Korsatko suggests that, as is the case for PGA and PLA, the release of drugs from PHB would be expected to be a function of its molecular weight. In vivo[151] (mouse) compatibility tests carried out with the PHB tablets showed satisfactory results.

Release work on microspheres of P(D,L)LA and PHB containing a low molecular weight drug has been reported by Bissery and co-workers.[153,154]

PHB and particularly its copolymer series with PHV, have reasonably good melt stability and can therefore be easily injection moulded (preferably with added inert nucleating agents such as talc and calcium carbonate). They should therefore be suitable for exploitation as devices for controlled release of bioactive molecules from melt pressed films or compressed matrices.[155]

1.3.2.7 Polyester Hydrogels

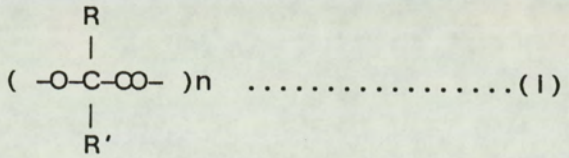
Heller[12] and Baker[32] have demonstrated macromolecular release from polyester hydrogels. These hydrogels are synthesised from linear pre-polymers of polyesters containing pendant or backbone unsaturation. The polymers with pendant unsaturation are cross-linked by free radical coupling of the unsaturation, and the backbone unsaturated polymers are cross-linked with N-vinylpyrollidone or acrylamide. Polyethylene glycol, along with its copolymers with fumaric acid, are the poly'esters' discussed.

Release of macromolecules e.g. bovine serum albumin occurs via bulk (homogeneous) erosion of the hydrogel matrix. The release rate depends on the cross-link density and double bond reactivity of the cross-linking agent. The authors report that as the hydrogel / release agent matrix is prepared under aqueous, neutral pH conditions at room temperature, it should be possible to incorporate thermally labile and pH sensitive macromolecules.

Another swellable hydrogel system consisting of a hydrophilic (e.g. poly vinyl alcohol) and a hydrophobic part (e.g. PLA) has been proposed by Churchill and Hutchinson,[156] but no experimental substantiation of the proposals has been given. However release of bovine growth hormone from a terpolymer of polyethylene glycol / P(D,L)LA / PGA was reported.

1.3.2.8 Other Ester containing Polymers

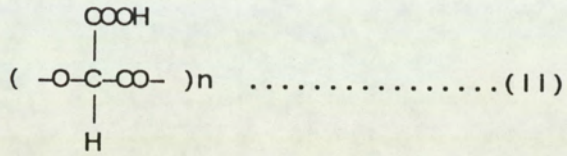
Poly alpha esters other than PGA and PLA can be prepared via the anhydrosulphite derivative of the appropriate alpha-hydroxy carboxylic acid. Suggestions that molecular weights of polymers produced by this route[110] are very low, over-simplify the problem since the molecular weights are dependent both on initiation technique and the particular anhydrosulphite involved. An outline of these ring opening processes is given, together with an overview of the thermal stability of the polymers in a recent review.[157] A consideration of the application of poly alpha esters to bone fixation and related uses has been reported by Vert et al.[90] Whilst it is true that the poly alpha ester series has not yet been explored for drug delivery, *in vitro* degradation work[158] has shown that, as expected, hydrolysis rates are affected by different substituents (R and R') in the poly alpha ester (I).



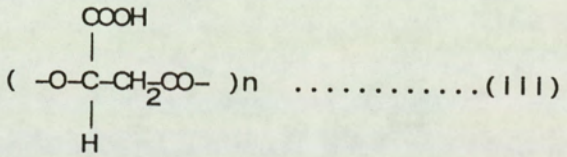
.....(I)

Increasing the size of R and R' decreases the hydrolysis rate and halogenation of pendant groups also affected the rate of hydrolysis.

A suitable poly alpha ester for drug release may be the hydrophilically substituted poly tartronic acid[159] (II), derived from the anhydrosulphite of the parent acid.



A close relative of tartronic acid is poly beta malonic acid (III).



It has been used for pendant chain drug delivery,[160,161] with a possibility of release by erosion, or by a combination of these two mechanisms. Vert et al[162] suggest that poly malonic acids with molecular weights greater than 25,000 Daltons could be used for macromolecular release since the polymer chain degrades slowly in neutral pH conditions.

Possible polyesters from acids in the Krebs cycle have been discussed by Wise,[163] and Bitritto et al[164] describe the microbial biodegradation of other hydroxy acid based polymers.

1.3.2.9 Surface Eroding Polymers

Two classes of polymers which undergo surface (heterogeneous) erosion are polyanhydrides[9,14,28,165-168] and poly ortho esters.[9,11,137,169-176]

Polyanhydrides are suitable for drug release, having very good biocompatabilities.[166]

Poly ortho esters can be subdivided into the commercially developed Chronomer (Alzamer) series,[137,169] and those that have been synthesised at SRI International by Heller et al.[172-176]

The poly ortho esters prepared in the Alzamer series for steroid release discussed by Gabelnick[137] show some *in vivo* irritation (rat, dog, monkey and human volunteers) and since they are stable in alkaline conditions, incorporation of a basic salt is needed in order that erosion may become a surface limited event.[11] However this produces a swelling type system with diffusion being the main release mechanism and the system exhibiting non-zero order release.

The SRI poly ortho esters on the other hand can use basic or acidic salts - with incorporation of small amounts of anhydrides into poly ortho esters being reported by Shih et al.[170] This overcomes the problem of basic salt incorporation, producing matrices which heterogeneously erode at variable erosion rates depending on the pKa of the parent acid of the anhydride used.

Partially esterified copolymers of methyl vinyl ether with maleic anhydride[11] also undergo surface erosion but their

dissolution and hence release characteristics are very pH dependant.

1.3.2.10 Related Polymeric Controlled Release Systems

An emerging application for polysaccharides is in the controlled release field, with several recent papers[177-180] discussing their potential. Schroder et al[180] propose a possible macromolecular release system.

There are obvious similarities between esters and amides; and other potential polymer systems for drug release are polyamides;[9,47,181] polyacrylamide;[11] poly amino acids;[182-184] poly peptides[185-187] and polyester-co-urethanes.[188]

1.4 FACTORS AFFECTING SELECTION OF EXPERIMENTAL SYSTEMS

For the *in vitro* study of macromolecular controlled release and the present study in particular, many factors have to be considered. After a careful consideration of the literature suitable polymer / macromolecular drug systems were chosen. The rationale behind the choice of polymers and macromolecular markers is given in the next two sections.

1.4.1 CHOICE OF POLYMER

As discussed in section 1.3, the general requirement for a biodegradable polymer is a hydrolysable constituent in the polymer

backbone or side chain. From the previous section it can be seen that controlled release of macromolecules from polymer matrices can be achieved by bioerosion. Indeed, for heterogeneously eroded polymers zero-order release rates have been obtained using polyanhydrides,[166,167] poly (ortho esters)[169-176] and partially esterified copolymers of methyl vinyl ether and maleic anhydride.[11]

Also apparent is the fact that work in this field is still in its infancy. Despite this, the aliphatic polyesters, which decompose to simple metabolites usually found in the body, have been demonstrated to act as effective matrices for a range of high molecular weight species.[33,125] The advantages and disadvantages of the various commercial bioabsorbable suture materials and other biodegradable polymers are summarised in Table 1.2.

Many polyesters were considered in my review of the literature. The lowest member of the series PGA is only readily available as a surgical suture which is highly crystalline and would hinder the incorporation of any potential drugs into a PGA matrix. The next polymer in this series is PLA and although this is available in a more amorphous form than PGA its cost would be limiting for this research work. The PHB/PHV 'Biopol' range of copolymers[152] on the other hand are available in bulk over a wide range of molecular weights and copolymer ratios. As the free acid is a normal constituent of human blood, degradation would be expected to produce non-toxic metabolites. An additional advantage of this copolymer range is that it was untried in its application

TABLE 1.2 Potential bioerodible polymers for controlled release matrices: Summary of advantages and disadvantages of currently available systems

POLYMER	ADVANTAGES	DISADVANTAGES
POLY CAPROLACTONE	Readily soluble in organic solvents at room temperature, copolymerises with other lactones and alpha esters.	Slow degradation (for homopolymer), low Tm, diffusional release of low molecular weight compounds.
POLY GLYCOLIC ACID	Degrades relatively quickly, copolymerises with PLA, inexpensive.	Too crystalline in suture form (only commercially available as a suture), difficult to solvate.
POLY LACTIC ACID	Degrades more quickly than PCL, copolymerises with PGA, PLA and other lactones. Proven use in controlled release field.	More hydrophobic than PGA, diffusional release of low molecular weight compounds Very expensive ($5 / g).

TABLE 1.2 (Cont)

POLYMER	ADVANTAGES	DISADVANTAGES
POLY HYDROXY BUTYRATE-co--HYDROXY VALERATE	Wide range of copolymers and molecular weights available. Novel w.r.t. biomedical applications.	Expensive (at the present time), only soluble in halogenated solvents. In vivo degradation poorly characterised. Slow in vitro hydrolysis.
POLY DIOXANONE	Relatively fast degradation rate, 'medium' crystallinity	Expensive (only available in suture form), specialised solvents needed
POLY TARTRONIC ACID	Novel polyester	High molecular weight polymers difficult to prepare and solvate.
POLY SACCHARIDE BLENDS	Inexpensive, easy variation of blend composition.	Poor blend homogeneity

to biomedical devices. This copolymer series was therefore chosen as the 'base' for fabrication of the matrices.

The polylactone series - especially poly caprolactone has been shown to release low molecular weight species[136,137] and some additional comparative release work was carried out using this polymer.

In addition the *in vitro* degradation of the commercial biodegradable suture materials Dexon (PGA), Vicryl (PGA co PLA) and PDS (polydioxanone) was evaluated for useful comparisons with literature and PHB-PHV degradation results.

1.4.2 CHOICE OF MACROMOLECULAR MARKER

In order to study the release of macromolecules from a bioerodible polymer a suitable 'drug' had to be found. Ideally a range of drugs with varying molecular weights would be used. A convenient method of assaying the 'drug' is by spectrophotometric determination and dyes with strong chromophores are suitable - having the advantage that they are generally less toxic than active drugs and are therefore easier to handle and process. They are also less expensive than active drugs but, as will be discussed in a subsequent chapter, many dyes are water soluble which causes matrix incorporation problems. Table 1.3 lists the advantages and disadvantages of some potential markers. A suitable range of macromolecular dyes are the O-fluoresceinylthiocarbamoyl

TABLE 1.3 Potential marker dyes for release studies

DYE	MOLECULAR WEIGHT /Daltons	ADVANTAGES	DISADVANTAGES
FLUORESCEIN	332	Reasonable water and organic solvent solubility, high extinction coeffic--ient, inexpensive.	Absorbs onto glass vessel walls.
BOVINE SERUM ALBUMIN	66,000	Good spectro--photometric assay, inexpensive.	Very poor organic solvent stability, thermally unstable, degraded at non neutral pH's.
POLY VINYL PYRROLIDONE	10,000 to 360,000	Water and organic solvent soluble	Poor assays available.
FITC DEXTRAN	4,000 to 150,000	Wide range available, reasonable assay.	Expensive, poor solvent stability, thermally unstable

-dextrans[189](FITC-dextrans) which are available with molecular weights from 4,000 to 150,000 and these and fluorescein itself (molecular weight 332) were used as high and low molecular weight markers respectively.

1.5 SCOPE OF WORK

The most commonly used biodegradable polymers are the absorbable surgical sutures based on simple poly alpha esters, or the lower members of the polylactone series. Applications for biodegradable / bioerodible polymers are extensive ranging from the relatively simple absorbable sutures to complex polymer / drug matrices for controlled release applications. In addition, drug delivery systems themselves cover a large area depending on the local environmental release conditions. However as has already been discussed there are relatively few biodegradable polymers available for possible controlled release applications testing, and most of these derive their suitability from their *in-vivo* presence as simple organic acids.

To add to this rather limited list of 'biopolymers' the potentially useful polyhydroxybutyrate-co-hydroxyvalerate series of polymers were considered for possible controlled release applications. At the onset of this work very little information was available on this copolymer range and it was presumed at this point that PHB/PHV polymers would behave in a similar way to their

polyester relatives PGA and PLA; with the literature giving a broad indication only of the expected behaviour of the PHB homopolymer alone.

The approach to the experimental work was two fold with primary studies assessing the *in vitro* breakdown of members of the PHB-PHV copolymer range, with comparative studies on other bioabsorbable polymers. The initial aim was to follow polymer hydrolysis by weight loss and water uptake of the various physical forms of the polymer samples with parallel studies of the degradation using other techniques as they became available.

Early reports of the mechanism of polymer biodegradation and bioerosion led to the simplified view of the removal of the outer layer of the polymer matrix by hydrolysis exposing a fresh inner surface i.e similar to the peeling of an onion, with no information on the molecular aspects of the degradation. It was hoped that a deeper understanding of the degradative pathway of PHB/PHV hydrolysis would aid the evaluation of subsequent work on release of a series of dyes (of varying molecular weight) from polymer films and tablets. It was also hoped that the interestingly novel and complex situation of modelling release of a polymeric material from a polymer could be addressed.

CHAPTER TWO

EXPERIMENTAL

2.1 MATERIALS

In the following subsections the various reagents and materials used in the experimental work are listed, with details of source and batch number (where applicable).

2.1.1 POLYMERS

For the structural formulae see Table 1.1 The surgical sutures and polycaprolactone were used without further purification.

POLY CAPROLACTONE (PCL): Obtained in pellet form, supplied by Aldrich Chemical Co. Inc. Polystyrene equivalent weight average molecular weight = 54,500, number average = 20,500.

POLYDIOXANONE (PDS): Obtained as a monofilament surgical suture,('PDS'), metric grade 2. Supplied by Ethicon Ltd, Edinburgh.

POLYGLYCOLIC ACID (PGA): Obtained as a multifilament surgical suture, ('Dexon'), metric grade 5. Supplied by Davis and Geck (Cyanamid of Great Britain Ltd) Gosport, UK.

POLYGLYCOLIC-co-LACTIC ACID (PGA/PLA): Obtained as a multifilament surgical suture, ('Vicryl'), metric grade 5. Supplied by ~~Davis and Geck~~ ETHICON LTD,

POLYHYDROXYBUTYRATE-co-HYDROXYVALERATE (PHB/PHV): Obtained in powder form as the 'Biopol' series of copolymers from Marlborogh Biopolymers Ltd, Stockton on Tees, Cleveland, UK. The grades used with their weight average molecular weights ($\bar{M}\bar{w}$) are summarised in Table 2.1.

The various PHB/PHV grades are abbreviated in this text as a shortened expression of polymer molecular weight e.g 350PHB (12%PHV) = polyhydroxybutyrate-co-valerate $\bar{M}\bar{w} = 3.5 \times 10^5$, valerate content = 12%. Similarly 36PHB (20% PHV) = polyhydroxybutyrate-co-valerate $\bar{M}\bar{w} = 3.6 \times 10^4$, valerate content = 20%. The polymers were used without further purification for cold compressed tablet and melt pressed disc work. For solvent casting, polymer solutions in chloroform (typically 15% w/v) were filtered through a number 4 pyrex glass sinter.

Table 2.1 Polyhydroxybutyrate-co-Valerate Polymer Grades used

MOLECULAR WEIGHT /10^3	VALERATE CONTENT/%	BATCH NUMBER
36	20	P/V/1 Bx T/9/4-2
100	12	P/V/2 Bx T/9/05/037-1
170	12	P/V/2 Bx T/9/05/037-3
300	20	Bx P/V/1-B
350	12	Bx P/V/2 (T/9/0.5-27)
400	7	Bx P/V/7 (EE)
750	10	P 7/46
800	0	Bx G/V/9 (EE)

2.1.2 MARKER DYES

FLUORESCEIN: Technical grade, supplied by BDH Chemicals Ltd. Poole, UK. Used without further purification (Structural formulae Fig 2.1a).

FITC-DEXTRAN DYE SERIES: Supplied by Sigma Chemical company Ltd. Poole UK, (Structural formulae Fig 2.1b). Table 2.2 summarises the various molecular weights used. All dyes were used as obtained.

Table 2.2 FITC DEXTRAN dyes used

GRADE	MOLECULAR WEIGHT	Moles of FITC label per mole of glucose residue
FITC 4	4057	0.004
FITC 20S	17500	0.004
FITC 40	41000	0.003
FITC 70	71600	0.004
FITC 150	156000	0.004

2.1.3 BUFFER SALTS

Three aqueous buffers were used to provide acidic, physiological and alkaline degradation conditions. These were based on citric acid, potassium phosphate and sodium carbonate respectively. All salts were used as supplied.

CITRIC ACID: Two sources a) Standard laboratory reagent grade obtained from Fisons Scientific Apparatus, Loughborough.

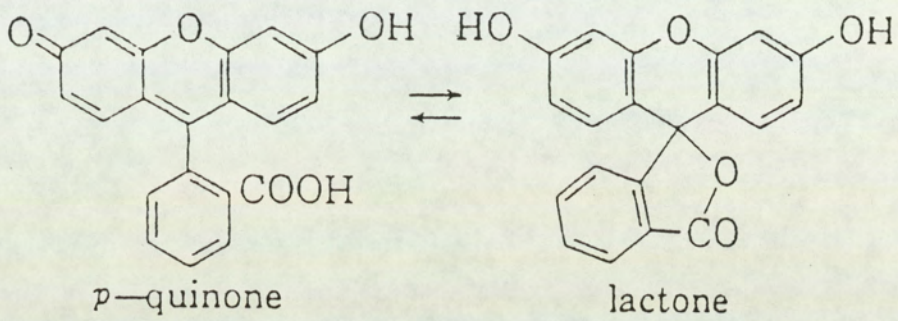


Figure 2.1a Molecular structures of solvated fluorescein dye

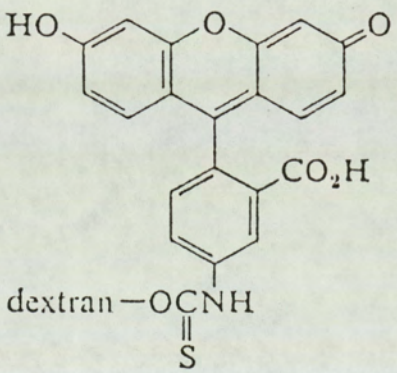


Figure 2.1b Molecular structure of the FITC dextran dyes

b) General purpose reagent grade obtained from Hopkin and Williams Ltd, Essex, UK.

POTASSIUM DIHYDROGEN ortho PHOSPHATE (Anhydrous): GPR grade supplied by BDH Chemicals Ltd. Poole, UK.

DIPOTASSIUM HYDROGEN ortho PHOSPHATE (Anhydrous): GPR grade supplied by BDH Chemicals Ltd. Poole, UK.

SODIUM CARBONATE: Analar grade, BDH Chemicals Ltd Poole, UK.

SODIUM HYDROGEN CARBONATE: Analar grade, BDH Chemicals Ltd. Poole, UK.

2.1.4 SOLVENTS

All solvents were used without further purification.

CHLOROFORM: HiperSolv HPLC grade (BDH).

ETHANOL: GPR grade (J.Burroughs Ltd).

METHANOL: GPR grade (Tennants Ltd).

TETRAHYDROFURAN (THF): GPR grade (BDH).

2.1.5 BIOCHEMICAL REAGENTS

These were used in the blood plasma degradation studies.

HEPARIN: Sodium salt grade II from Porcine Intestinal Mucosa (Sigma), Activity = 156.3 USP/mg. The heparin was used as obtained.

ISOTONIC SALINE: Type 'Amidose' from Amisol Laboratories, Paris. France.

2.1.6 PHARMACEUTICAL EXCIPIENTS

These were used in the cold compressed tabletting studies, all were used as supplied.

AMBERLITE: (Cholestramine resin) Supplied by Rohm and Haas Ltd Croydon, UK.

HYDROXYPROPYLMETHYLCELLULOSE (HPMC): Premium grades E4M and E15 (Dow Chemical Co. Ltd.).

LACTOSE: 'Fast flo' grade (Formost McKessen).

MICROCRYSTALLINE CELLULOSE (MCC): Avicel PH102 grade (Honeywill and Stein).

CARBOXYVINYL POLYMER (poly acrylic acid): Grades 'Carbopol' 934 and 941 (B.F. Goodrich).

2.2 BUFFER RECIPES

Buffers were made according to methods listed by Dawson[190] in 1, 2, or 16 litre quantities in freshly distilled water.

SGN (simulated gastric fluid) consisted of: Sodium Chloride (2.0 g/litre) with concentrated hydrochloric acid (10N, 7 mls/litre). The ready made solution was supplied on site at Pfizer Central Research, Sandwich, Kent.

pH 2.3: This was made up in mulitiples of 20 ml batches, each batch consisting of: 0.4 mls 0.2M dipotassium hydrogen ortho phosphate, plus 19.6 mls 0.1M citric acid.

pH 7.4: This was made up in mulitiples of 200 ml batches, each batch consisting of: 81 mls 0.2M dipotassium hydrogen ortho

phosphate, plus 19 mls 0.2M potassium dihydrogen ortho phosphate made up to 200 mls with distilled water.

pH 10.6: This was made up in mulitiples of 10 ml batches, each batch consisting of: 9 mls 0.1M sodium carbonate, plus 1 ml 0.1 M sodium hydrogen carbonate.

The pH values of the above solutions were measured at various temperatures using an EIL 3055 pH meter (Kent Industrial Measurements Ltd). The variations of the pH's of the solutions with temperature is given in Table 2.3.

Table 2.3 Variation of Buffer pH with Temperature

Nominal recipe pH	pH @20°C	pH @37°C	pH @70°C
2.3	2.48	2.30	2.27
7.44	7.50	7.48	7.41
10.6	10.86	10.58	10.21

2.3 EXPERIMENTAL TECHNIQUES

2.3.1 POLYMER MATRIX PREPARATION

Three methods were used to produce various physical forms of polymer and polymer/dye matrices. These were: Solvent casting (for film production), melt pressing (to form discs) and cold compression (to fabricate tablets).

2.3.1.1 Solvent Casting

SOLUTION PREPARATION: A) Polycaprolactone / fluorescein: Solutions were typically prepared by dissolving PCL (1.00 to 2.00g) and fluorescein ($3.3x10^{-3}$g to $3.7x10^{-3}$g) in a mixture of THF (7.0 mls) and a co-solvent (5.0 mls). The co-solvent being either cyclopentane, cyclohexane or cycloheptane.

B) PHB-co-PHV: Solutions were prepared by typically dissolving 0.40 to 1.00g of polymer in 4.0 to 5.0 mls of chloroform. The resulting solutions were filtered using a sintered filter (Pyrex number 4).

C) PHB-co-PHV / dye: Two dyes were used and solution preparation varied for each type. (i) Fluorescein: Solutions were typically prepared by dissolving 0.0200g of dye in 2.8 mls of THF and adding this to 5 mls of a 15% solution of polymer dissolved in chloroform.

(ii) FITC Dextran: Solutions were prepared using a multisolvent system. A typical polymer / dye system had two component solutions. The first consisting of polymer (0.5g) dissolved in 5.0 mls of chloroform with 2.0 mls of THF and 0.4 mls of ethanol added; the second containing 0.0330g of FITC dextran dye dissolved in 0.5 mls of distilled water with added ethanol (1.8 mls in 0.6 ml aliquots) and THF (0.5 mls in 0.1 ml aliquots). The two solutions were gently warmed in a water bath and mixed.

FILM FORMATION: Polymer and polymer / dye solutions were cast onto a mould consisting of a Melinex sheet onto which four glass slides

were attached (by adhesive) at right angles to each other. After solvent evaporation at room temperature in a fume cupboard the matrices were removed by cutting around the edge of the resulting film with a sharp knife. Latter films were prepared by casting solutions onto a carefully cleaned glass petri dish at room temperature. Film thicknesses ranged from 0.07 to 0.20mm, and were typically 0.12 to 0.15mm.

2.3.1.2 Melt Pressing

Polymer discs were prepared by melt pressing powdered polymer in a mould between two metal plates, with a protective cellophane layer on either side of the mould. The mould consisted of a stainless steel plate 2mm thick with 1cm and 2cm circular pieces cut out at regular intervals. Discs were fabricated by filling the individual circular moulds with dry polymer powder which was then pre-heated (to 140^{o}C for the 3x10^{5}molecular weight 20% valerate copolymer, 185^{o}C for the high molecular weight (8x10^{5}) homopolymer), on an upstroking hydraulic press (Bradley and Turton Ltd. Kidderminster UK.) for two and a half minutes (no applied pressure), followed by two minutes at an applied pressure of 25 tons. The press was cooled to 20^{o}C and the discs were left in the mould to nucleate before removal.

2.3.1.3 Cold Compression

Powdered blends of PHB/PHV polymer (350PHB (12%PHV) and 300PHB (20%PHV)), FITC dextran dye and various excipients were prepared by sieving the components through a 150 um standard sieve and mixing them together in a glass vial on a Coulter mixer for at least four hours. The typical batch size was 2g and the normal dye loading in the blends was 7.5% w/w.

Tabletting of the mixed blends was by manual loading of approximately 100mg of a blend into the die of a single punch tablet press (Manesty F3) fitted with either 6mm or 8.5mm normal concave tooling. A 2% w/v solution of stearic acid dissolved in chloroform was used to lubricate the tooling and tablet compaction pressure was typically 130 Mpa (as measured on an instrumented Manesty F3 tabletting machine). Tablet hardness was measured using a Schleuniger-2E tablet hardness machine.

2.3.2 MATRIX DEGRADATION CONDITIONS

Three *in vitro* techniques were used to evaluate the degradative breakdown of the PHB/PHV matrices and the other biodegradable polymers studied.

2.3.2.1 Buffer Degradation

The primary study of polymer degradation behaviour was the study of matrix resistance to buffer hydrolysis. Various buffers were used (see section 2.2) at two temperatures - physiological

(37°C) and for accelerated degradation studies, 70°C. Samples were immersed in glass jars containing 40 mls of freshly prepared buffer, which was replaced weekly. Gravimetric and surface techniques, which are outlined in section 2.3.3 were used to monitor polymer degradation.

2.3.2.2 Pre-treatment with an Oxygen Plasma

To measure the effect of initial surface rugosity on the rate of degradation, several melt pressed discs, polymer grades 300PHB (20% PHV) and 350PHB (12% PHV), were exposed to an oxygen plasma for 33 minutes in a Nanotech Plastech 250 parallel plate plasma reactor. Reactor conditions were: Operating frequency 13.56 MHz, radio frequency power 100 Watts, at ambient temperature. Oxygen flow rate was 10 mls / minute and the pressure in the reactor was approximately 0.3 Torr.

2.3.2.3 Blood Plasma Degradation

This was used as a degradative method in an attempt to provide a better *in vitro* model than the aqueous buffer hydrolysis studies.

Blood plasma samples were prepared by centrifuging approximately 20 mls of human blood plasma in a heparinised tube (heparin activity 5000 units per ml) for five minutes at 3000 rpm. The supernatent plasma was pipetted off into small glass vials, 3 mls to each sample. After immersion for 15 hours the samples were

thoroughly washed with distilled water and the hydrated and dry weights determined as described in section 2.3.3.3.

2.3.3 ANALYTICAL TECHNIQUES

Briefly outlined in this section are the various analytical methods used to follow polymer degradation and dye release.

2.3.3.1 Spectrophotometry

Ultra violet / visible spectra of dye solutions were obtained using either a Perkin Elmer 137 UV spectrophotometer or a Uvikon 810 Kontron spectrophotometer.

2.3.3.2 Dye Calibration

For the calculation of the amount of dye released during dissolution the max the extinction coefficient values for each dye had to be determined using either of the spectrophotometers mentioned above. Table 2.4 summarises these values for the various dyes as derived from the spectra taken on the Uvikon spectrophotometer using the Beer Lambert law stated in Equation 2.1 .

$$A = E.C.l \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 2.1$$

Where: A = Absorbance

E = The extinction coefficient (expressed as the value for a 1% w/v/ solution in a 1 cm cell i.e. the ${}^{1}_{1}E$)

C = The concentration (expressed in this work as % w/v)

l = The cell path length (= 1cm in these studies)

The corresponding values determined on the UV 137 Perkin Elmer spectrophotometer are listed in Table 2.5.

2.3.3.3 Gravimetric

Weight loss and buffer uptake of polymer matrix samples subjected to hydrolytic or blood plasma degradation were followed by the measurement of the hydrated and dry sample weights. Samples were periodically removed from the buffer solutions and thoroughly washed with distilled water. After removal of surface water, the samples were weighed to give the 'wet' weight (Equation 2.2). The polymer samples were then dried in a vacuum oven at 80^{o}C for 8 hours and weighed to obtain the dehydrated or 'dry' weight (Equation 2.3)

$$\text{HYDRATED ('Wet') WEIGHT} = \frac{\text{Weight of hydrated sample @ time t}}{\text{Weight of dehydrated sample @ time 0}} \times 100\% \quad \ldots\ldots \quad 2.2$$

$$\text{DEHYDRATED ('Dry') WEIGHT} = \frac{\text{Weight of dehydrated sample @ time t}}{\text{Weight of dehydrated sample @ time 0}} \times 100\% \quad \ldots \quad 2.3$$

Table 2.4 Spectrophotometric Data for the Marker Dyes Determined on the Kontron Uvikon 810 Spectrophotometer

DYE	pH	max/nm	E value	secondary max/nm	E^{1}_{1} value
FLUORESCEIN	1.3	437.2	1370	---	---
	7.4	490.1	1135	237.8	620
	10.6	490.2	2758	238.7	1483
FITC 4	1.3	440.3	8.26	---	---
	2.3	237.7	6.72	439.5	5.26
	7.4	493.7	13.35	238.5	9.13
	10.6	491.1	14.97	238.6	10.54
FITC 40	1.3	440.3	23.18	---	---
	2.3	237.7	15.58	439.5	13.26
	7.4	491.9	29.41	238.6	21.24
	10.6	490.2	60.89	238.9	31.79

Table 2.5 Spectrophotometric Data for the Marker Dyes Determined on the UV 137 Perkin Elmer Spectrophotometer

DYE	pH	max/nm	$^{1}_{1}E$ value
FLUORESCEIN	7.4	492	416
FITC 4	2.3	441	1.3
	7.4	492	2.9
	10.6	492	3.67
FITC 20	2.3	440	5.6
	7.4	492	12.5
	10.6	492	16
FITC 40	2.3	445	1.76
	7.4	492	6.16
	10.6	492	6.55
FITC 70	2.3	445	1.41
	7.4	492	4.1
	10.6	492	4.6
FITC 150	2.3	445	1.3
	7.4	492	4.3
	10.6	492	6.6

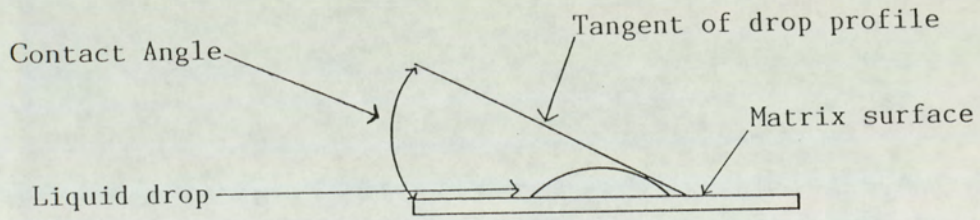
2.3.3.4 Surface Energy

This was calculated from the contact angle measurements of two standard liquids on sample surfaces. The two liquids – water and methylene iodide have inherent differences in their polar and dispersive surface free energy components.

From the measurement of contact angle values of both liquids on a sample surface the polar (γp) and dispersive (γd) components of the surface free energy can be calculated using the Owens and Wendt method.[191]

The contact angle (θ) of a liquid drop on a solid surface can be defined as the angle measured through the liquid between the surface and a tangent drawn to the profile drop at the point of liquid solid contact.[192] This is illustrated in Figure 2.2.

Figure 2.2 Illustration of the contact angle of a liquid on a solid surface



Measurements were made after thoroughly cleaning polymer matrix samples with distilled water and drying (see section 2.3.3.3). The samples were then mounted on a microscope slide and small drops of water and methylene iodide were placed onto the

matrix surface. The contact angle profiles were observed through a cathetometer and values were determined at each side of two to three drops of the two liquids. The polar and dispersive components of the surface free energies were then calculated using a computer program.[193]

2.3.3.5 Goniophotometry

This is a technique for monitoring changes in surface gloss. The intensity of light reflected from a given surface is measured as a function of viewing angle. The apparatus used in this study was an automated goniophotometer which had been considerably modified.[194] Figure 2.3 illustrates the experimental set up. Samples were mounted behind a metal plate (with a 2 x 3 mm slit) which was at 45 degrees to a beam of collimated light. The intensity of surface reflected light was measured by a photocell at angles between 0 and 138 degrees to the sample surface. The data was relayed to a Sharp MZ 80 microcomputer and spectra of incident light intensity (I) against viewing angle (α) were plotted by the computer together with the calculated characteristic parameters that can be derived from goniophotometric spectra.

2.3.3.6 Scanning Electron Microscopy

A detailed examination of polymer matrix morphology and topography was made using a Stereoscan 5150 scanning electron

Figure 2.3 Diagramatic representation of a Goniophotometer[194]

A = Mercury vapour lamp; B = Wavelength filter; C = Shutter unit; D = Series of neutral filters; E = Collimating tube; F = Polariser and variable slit aperture; G = Mounted specimen; H = Photocell; I = Photocell analyser and variable slit aperture

microscope. Samples were prepared by depositing a layer of gold onto the sample surface by a gold spluttering technique.

2.3.3.7 Gel Permeation Chromatography

Polymer molecular weights and molecular weight distributions were determined by gel permeation chromatography. The apparatus consisted of a Rheodyne P/N 7125047 injector (Anachem House, Luton, UK.) and a Waters Associates chromatography pump (model M6000A), with a 'train' of microstyragel columns (pore sizes - 10^2, 10^3, 10^4 and 10^5 Angstroms). The polymer samples were detected using an ultra-violet detector (Applied Chromatography Systems Ltd) and the eluting solvent was chloroform (flow rate 1ml per minute). Samples were dissolved in chloroform (typically 0.5% w/v) and pre-filtered through a millipore filter. 100µl aliquots of the polymer solution were then injected onto the columns.

A set of monodisperse polystyrene standards were used to calibrate the columns for molecular weight determinations, and the chromatograms were statistically analysed to give the number and weight average polystyrene eqivalent molecular weights.

The molecular weights were calculated assuming there were no interactions between the polymer and the solvent or the polymer and the packing material, and no account was made for band broadening corrections. Reference should be made to Yau[195] for further details.

2.4 DYE DISSOLUTION: IN VITRO MODELLING

The major difficulty in constructing an *in vitro* model is in mimicking *in vivo* i.e. body conditions. The main points to be considered are the pH of the dissoluting medium, the experimental apparatus and the method of dye determination.

2.4.1 pH

Buffer solutions are used to maintain a constant pH Physiological pH is 7.4 and many buffers and solutions have been used in *in vitro* models e.g. isotonic saline,[144] Sorensons buffer,[55] citrate/phosphate buffers[86] and Ringers solution[196,197] to mention a few.

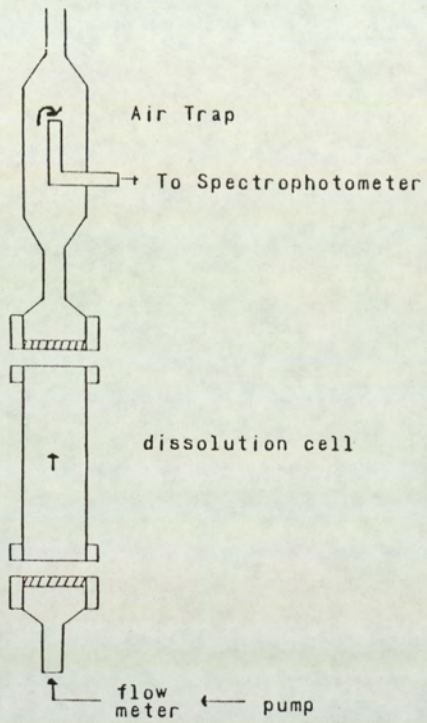
2.4.2 EXPERIMENTAL APPARATUS

There are basically two types of apparatus used for dye/drug release studies: A) Continuous flow and B) Rotation systems.

Continuous flow systems have been discussed by Tingstad and Riegelman,[198-201] and Gould.[202] The basic experimental set up is described in Fig 2.4,[198] the basic advantages of this type of system are firstly that 'sink' conditions are easily achieved, that is the drug does not reach its saturation concentration in the elution medium (in these systems it is usually less than 10% of the drugs solubility in the solvent), and secondly, there are only two parameters to be considered in the control of the dissolution

process; The cross sectional area of the dissolution cell and the flow rate.

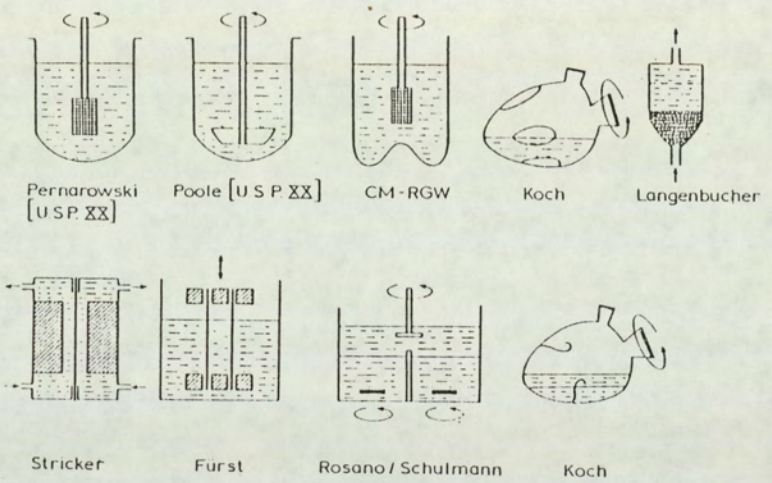
Figure 2.4 Continuous Flow Apparatus[198]



The disadvantages of continuous flow systems are that many drugs have a low aqueous solubility, hence large flow rates are needed, with the consequent difficulty of measuring the drug concentration in these very dilute solutions. Another problem with high flow rates, is the difficulty in obtaining laminar flow around the drug matrix. Tingstad and Riegelman[200] suggest that a layer of fine glass beads placed at the bottom of the dissolution cell enables flow rates of up to 80mls per minute for a dissolution chamber of diameter 2.5 cm.

Rotation systems discussed by Gould[202] are widely used in the pharmaceutical industry as they are a quick method for determining release rates and are easily automated. Some examples are given in Fig 2.5.[203]

Figure 2.5 Examples of Rotating Dissolution Apparatus[203]



Against the advantages of rotation systems is the fact that sink conditions are hard to achieve in these 'stew' methods and if a basket is used to hold the drug matrix, variable shear rates over the tablet can be obtained. In addition, there are more external parameters in this type of apparatus than for the continuous flow systems e.g. speed, position and type of stirrer, basket 'rattle', motor vibrations etc.

However, for subcutaneous implants, there is evidence that sink conditions do not occur, with the hydrodynamic boundary layer reported[204] as being approximately 100 μm. Hence, a compromise

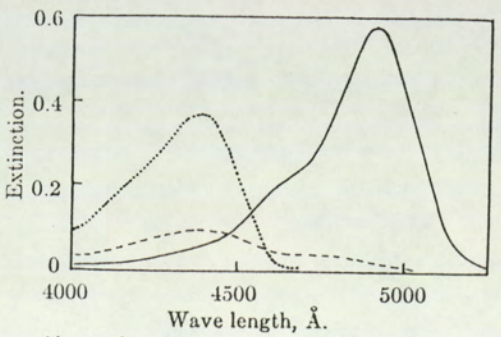
between perfect 'sink' conditions (as illustrated by the standard pharmaceutical dissolution techniques) and the release rate limiting 'static' conditions (where there is little external removal of released dye), has to be reached in order to make valid *in vitro* models.

2.4.3 Dye determination

Most work in the literature uses either radioanalysis - H^{22} or ^{14}C,[97] or spectrophotometric determination of a dye or protein.[21] Radioassay methods have also been employed in studies of polymer degradation.[88] On the grounds of convenience it was decided to use marker dyes for release studies with quantitative release being followed using spectrophotometric determination of the dissolution medium.

In the case of water soluble dyes, pH is usually very important and fluorescein, for example, has a widely varying UV / visible absorbance depending on the pH of the solution. Figure 2.6 shows[205] this shift in spectrum profile with pH.

Figure 2.6 Spectrophotometric profiles of Fluorescein as a function of pH[205]



Absorption of 8 μM fluorescein in water: dotted line, pH 1; dashed line, pH 3; full line, pH 8.

2.4.4 Experimental systems

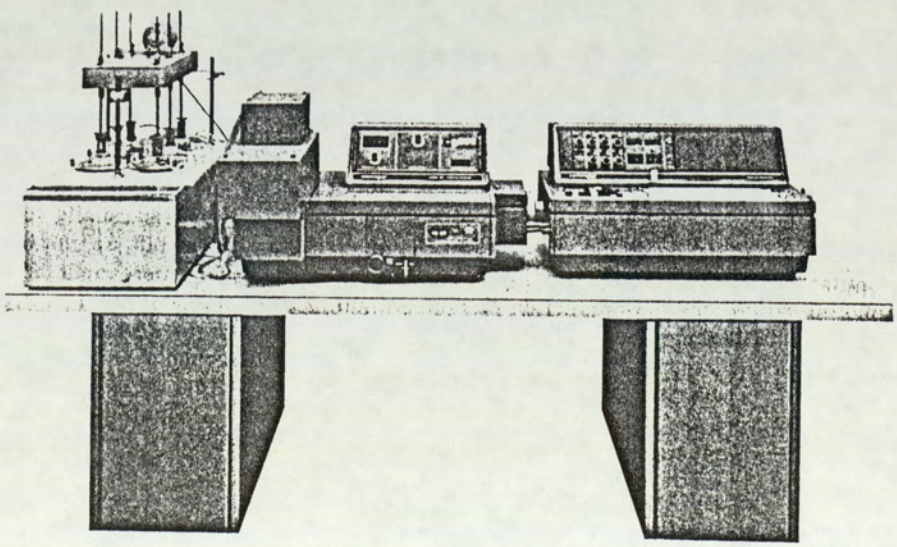
From the above discussion of *in vitro* modelling, release of dye from polymeric matrices was followed under two extreme conditions - a 'static' or 'stew' method and a standard USP (United States Pharmacopoeia) dissolution method which is used in the pharmaceutical industry for accurate determination of drug release from tablets.

In the static experiments, samples were placed in glass jars (for the polymer films) or boiling tubes (for the polymer tablet release studies) containing buffer (25mls for tablet and FITC dextran film dissolution, and 75mls for the fluorescein films). The containers were maintained at 37 ± 0.1^{o}C in a water bath. 5ml aliquots were taken periodically (with concurrent replacement of buffer to maintain constant volume) and the dye concentration determined spectrophotometrically. Before sampling, the containers were inverted to disperse any 'dye pocket' which had been formed.

The other dissolution method used was the standard USP XXI pharmaceutical tablet (basket) dissolution method which maintains 'sink' conditions throughout the experiment. The apparatus (an example of which is outlined in Figure 2.7[206]), consisted of a thermostated water bath maintained at 37 ± 0.1^{o}C (Copley model, Hanson Research CA,USA.) in which were placed six 900 ml glass flasks each covered with a perspex disc containing holes for a stirrer and inlet / outlet auto-sample tubes. The variable speed control basket stirring mechanism (Copley dissolution drive

control, Hanson Research CA, USA.) was normally set to 100 rpm with samples being placed in the dissolution basket 2 cm from the bottom of the glass flasks. Dye release was followed using a Uvikon 810 spectrophotometer (Kontron Instruments, St Albans, UK.) which was fitted with a programmable cell changer for automatic analysis for up to six samples, the spectrophotometer being controlled by a Commodore PET 4032 microcomputer. A small loop of the dissolution buffer was circulated through the flow cells in the spectrophotometer using a peristaltic pump (Watson-Marlow Camline model 502S), and the measured absorbances (assuming the dissolution medium in the flow cells was at room temperature), were stored in the computer and were printed out along with the calculated dissolution parameters at the end of each experimental run.

Figure 2.7 A typical USP Dissolution set up[206]



CHAPTER THREE

POLYMER CHARACTERISATION BY MEASUREMENT OF BULK DEGRADATION

3.1 INTRODUCTION

Outlined in this chapter is the study of the rate and nature of the degradation of the PHB/PHV copolymer series, together with a study of the hydrolytic degradation of several commercially available suture materials.

3.2 DEGRADATIVE METHODS

Hydrolytic degradation of the various physical forms of the PHB/PHV polymers was carried out in a range of aqueous buffer solutions (pH 2.3 to 10.6) at two temperatures 37^{o}C and 70^{o}C. The films tested were typically 2cm square, weight 0.0700g and thickness 0.02 mm. The melt pressed discs were typically 2cm in diameter, weight 0.6600g and thickness 0.50 mm. Test samples were removed weekly and the wet and dry weights determined as described in section 2.3.3.3. Suture degradation was carried out on 50 cm lengths (Typical weights 0.060g (PDS) to 0.12g (Vicryl)), and the *in vitro* breakdown was monitored in a similar fashion to the PHB/PHV copolymer samples.

3.3 RESULTS

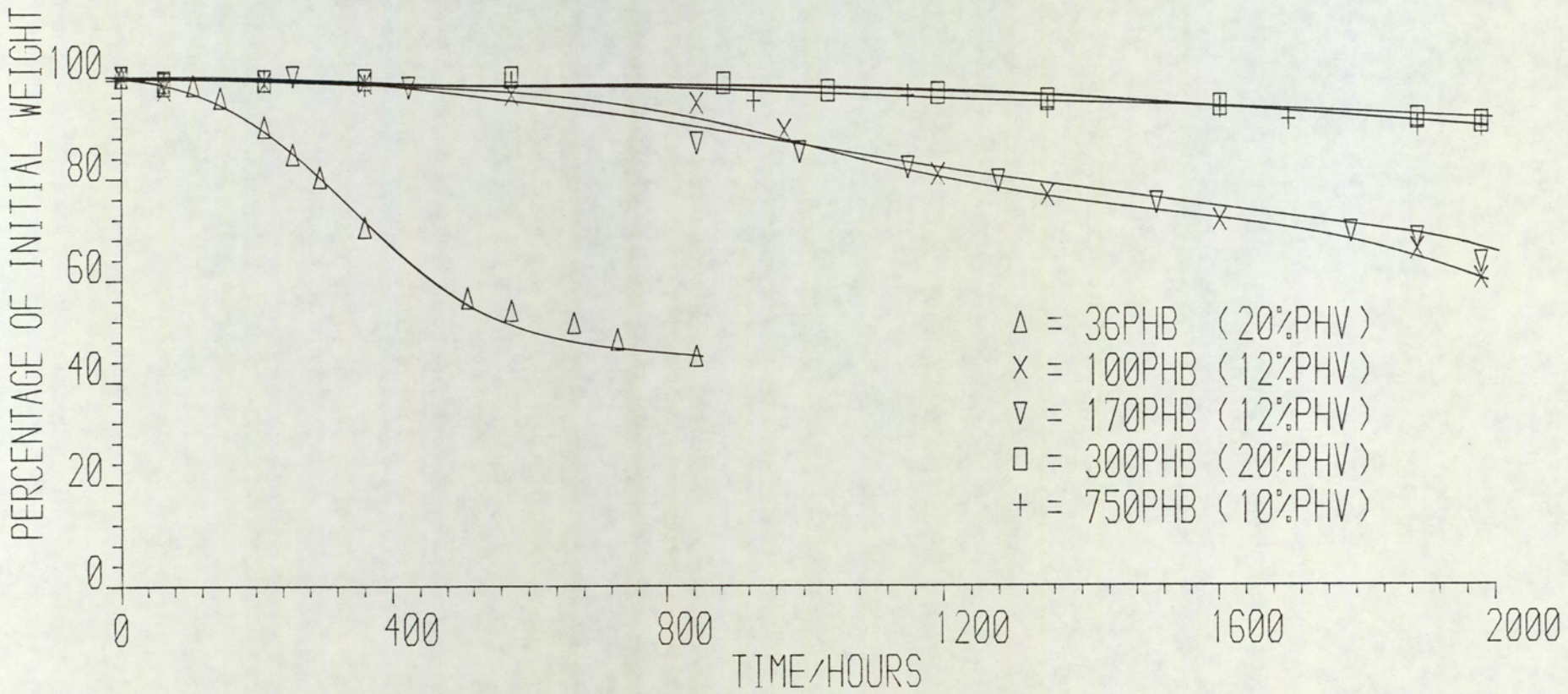
The degradation process as measured by polymer matrix weight loss can be represented as the percentage of the initial

(dry) weight remaining at time t. For the purpose of presenting the hydrolytic degradation data, plots are made of dry weight (i.e weight remaining) against time (hours).

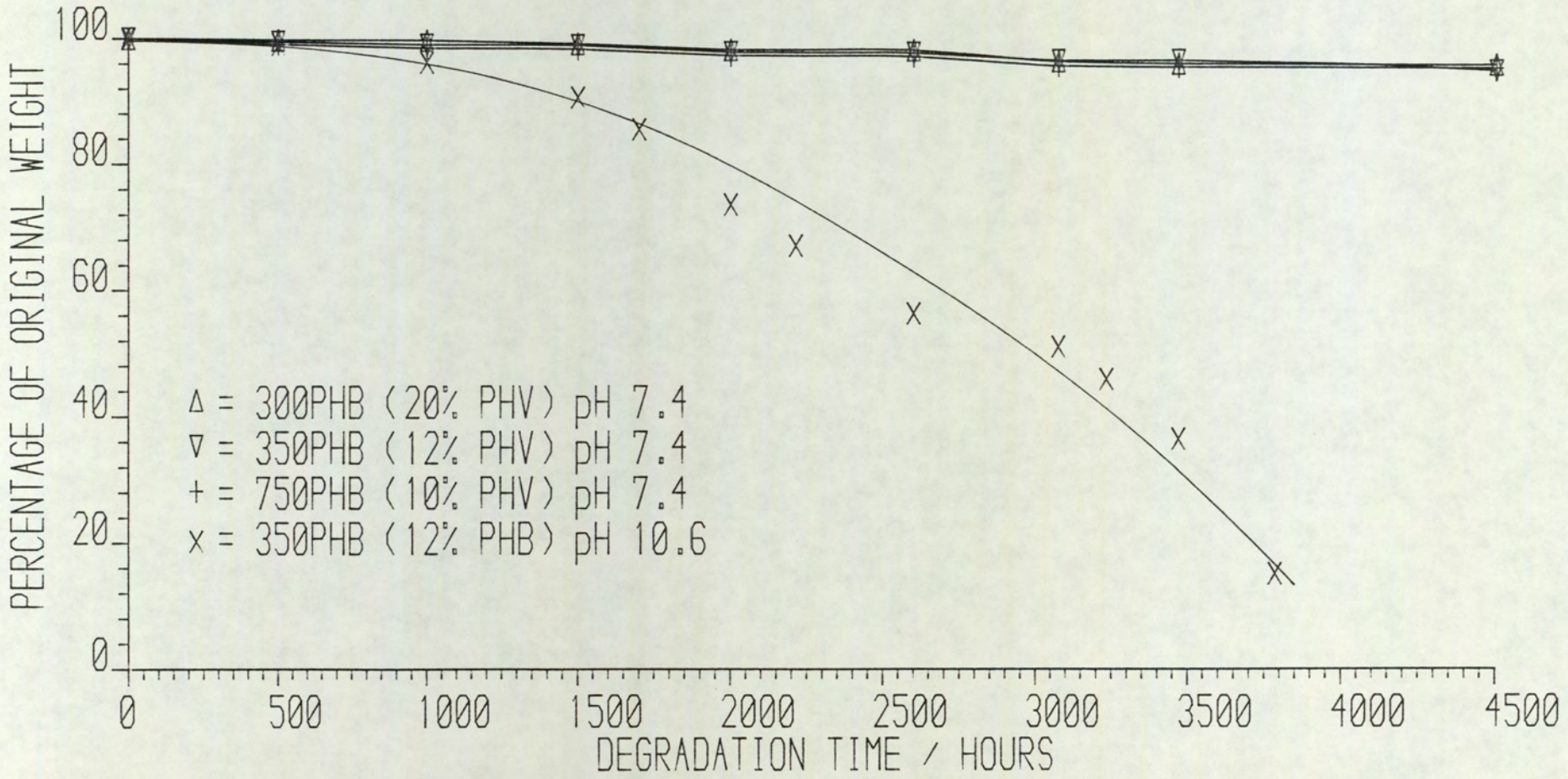
3.3.1 FILM WEIGHT LOSS

Initial hydrolysis studies were carried out on solvent cast polymer films in a pH 7.4 buffer at 70°C (to facilitate accelerated degradation) for 2000 hours (n=1 to 2). Graph 3.1 shows the weight loss profile of the films as a function of degradation time. The dependence of the degradation rate on polymer molecular weight at the same copolymer composition is clearly shown in a comparison of the 36PHB and 300PHB (both 20%PHV) profiles. The traces are characterised by an initial slow weight loss followed by a secondary enhanced degradation phase. The results of a full study of film degradation in a series of buffers at physiological temperature and for the 300PHB (20%PHV) and 750PHB (10%PHV) polymer grades under accelerated (70°C) conditions is presented in Graphs 3.2 to 3.4, with the times for 10% and 50% weight loss summarised in Table 3.1. The results for one particular polymer grade (350PHB 12% PHV) are listed in Table 3.2. From this data the increasing stability to hydrolytic attack as polymer molecular weight increases is borne throughout most of the (buffer) degradation conditions used. This is equally true at either of the two temperatures used, with molecular weight overiding differences in copolymer composition. Increasing the

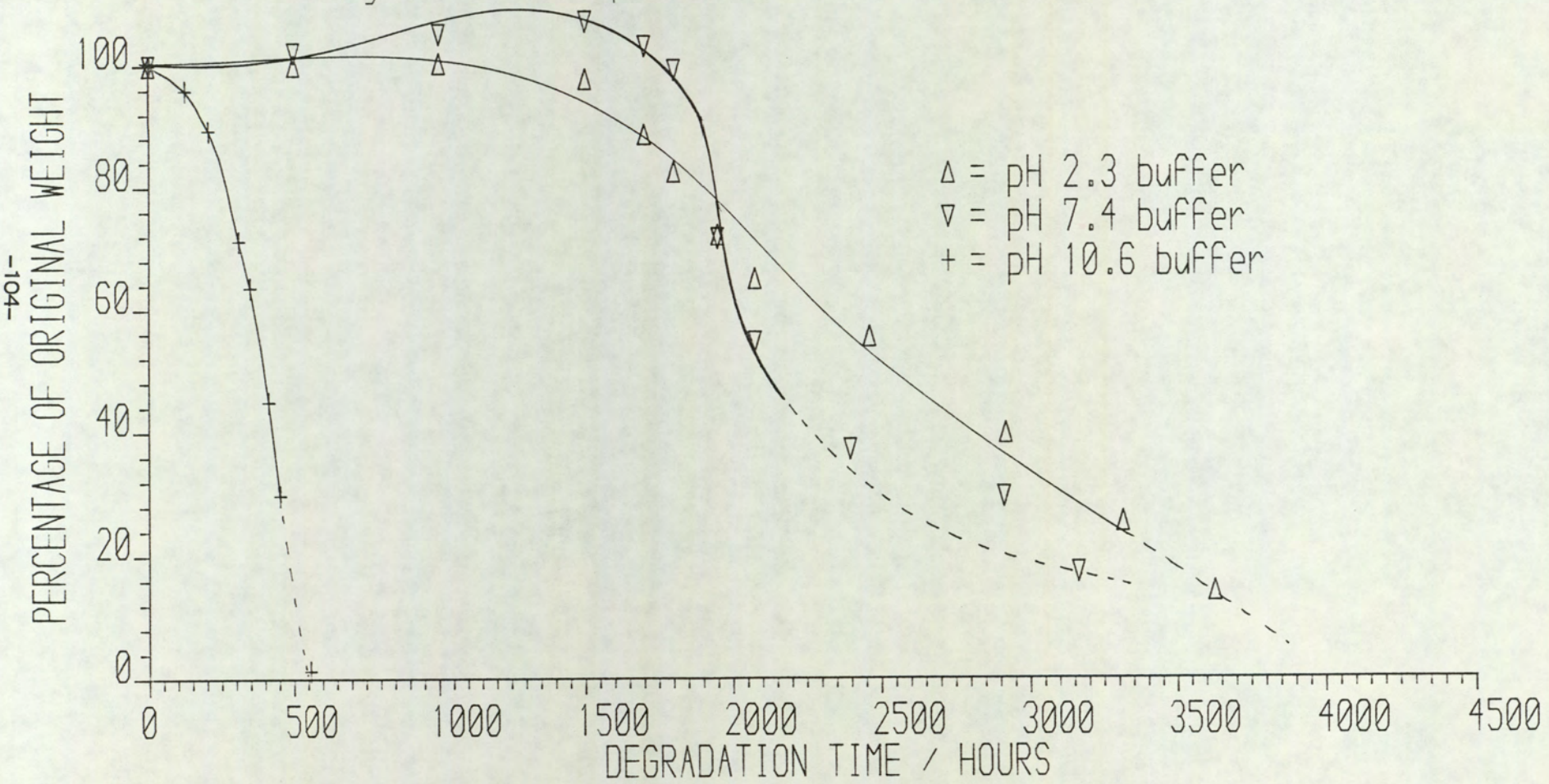
GRAPH 3.1 Weight Loss of PHB/PHV Solvent Cast Films in a pH 7.4 Buffer at 70°C



GRAPH 3.2 PHB/PHV Film Weight Loss
in pH 7.4 and 10.6 Buffers @ 37°C



GRAPH 3.3 300PHB (20% PHV) Film
Weight Loss in aqueous buffers @ 70°C



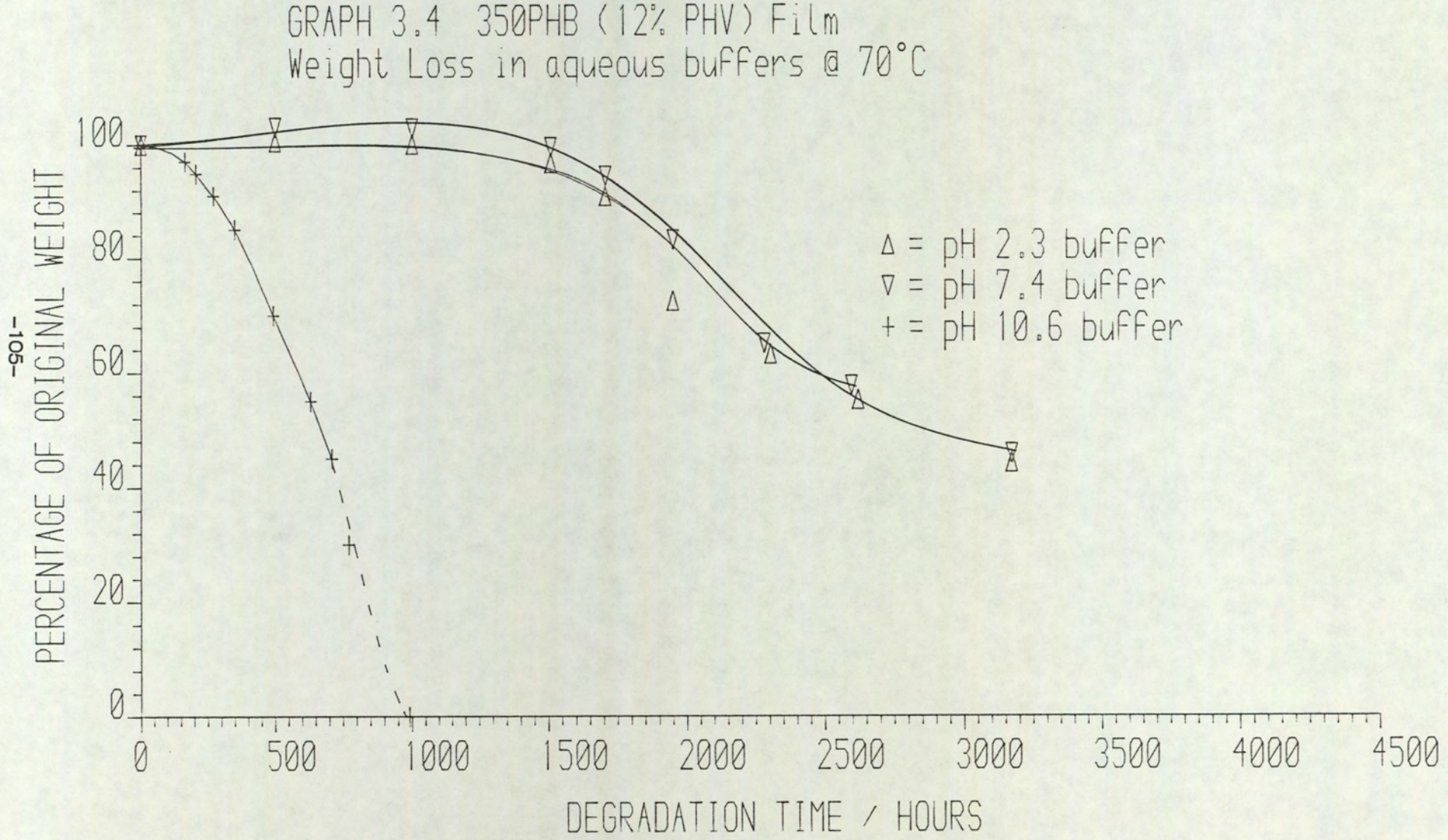

GRAPH 3.4 350PHB (12% PHV) Film
Weight Loss in aqueous buffers @ 70°C
PERCENTAGE OF ORIGINAL WEIGHT
100
80
60
40
20
0
0
500
1000
1500
2000
2500
3000
3500
4000
4500
DEGRADATION TIME / HOURS
Δ = pH 2.3 buffer
▽ = pH 7.4 buffer
+ = pH 10.6 buffer

Table 3.1 Weight loss Parameters for PHB/PHV solvent cast films under various degradation conditions

SAMPLE		DEGRADATION CONDITIONS		DEGRADATION PARAMETERS	
Molecular Weight/10^{-5}	% PHV	pH	TEMP / ^{o}C	$t_{10\%\ loss}$ / Hours	$t_{50\%\ loss}$ / Hours
3.0	20	7.4	37	(4.73% @ 5475)	
3.5	12	7.4	37	(5.63% @ 5475)	
4.0	7	7.4	37	(2.3% @ 2360)	
7.5	10	7.4	37	(6.34% @ 5475)	
8.0	0	7.4	37	(1.8% @ 2360)	
3.5	12	10.6	37	1530	3080
4.0	7	10.6	37	1225	(15% @ 2360)
8.0	0	10.6	37	1125	2100
3.0	20	2.3	70	1680	2550
3.5	12	2.3	70	1725	2875
4.0	7	2.3	70	(5.5% @ 2360)	
7.5	10	2.3	70	2050	(20% @ 2390)
8.0	0	2.3	70	1375	(31% @ 2360)

Table 3.1 (Cont)

SAMPLE		DEGRADATION CONDITIONS		DEGRADATION PARAMETERS	
Molecular Weight/10^{-5}	% PHV	pH	TEMP / ^{o}C	$t_{10\%\ loss}$ / Hours	$t_{50\%loss}$ / Hours
0.36	20	7.4	70	200	400
1.0	12	7.4	70	825	2000
1.7	12	7.4	70	800	(37% @ 1980)
3.0	20	7.4	70	1850	2200
3.5	12	7.4	70	1860	2925
4.0	7	7.4	70	(2.2% @ 830)	
7.5	10	7.4	70	1950	2400
8.0	0	7.4	70	2075	(26% @ 2360)
3.0	20	10.6	70	200	400
3.5	12	10.6	70	275	630
4.0	7	10.6	70	490	1600
7.5	10	10.6	70	175	580
8.0	0	10.6	70	<<214	<214
7.5	10	7.4	100	~60	~120

alkalinity of the buffer increases the rate of hydrolysis. This is examplified in Table 3.2 which shows a five fold increase in degradation rate from pH 2.3 to pH 10.6 at 70°C. A close approximation exists between the low temperature alkaline conditions and the acid degradation at the higher temperature. The high molecular weight 7% hydroxyvalerate copolymer and the very high molecular weight homopolymer film samples show the expected greater stability to degradative attack than the lower molecular weight higher valerate films. However some of these higher molecular weight samples showed a faster degradation rate under the lower temperature degradation conditions. This is presumably due to the poor film formation of these polymer blends as the high molecular weight / low copolymer films used in the low temperature study were translucent as opposed to the clear films formed by the 300PHB (20%PHV) and 350PHB (12%PHV) grades. High temperature studies on the degradation of the 750PHB 10% valerate copolymer under the extreme conditions of pH 7.4 100°C show the expected rapid weight loss. Where differences in copolymer ratio can be observed for example between the 300PHB 20% valerate, 350PHB 12% valerate and the 400PHB 7% valerate polymer grades, a decrease in stability with increasing valerate content occurs over and above that associated with the small changes in molecular weight. Data from Table 3.1 shows that the times for 10 and 50% weight loss are consistently lower for the lower valerate copolymers. This is

Table 3.2 Weight loss of a solvent cast 350PHB film as a function of pH and temperature.

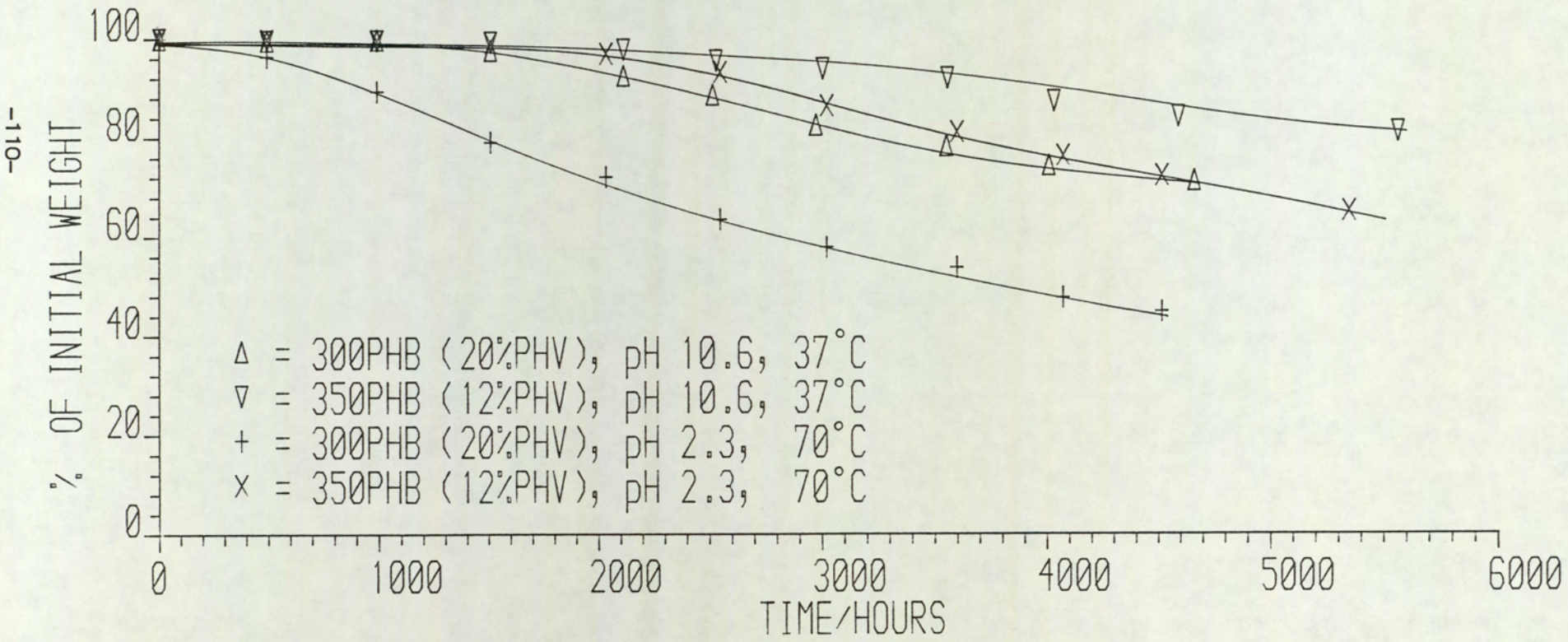
DEGRADATION CONDITIONS		$t_{10\%\ loss}$	$t_{50\%\ loss}$
pH	TEMP / ^{o}C	/ Hours	/ Hours
7.4	37	(5.6% @ 5475)	-
10.6	37	1550	3080
2.3	70	1725	2875
7.4	70	1870	2925
10.6	70	275	630

expected as the lower valerate members of the PHB/PHV series are inherently more crystalline[207] and would be expected to degrade more slowly as the amorphous regions of crystalline polyesters have been shown to preferentially degrade.[82]

3.3.2 MELT PRESSED DISC WEIGHT LOSS

Similar hydrolysis studies to those performed on solvent cast films were carried out on melt pressed discs (n=1 to 2) of PHB/PHV copolymers for up to 6000 hours. Graph 3.5 shows the weight loss of the discs as a function of degradation time for two degradative conditions (37^{o}C, pH10.6 and 70^{o}C, pH 2.3). Table 3.3 presents the degradation parameters of all the range of discs studied, in a similar fashion as the data for the solvent cast films. Table 3.4

GRAPH 3.5 Weight Loss of PHB/PHV Discs
in Various aqueous Buffer Conditions



lists the same weight loss parameters for one particular grade of hydroxybutyrate copolymer - PHB300K (20% valerate)

The data again shows the dependence on the rate of degradation of molecular weight and pH, with increasing alkalinity and temperature enhancing the degradation rate as seen in Table 3.3. Plates 3.1 and 3.2 show 300PHB (20%PHV) melt pressed discs at various stages of degradation, with the original discs on the left hand side of each photograph. Note the small difference in appearance between the middle discs in the two pictures, even though they have been subjected to differing hydrolysis conditions. Plate 3.1 shows discs that have been degraded in a pH 10.6 buffer at 37^{o}C and Plate 3.2 illustrates the effects of a pH 7.4 buffer at 70^{o}C on the disc matrices. It is only when the weight loss is greater than 30% that an obvious change in matrix appearance occurs. The right hand disc in Plate 3.2 shows one such disc degraded in a pH 7.4 buffer for 3200 hours at 70^{o}C with an associated weight loss of 58%. The substantially degraded disc on the right hand side of Plate 3.1 shows the remains of a 300PHB (20%PHV) disc (pre-treated with an oxygen plasma) after buffer degradation for greater than 6000 hours. It is apparent that the effects of prolonged hydrolytic degradation on the melt pressed discs under both conditions is similar, the main difference being the time scale.

At physiological temperatures the discs degrade relatively slowly with the acidic and physiological buffers having little

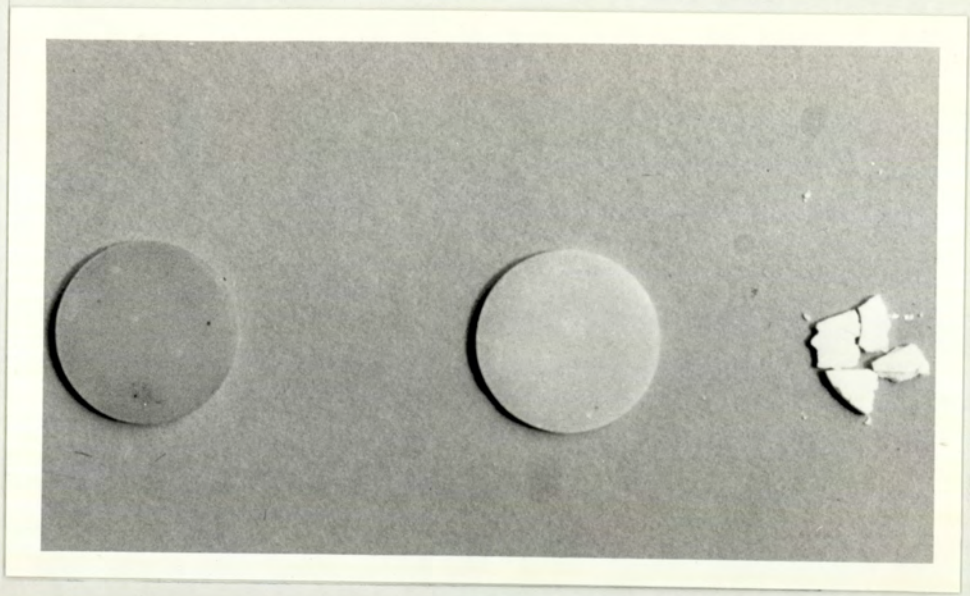


Plate 3.1 Melt Pressed 300PHB (20%PHV) Discs: Undegraded (Left);
pH 10.6 / 37°C / 2300 Hours, 9.8 % Weight loss (Middle);
pH 10.6 / 37°C / 6600 Hours, 53 % Weight loss (Right). (X 1.5)

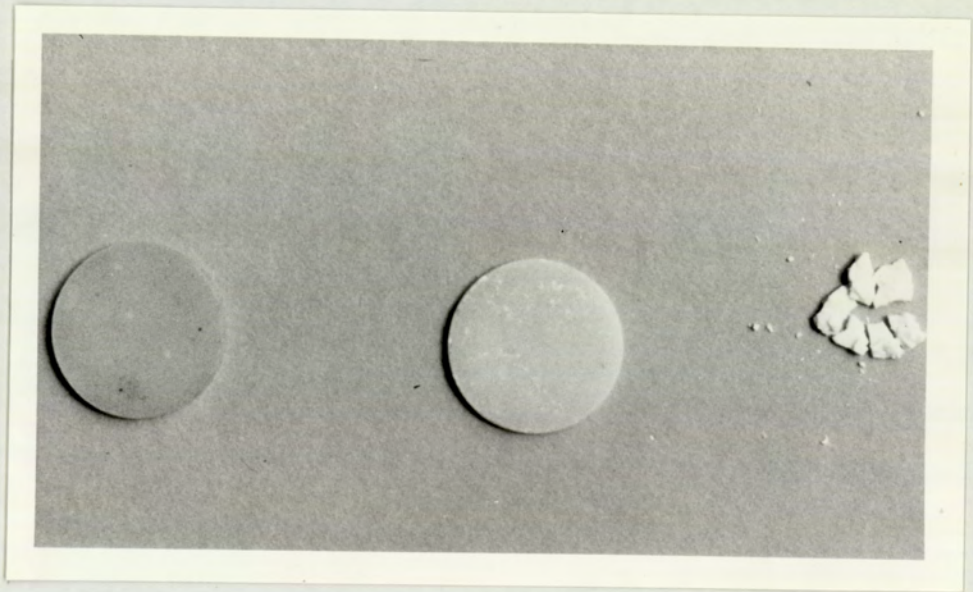


Plate 3.2 Melt Pressed 350PHB (12%PHV) Discs: Undegraded (Left);
pH 7.4 / 70°C / 2100 Hours, 12.3 % Weight loss (Middle);
pH 7.4 / 70°C / 3200 Hours, 58 % Weight loss (Right). (X 1.5)

Table 3.3 Weight loss Parameters for PHB/PHV melt pressed discs under various degradation conditions

SAMPLE		DEGRADATION CONDITIONS		DEGRADATION PARAMETERS	
Molecular Weight/10^{-5}	% PHV	pH	TEMP / ^{o}C	$t_{10\%}$ loss / Hours	$t_{50\%}$ loss / Hours
3.0	20	2.3	37	(0.76% @ 5425)	
3.5	12	2.3	37	(0.89% @ 5425)	
3.0	20	7.4	37	(1.2% @ 5560)	
3.5	12	7.4	37	(1.1% @ 5560)	
4.0*	7	7.4	37	(0.5% @ 1080)	
8.0*	0	7.4	37	(0.27% @ 1080)	
3.0	20	10.6	37	2450	(29% @ 4660)
3.5	12	10.6	37	3750	(19% @ 5560)
3.0	20	2.3	70	975	3750
3.5	12	2.3	70	2750	(35% @ 5350)
4.0*	7	2.3	70	(0.77% @ 864)	
8.0*	0	2.3	70	(0.70% @ 864)	
3.0	20	7.4	70	2020	3080
3.5	12	7.4	70	2600	3140
4.0	7	7.4	70	2170	(12% @ 2290)
8.0	0	7.4	70	(1.4% @ 2290)	

* = reference 208

Table 3.3 (Cont) Weight loss Parameters for PHB/PHV melt pressed discs under various degradation conditions

SAMPLE		DEGRADATION CONDITIONS		DEGRADATION PARAMETERS	
Molecular Weight/10^{-5}	% PHV	pH	TEMP / ^{o}C	$t_{10\%}$ loss / Hours	$t_{50\%}$ loss / Hours
3.0	20	10.6	70	200	500
3.5	12	10.6	70	390	1230
4.0	7	10.6	70	470	875
8.0	0	10.6	70	250	700

Table 3.4 Weight loss of a melt pressed 300PHB disc as a function of pH and temperature.

DEGRADATION CONDITIONS		$t_{10\%}$ loss	$t_{50\%}$ loss
pH	TEMP / ^{o}C	/ Hours	/ Hours
2.3	37	(0.7% @ 5425)	
7.4	37	(1.2% @ 5560)	
10.6	37	2450	(29% @ 4660)
2.3	70	975	3750
7.4	70	2020	3080
10.6	70	200	500

effect - there being only 0.6 to 1% weight loss respectively after 4000 hours. The differences in copolymer composition are again noticeable. Comparing the 12% and 20% valerate (molecular weight ($\overline{Mw}$) approximately $3x10^5$) melt pressed disc samples shows that the lower valerate samples in some cases exhibit twice the resistance to hydrolytic attack. The polymer samples used to fabricate the high molecular weight 7% hydroxyvalerate and homopolymer discs were non-nucleated and a lower disc crystallinity resulted from an inadequate cooling time during disc preparation. This reduction in disc crystallinity is reflected in the degradation parameters contained in Table 3.3 which show a faster ten and fifty percent loss for these samples than for the highly crystalline 350PHB (12%PHV) and 300PHB (20%PHV) melt pressed discs.

The similarities between the low temperature alkali and high temperature acidic conditions exhibited in the solvent cast film samples is less pronounced for the melt pressed discs, presumably due to larger differences in physical form (especially crystallinity) that exist between the disc samples.

3.3.3 TABLET WEIGHT LOSS

The degradation parameters of the hydrolysis studies on cold compressed tablet samples as measured by weight loss are summarised in Table 3.5. Care must be taken as all the tablets contain soluble dyes, and excipients that either swell or are dissolved. However recorded dry weights of greater than the

combined excipient and / or dye loading show true loss by hydrolysis providing the tablets do not disintegrate. These 'true' weight losses are expressed in Table 3.5.

Weight loss of the tablet samples is faster than the other physical forms of the PHB/PHV copolymer series with a t_{10}% loss for the 300PHB (20%PHV) tablets containing FITC40 and 7.5% lactose being 30% faster than the equivalent solvent cast film. The difference between the degradation rates of the tablets and the film and disc PHB/PHV forms is most marked in the extreme hydrolysis conditions of a pH 10.6 buffer at 70^{o}C. In this media the tablets degrade several times faster than films or discs made from the same grade of copolymer. This is probably due to the higher surface area available for degradative attack for the tablets since they are very much more porous than the other physical forms of polymer tested. However, as already stated the weight loss measurements presumed that true matrix degradation can only be observed at weight losses greater than the excipient plus dye loadings. This is almost certainly not true, with polymer degradation occurring (to a small extent) before this stage of the weight loss is reached; but this comparative method is only used here to enable simple contrasts between the various other types of PHB/PHV matrix.

Table 3.5 Weight loss Parameters for Cold Compressed Tablets Degraded under various Conditions

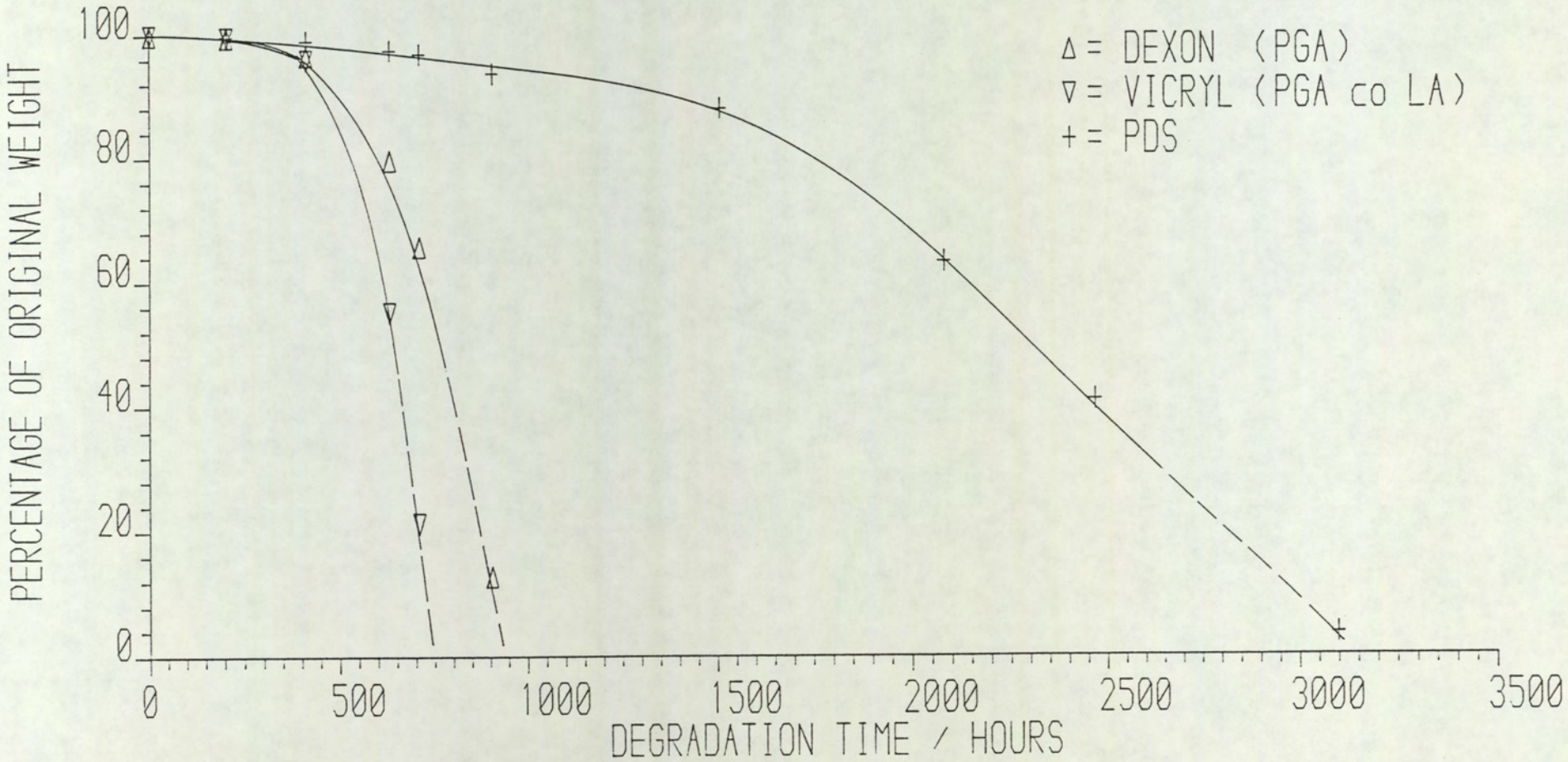
TABLET BLEND			DEGn CONDITIONS		DEGn PARAMETERS	
Polymer	Dye	Excipient	pH	TEMP / ^{o}C	$t_{10\%}$ loss / Hours	$t_{50\%}$ loss / Hours
300PHB	FITC40	'Base'	7.4	37	(6% @ 2900)	
300PHB	FITC4	2.5% Lactose	7.4	37	(6% @ 2900)	
300PHB	FITC40	7.5% Lactose	7.4	70	1270	(20% @ 2900)
350PHB	FITC40	7.5% MCC	7.4	70	(5% @ 2900)	
350PHB	FITC4	3% pH 10.6	7.4	70	970	(22% @ 2900)
300PHB	FITC40	25% Lactose	10.6	70	~45	~80
350PHB	FITC40	12.5% Lactose	10.6	70	~45	~75
350PHB	FITC4	7.5% Lactose	10.6	70	~65	~95

pH 10.6 = Alkaline buffer salts, MCC = microcrystalline cellulose

3.3.4 SUTURE WEIGHT LOSS

For comparative hydrolytic degradation studies commercial suture materials (Dexon, Vicryl and PDS) were subjected to the same buffer degradation as the PHB/PHV copolymer samples (n=1 to 4). Graph 3.6 illustrates the weight loss of the three different sutures in a pH 7.4 buffer at physiological temperature (not all the experimental points are shown). Times for 10% and 50% weight loss for the differing degradation conditions are listed in Table 3.6.

GRAPH 3.6 Weight loss of Various Biodegradable Surgical Sutures in a pH 7.4 Buffer @ 37°C



The data shows the expected increase in the degradation rate with increasing alkalinity for the two poly alpha ester suture materials Dexon (PGA) and Vicryl (PGA co PLA). The Polydioxanone (PDS) sutures however, show a faster rate of acidic hydrolysis than alkali or pH 7.4 hydrolysis - the times for 50% weight loss being 975, 2375 and 1120 hours respectively. This observation is as yet unreported.

The degradation of the sutures at the higher temperature used for the buffer hydrolysis studies (70^{o}C) was too fast to accurately measure, with times for 50% weight loss varying from around three days for Vicryl and Dexon to approximately six days for the PDS samples.

3.3.5 EFFECT OF OXYGEN PLASMA PRE-TREATMENT

The degradation parameters for melt pressed disc samples that had been exposed to an oxygen plasma prior to hydrolysis are summarised in Table 3.7. The degradation profiles are illustrated in Graph 3.7 (pH 10.6 37^{o}C) for two polymer grades - 300PHB (20% PHV) and 350PHB (12%PHV). The graph also contains additional points corresponding to the equivalent non-plasma etched disc samples.

The plasma treated discs exhibit the usual enhanced alkaline degradation that is associated with the various physical forms of

GRAPH 3.7 Effect of Pre-treatment with an Oxygen Plasma on the Weight Loss of PHB/PHV Discs in a pH 10.6 Buffer at 37°C

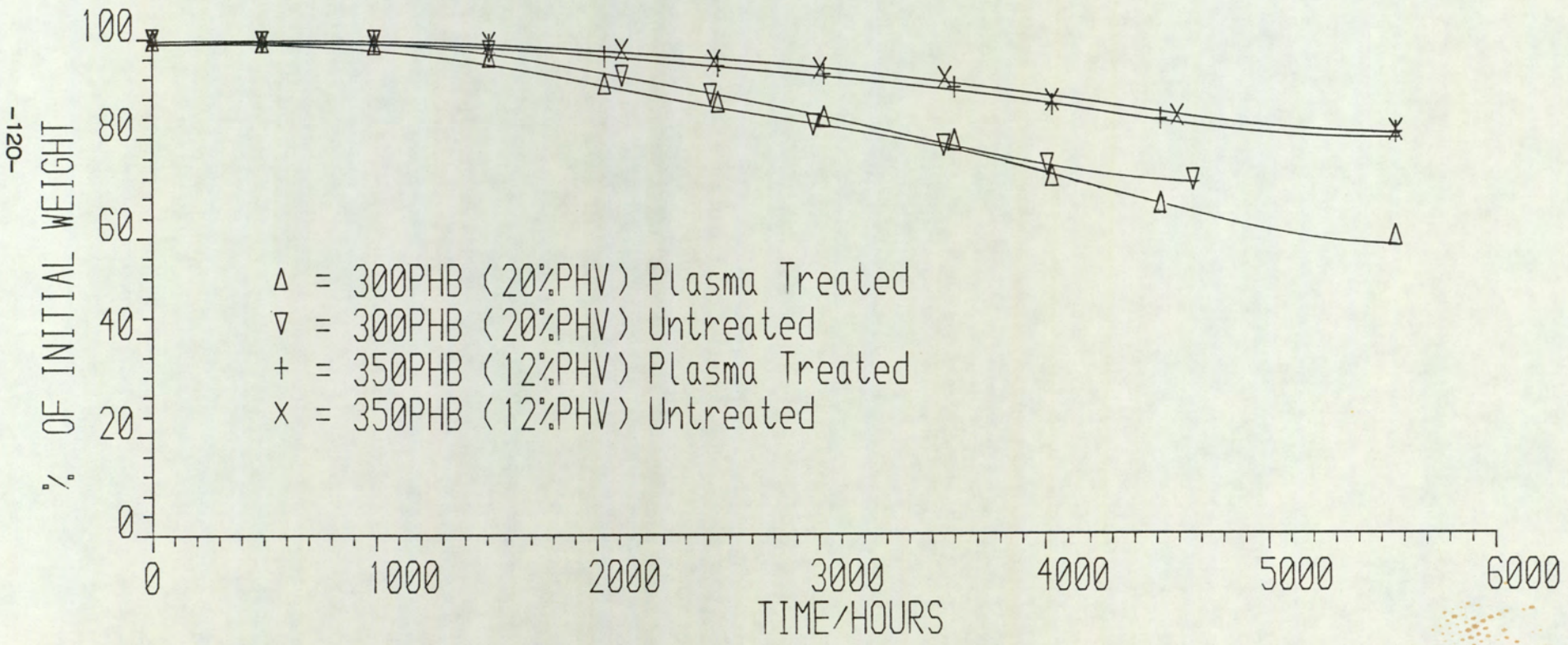


Table 3.6 Weight loss Parameters for Commercial Suture Materials under various Degradation Conditions

SUTURE	DEGRADATION CONDITIONS		DEGRADATION PARAMETERS	
	pH	TEMP / ^{o}C	$t_{10\%\ loss}$ / Hours	$t_{50\%\ loss}$ / Hours
Vicryl	2.3	37	600	1320
Dexon	2.3	37	785	1805
PDS	2.3	37	610	975
Vicryl	7.4	37	460	650
Dexon	7.4	37	560	780
PDS	7.4	37	1200	2375
Vicryl	10.6	37	~20	~160
Dexon	10.6	37	~20	~150
PDS	10.6	37	640	1120
Vicryl	7.4	70	~10	~65
Dexon	7.4	70	~15	~100
PDS	7.4	70	~80	~140

PHB/PHV copolymer matrices, with the data showing that under *in vitro* physiological degradation conditions there is very little difference between the samples exposed to the plasma and the untreated discs. At elevated pH's however, there is a noticeable difference between the treated and untreated samples with the surface etched discs showing a faster weight loss presumably due to the increase in matrix surface area resulting from the oxygen plasma treatment.

Table 3.7 Weight Loss Parameters for Oxygen Plasma Treated Melt Pressed Discs Under Various Hydrolytic Degradation Conditions at 37°C

SAMPLE		DEGRADATION CONDITIONS	DEGRADATION PARAMETERS			
Molecular Weight/10^{-5}	% PHV	pH	$t_{1\%}$ loss / Hours	$t_{5\%}$ loss / Hours	$t_{10\%}$ loss / Hours	$t_{50\%}$ loss / Hours
3.0	20	7.4	4700	(1.2% @ 5560)		
3.5	12	7.4	5230	(1.1% @ 5560)		
3.0	20	10.6	1000	1730	2200	6300
3.5	12	10.6	1445	2350	3550	(40% @ 6600)

3.3.6 BLOOD PLASMA DEGRADATION

In order to attain a better *in vivo* / *in vitro* comparison several melt pressed discs were immersed in approximately 3 mls of human blood plasma for 15 hour intervals. The study was followed for 370 hours for most of the samples, with one disc degraded for 600 Hours.

The weight loss of the plasma treated discs is illustrated on Graph 3.8 with a comparison of the extent of weight loss for plasma treated and untreated discs given in Table 3.8.

The results show that blood plasma treated discs degrade marginally faster than those subjected to a pH 7.4 buffer @ 37^{o}C. Another comparison that can be tentatively be made taking into account the small changes in weight loss observed is that the oxygen plasma pretreated 300PHB (20%PHV) discs also show a slight increase in the rate of hydrolysis for blood plasma treated samples over that of the pH 7.4, 37^{o}C conditions (0.37% for the blood plasma sample against 0.31% for the buffer treated sample after approximately 300 hours). This emphasises the effect that pre-roughening of the matrix surface has on the degradation rate of the matrices.

3.3.7 WATER UPTAKE STUDIES

In addition to the weight loss studies monitored during the hydrolytic breakdown of the PHB/PHV copolymer samples, the extent of matrix hydration by the degrading medium i.e. the 'wet' weight

GRAPH 3.8 Weight Loss of PHB/PHV Discs in Blood Plasma at 37°C

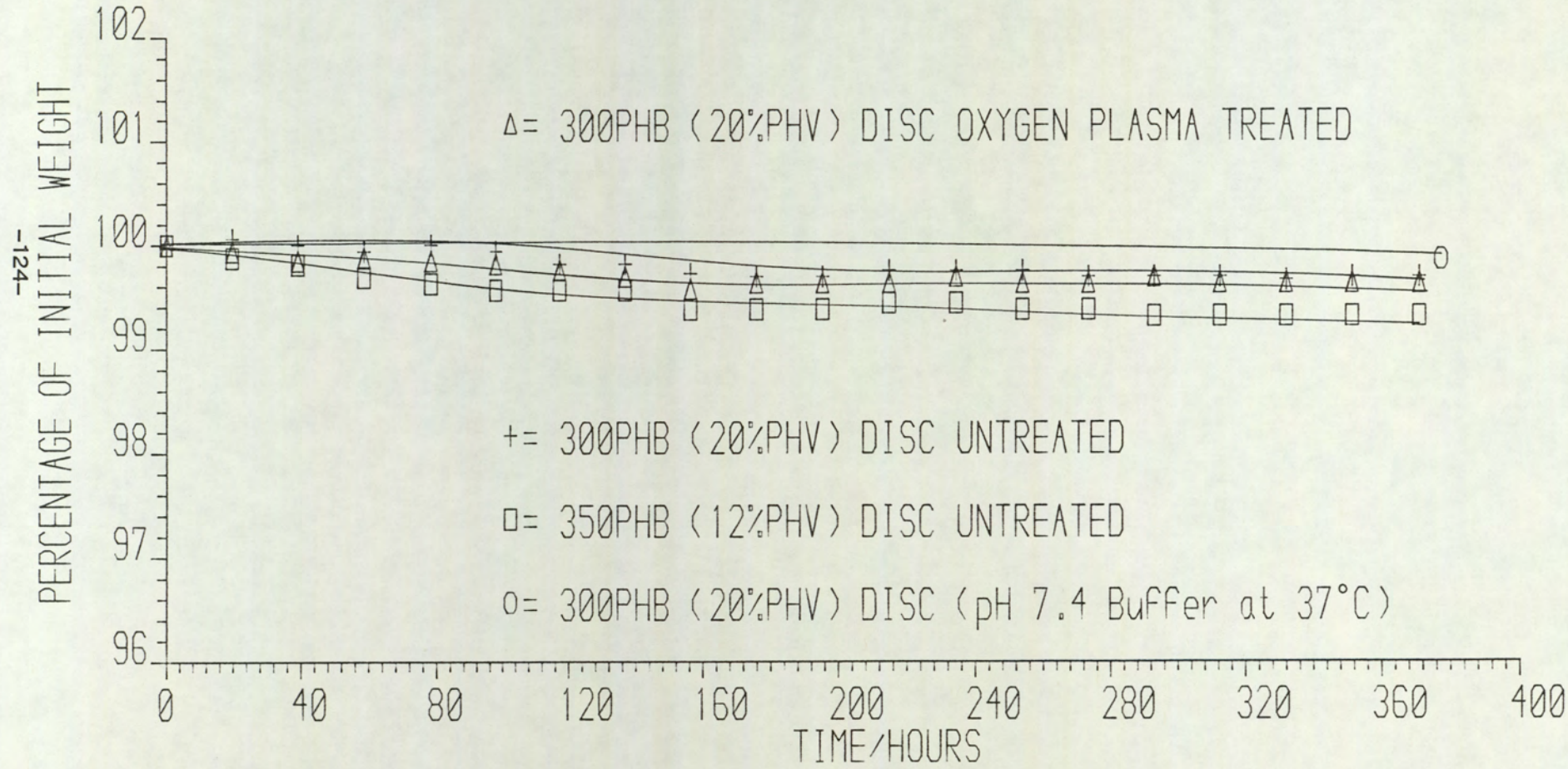


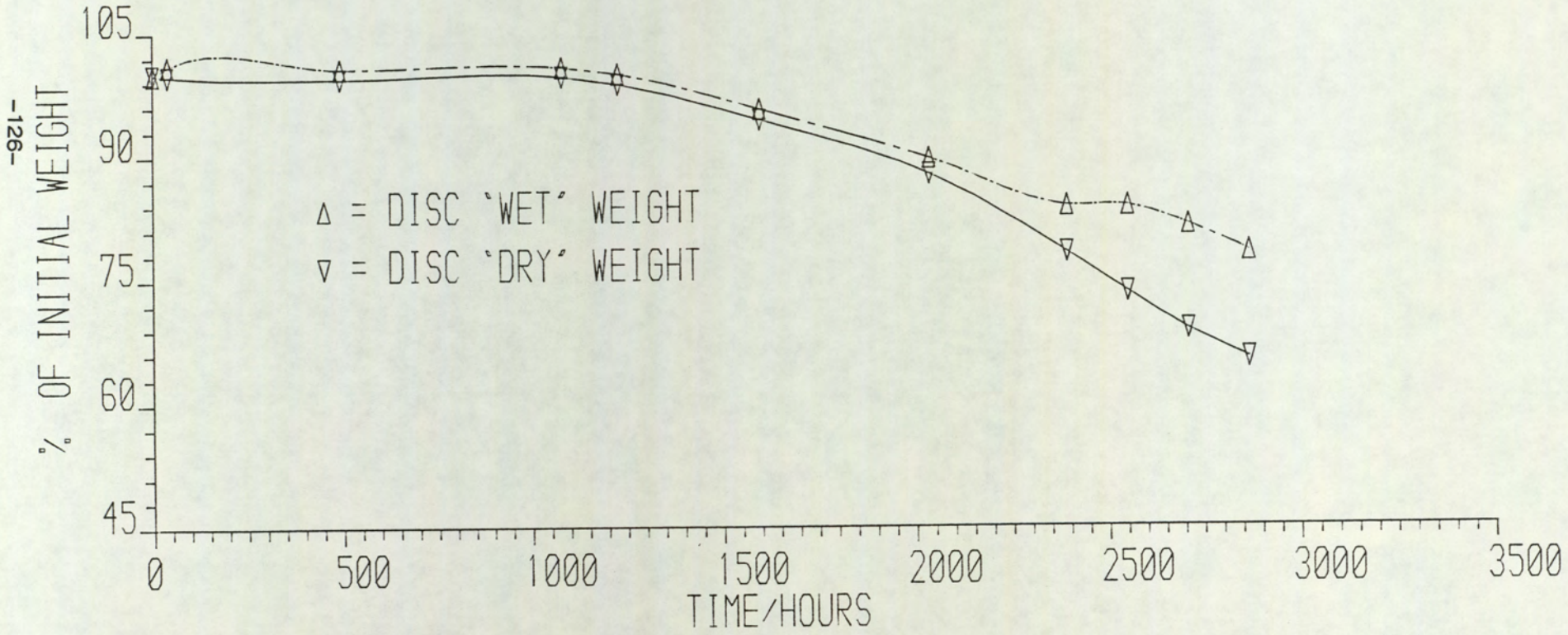
Table 3.8 Weight Loss Parameters for Blood Plasma Treated Discs and Discs Degraded in a pH 7.4 Buffer @ 37°C

SAMPLE		DEGRADATION CONDITIONS			DEGRADATION PARAMETERS
Molecular Weight/10^{-5}	% PHV	O_2 Plasma Pretreated	pH 7.4 / 37°C	Blood Plasma	Weight loss / % @ Time t / Hours
3.0	20	+	–	+	0.37 @ 370
3.0	20	–	+	–	0.41 @ 310
3.0	20	–	+	–	0.58 @ 630
3.0	20	–	–	+	0.31 @ 370
3.0	20	–	–	+	0.77 @ 608
3.0	20	–	+	–	0.73 @ 608
3.5	12	+	–	+	0.69 @ 370
3.5	12	–	+	–	0.45 @ 413
3.5	12	+	+	–	0.32 @ 413

+ = Degradation Treatment; – = N/A.

was measured. Graph 3.9 plots the hydrated and dehydrated weights of a 300PHB (20% PHV) melt pressed disc degraded in a pH 7.4 buffer at 70°C. At enhanced degradation times the difference between 'wet' and 'dry' weights increases significantly indicating an enlargement of internal surface area and matrix porosity as the (dry) weight loss surpasses 20%.

GRAPH 3.9 Water Uptake of a 300PHB (20%PHV)
Melt Pressed Disc in a pH 7.4 Buffer at 70°C



3.3.8 GPC STUDIES

Figures 3.1 and 3.2 show the gel permeation chromatograms of an undegraded and substantially degraded (100°C, pH 7.4 aqueous buffer for 190 hours with an associated weight loss of 80%) 750PHB (10%PHV) sample. It appears that the polymer eventually degrades to its oligomers as indicated by the discrete peaks observed at the low molecular weight end (RHS) of the spectra.

3.4 DISCUSSION

The buffer degradation studies on a series of PHB/PHV copolymer samples of varying physical form show weight loss profiles which are characterised by an initial slow plateau period followed by a secondary accelerated degradative phase. The rate of polymer matrix hydrolysis depends on polymer molecular weight and copolymer composition with an increasing resistance to hydrolytic attack as molecular weight increases together with a corresponding decrease in the degradation rate as the valerate content of the copolymers is increased. Increasing the alkalinity of the buffer enhances the degradation rate which indicates ester hydrolysis as the mechanism of degradation. A change in temperature from physiological to 70°C generally increases the hydrolysis rate some 30 to 100 fold depending on the physical form of the matrix. It must be noted however that a slight increase in the rate of degradation would be expected as there is a small pH increase as

Figures 3.1 and 3.2: GPC chromatograms of an undegraded (Fig.3.1) and a substantially degraded (Fig 3.2 - pH 7.4 buffer at 100°C, for 90 hours) 75OPHB (10%PHV) solvent cast film.

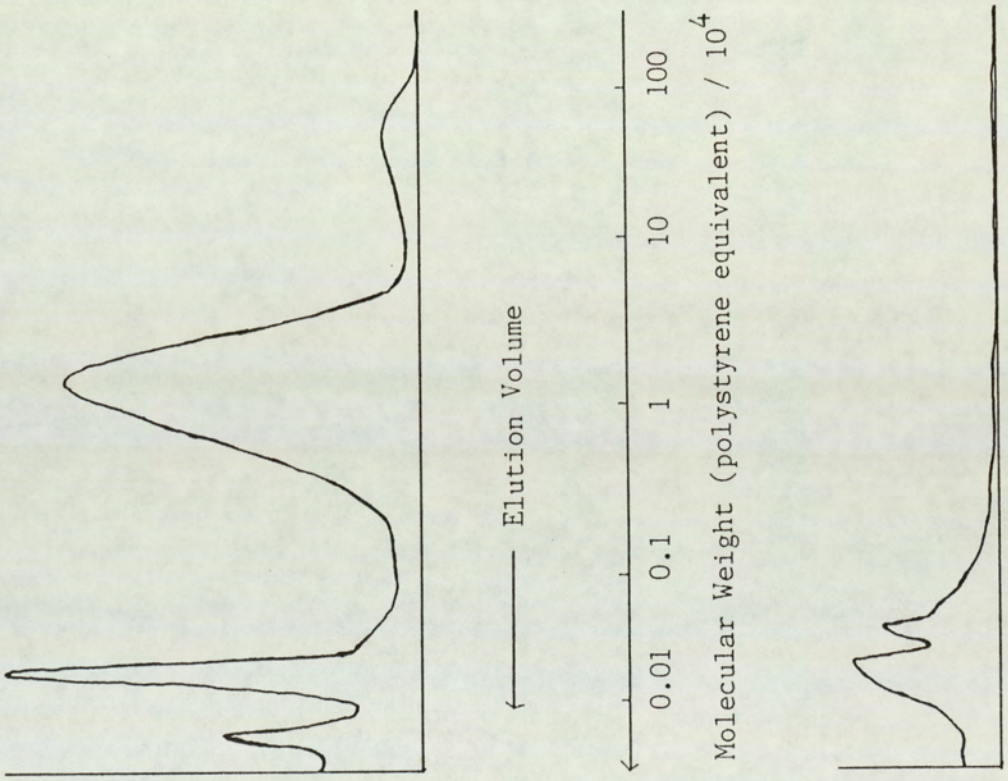


Fig 3.1

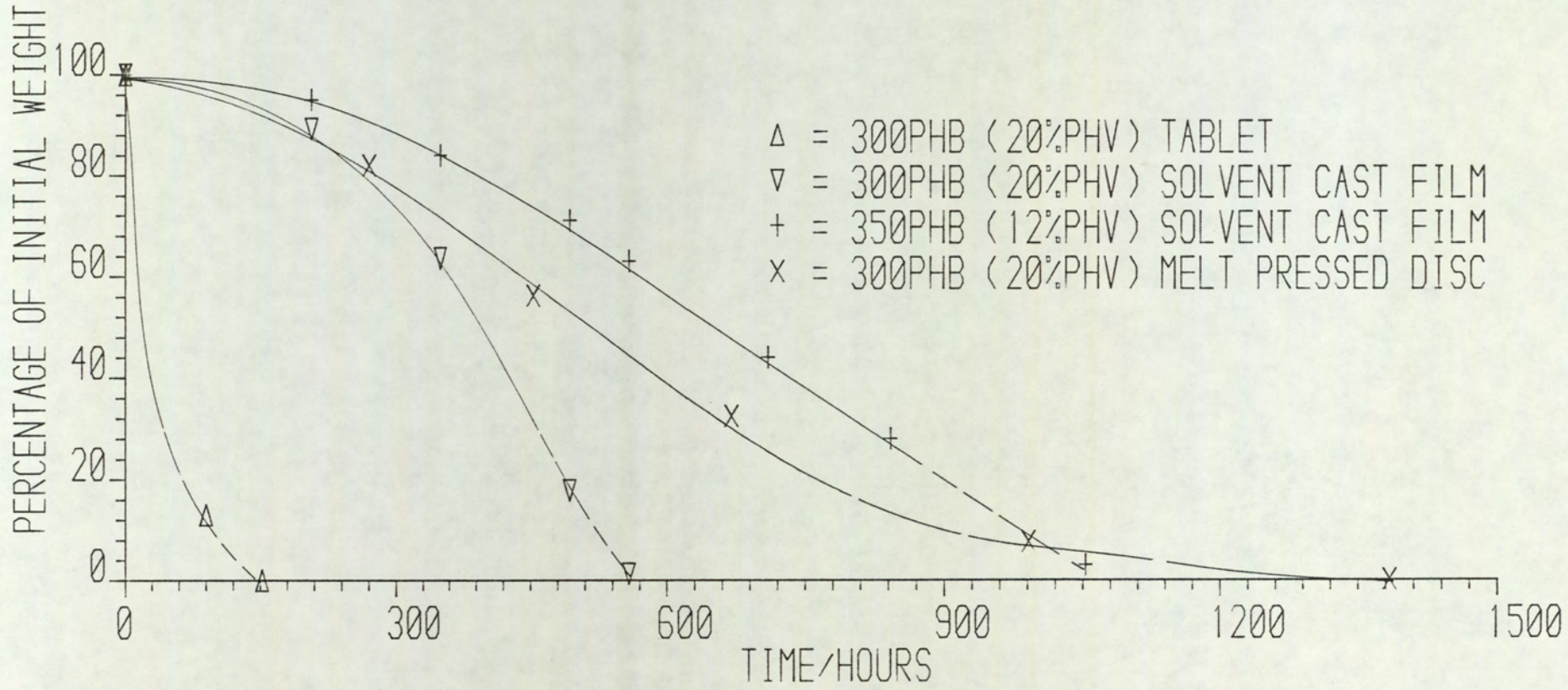
Fig 3.2

the temperature is raised. Some useful comparisons can be made between the lower temperature alkaline buffer studies (37^{o}C, pH 7.4) and the high temperature acidic buffer studies (70^{o}C, pH2.3).

Accurate studies of the degradation are possible until enhanced breakdown of the matrix occurs, which varies from 10 to 20% onwards for the solvent cast films to 20 - 45% onwards for the melt pressed discs depending on the degradation conditions. Further weight loss data serves as an indication of the durability of the samples to hydrolysis rather than an accurate measurement of matrix weight loss since catastrophic fragmentation of samples occurs, which leads to errors in sample analysis.

A comparison of the relative rates of degradation of the three physical forms of the PHB/PHV copolymer series tested in a pH 10.6 buffer maintained at 70^{o}C, is shown in Graph 3.10. The various types of matrix have differing stabilities to hydrolytic attack, the cold compressed tablets being the least stable, with an increase in stability to solvent cast films through to melt pressed discs. This change in degradation rate reflects differences in two inherent physical properties of the three matrix types; these being polymer crystallinity and matrix compaction. An increase in crystallinity occurs over the matrix series of powdered PHB/PHV (cold compressed tablets) to solvent cast films and melt pressed discs. This trend explains in part, the increased resistance to hydrolysis of the discs and films compared to the tablet type of matrix. Changes in compaction of

GRAPH 3.10 Comparison of the Degradation Rates of Various Physical Forms of PHB/PHV copolymers in a pH 10.6 Buffer at 70°C



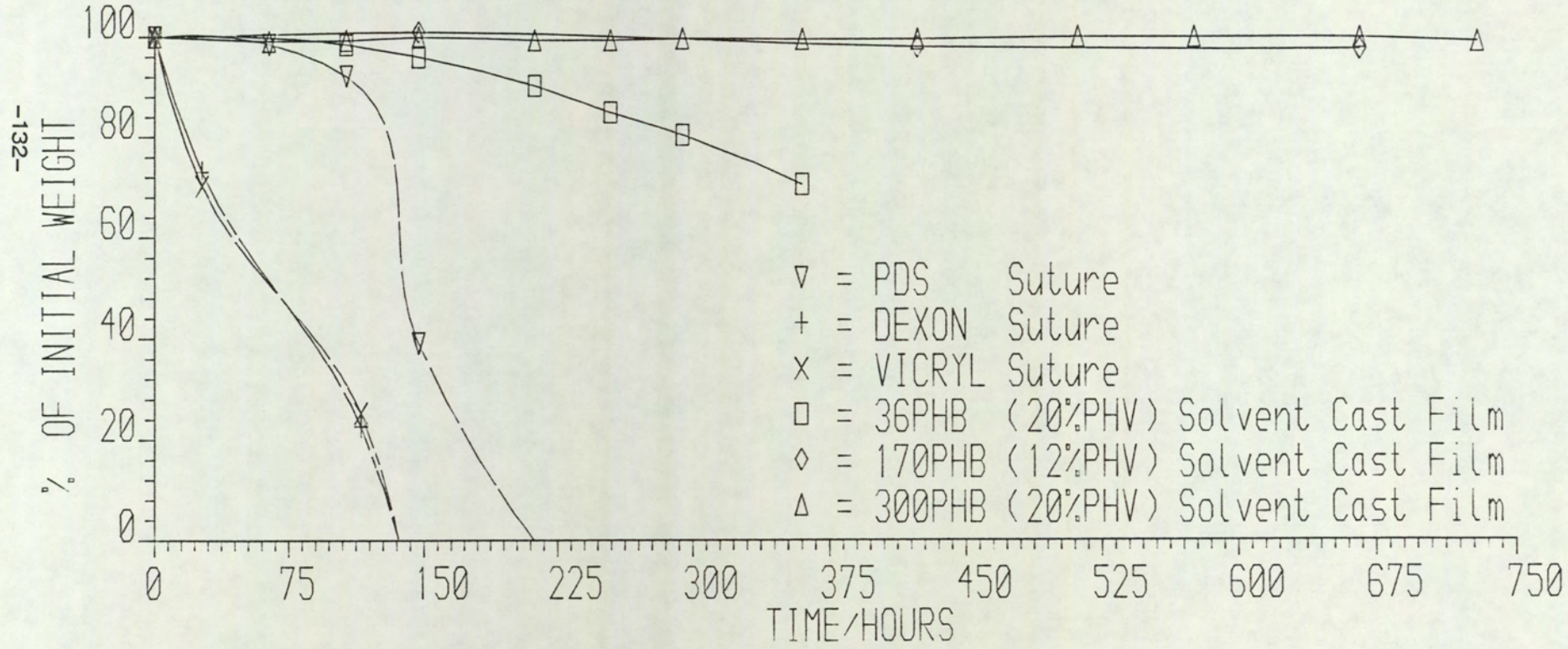
the polymer chains due to the method of fabrication are also evident. The cold compressed tablets are a physical blend and have a low compaction. The solvent cast films however exhibit better homogeneity but are less crystalline and compacted than the melt pressed discs which have been thermally processed under pressure.

Further evidence of this compaction theory derives from the buffer hydrolysis studies by Yasin[208] on injection moulded PHB/PHV pieces (c.f. Table 3.9), which show an increase in stability to hydrolysis over that of the melt pressed discs examined in this work. Injection moulded pieces are prepared under more vigorous conditions (higher temperature and pressure) than the discs and would therefore be expected to have a higher crystallinity and compaction which would lead to the observed slower rate of hydrolysis.

Comparison of the breakdown of the PHB/PHV copolymer series with commercial bioabsorbable suture materials is shown in Graph 3.11. The plots show a considerable increase in degradation rate from the least stable of the PHB/PHV series tested - the mechanically inadequate 36PHB (20% PHV) to PDS, the most stable suture.

Differences in the rates of suture degradation show that the PLA/PGA copolymer - Vicryl degrades faster than the PGA homopolymer suture - Dexon. As the sutures are of similar molecular weights this difference is presumably due to the large crystallinity contrast between the highly crystalline homopolymer

GRAPH 3.11 Weight Loss Comparison of PHB/PHV Matrices and Commercial Sutures in a pH 7.4 Buffer at 70°C



and the less crystalline copolymer, which overwhelms any stability that the more hydrophobic lactic acid units would be expected to show in the PLA/PGA copolymer. The increase in stability of PDS relative to the two poly alpha ester suture materials is seen not only in the lower rate of hydrolysis, but also by its ability to form monofilaments as opposed to the multifilamental form of Dexon and Vicryl. However a contribution to the increase in degradation rate of the multifilament sutures over that of the monofilamental PDS can be attributed to the higher surface area of polymer exposed to the degrading medium.

The deduced order of suture hydrolytic stability compares well with similar literature work on buffer hydrolysis of PDS,[49-55] Vicryl.[102-108] Reported work on the hydrolysis of Dexon by Chu[59] and others[63] shows a somewhat slower rate of degradation than that determined in these studies. This can be attributed to the differences in hydrolytic media used, with Chu using distilled water[78,82] (pH=6.8) which became more acidic as the (acidic) degradation products of the PGA were dispersed into the water. Reed[63] on the other hand used a pH 7.0 buffer system as opposed to the pH 7.44 buffer used in these studies. The accelerated degradation of the PDS sutures in acidic conditions is previously unreported and a simple ester hydrolysis mechanism can not account for this. The overall order of suture stability appears to be PDS > Dexon > Vicryl although true comparisons at equivalent molecular weights, crystallinities and suture form are impossible to attain,

as are comparative literature results at equivalent degradation conditions!

The complexities of collating the various rates of hydrolysis for the various biodegradable polymers used in this work are further illustrated when the times for 10% and 50% weight loss for the suture materials are contrasted with the various physical forms of the PHB/PHV copolymer series. Table 3.9 lists these degradation times for all the samples studies under one specific set of hydrolysis conditions - pH 7.4 buffer at the accelerated temperature of 70^{o}C.

True comparisons between the various polymer forms can only be made when the molecular weight, copolymer composition (where applicable) and mechanical properties are equivalent. However if one considers the most suitable candidate of the PHB/PHV series for comparisons with the suture materials - the 36PHB (20% PHV), it has very poor mechanical properties, hence relevant correlations can only be made on polymer samples having suitable molecular weights and physical properties for device fabrication. In the case of the PHB/PHV series the most suitable polymer grades for film and disc fabrication are those with valerate contents of between 10 and 20%, with a molecular weight ($\overline{Mw}$) of at least $2x10^{5}$.

Assuming this criterion for comparisons is valid, it can be seen that PHB/PHV copolymers are very much more stable to hydrolysis than commercially available bioabsorbable materials. This stability applies even in the more applicable blood plasma in

Table 3.9 Weight loss Parameters for PHB/PHV Copolymer Samples and Commercial Sutures in a pH 7.4 Buffer @ 70°C

SAMPLE				DEGRADATION PARAMETERS	
Polymer	Physical Form	Molecular Weight/10^{-5}	% PHV	$t_{10\%}$ loss / Hours	$t_{50\%}$ loss / Hours
Vicryl	Suture	~0.4	N/A	~10	~65
Dexon	Suture	~0.4	N/A	~15	~70
PDS	Suture	~0.6	N/A	~80	~140
PHB/PHV	Film	0.36	20	200	400
PHB/PHV	Film	1.0	12	825	2000
PHB/PHV	Film	1.7	12	800	(37% @ 1980)
PHB/PHV	Film	3.0	20	1850	2300
PHB/PHV	Film	3.5	12	1880	2900
PHB/PHV	Film	4.0	7	(2.2% @ 1010)	
PHB/PHV	Film	7.5	10	1950	2400
PHB/PHV	Film	8.0	0	2075	(26% @ 2360)
PHB/PHV	Disc	3.0	20	2020	3080
PHB/PHV	Disc	3.5	12	2800	3125
PHB/PHV	Disc	4.0	7	2170	(12% @ 2290)
PHB/PHV	Disc	8.0	0	(1.4% @ 2290)	
PHB/PHV	I.M.*	3.0	20	2200	(15% @ 2450)
PHB/PHV	I.M.*	3.5	12	1900	(22% @ 2450)

*Results from a study by Yasin[209] (I.M. = Injection Moulded sample)

vitro model where the rate of hydrolysis is very slightly increased. As discussed in section 1.3.2.6 the literature[148-153] gives very little information about the rate and nature of the *in vivo* degradation rate of the range of PHB/PHV copolymers, with work by Korsatko[151,152] showing slow degradation both *in vivo* and *in vitro*.

The secondary phase of the weight loss profiles is characterised by a large increase in porosity (as determined by the water uptake studies on the melt pressed disc samples) indicating extensive degradation throughout the bulk of the matrix i.e. homogeneous degradation. This bulk degradation is a particularity of the polyester series and attempts to predict the extent of degradation of the bulk using surface techniques are discussed in the next chapter.

CHAPTER FOUR

SURFACE CHARACTERISATION OF POLYMER DEGRADATION

4.1 INTRODUCTION

A full evaluation of the bulk hydrolytic degradation of the PHB/PHV copolymer series is given in the previous chapter. This chapter describes three experimental techniques - goniophotometry, surface energy measurement and scanning electron microscopy (SEM), which very accurately measure matrix physical properties and map the surface topography. Also discussed are the attempts to correlate the surface data at various stages of polymer matrix degradation using these methods in order to give an accurate picture of the extent of the bulk degradation.

4.2 DEGRADATION CONDITIONS

Hydrolytic degradation by aqueous buffers, of films and melt pressed discs fabricated from PHB/PHV copolymers were carried out as outlined in section 3.2

4.3 SURFACE TECHNIQUES

Three different methods were used to examine polymer matrix surfaces. Two of these had the advantage of being non-destructive, the third - SEM, involved preparation by coating the test samples with a layer of gold, hence no further hydrolytic studies were possible on these samples.

4.3.1 GONIOPHOTOMETRY

4.3.1.1 Introduction

Goniophotometry measures the intensity of light reflected from a surface as a function of viewing angle. Light is reflected from a surface by two different processes: Either by surface reflection at the surface / air boundary, or by reflection of light which has been scattered by penetration of the sample surface. Surfaces can be said to exist between two extremes: a) as a surface which behaves as a perfect mirror i.e. one which scatters no light and reflects all light at the incident angle (this is termed the 'specular reflectance'), and b) as a surface which scatters reflected light at an equal intensity in all directions (this is termed 'diffusive reflectance').

A useful parameter for categorising surfaces is the measurement of surface gloss.[194,210] The 'gloss factor' (GF) of a sample can be quantitatively defined by goniophotometry. It is expressed as the difference between the maximum angular reflectance (I_s, which occurs at the angle of incidence) and the diffuse reflectance (Id, which is the reflection normal to the sample surface), divided by the Peak Width at Half Height ($W_{1/2}$). This relationship which represents the maximum difference between the three measured parameters, is summarised in Equation 4.1, with an idealised goniophotometric spectrum illustrating the different parameters that can be derived presented in Fig 4.1.

$$G.F = \frac{(I_s - I_d)}{W_{1/2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 4.1$$

Goniophotometry is an accurate technique and can indicate changes in the size of surface features of less than the wavelength of light. As a surface deteriorates/degrades surface rugosity increases. During the initial stages of degradation when surface defects are less than the wavelength of light there is an associated drop in the measured specular intensity of a goniophotometric spectra with no change in peak profile i.e. $W_{1/2}$ and Id are approximately the same. As the size of surface imperfections increases to around the wavelenth of light spectrum peak broadening occurs and this is quantitatively seen as an increase in the peak width half height, with a continuing diminishment of I_s and an increase in I_d. A spectrum illustrating this stage of degradation is represented in Fig 4.2. Further degradation distorts the previously symmetrical peak with the I_d value approaching the I_s value as the surface nears a 'matt' appearance. A representative spectrum of this stage of degradation is presented in Fig 4.3.

Another useful parameter derived from goniophotometric spectra is the maximum peak height (= $I_s - I_d$) which gives a more simplified indication of the extent of surface gloss than the GF value.

Figure 4.1 A typical Goniophotometric Spectrum indicating the various derived parameters[194]

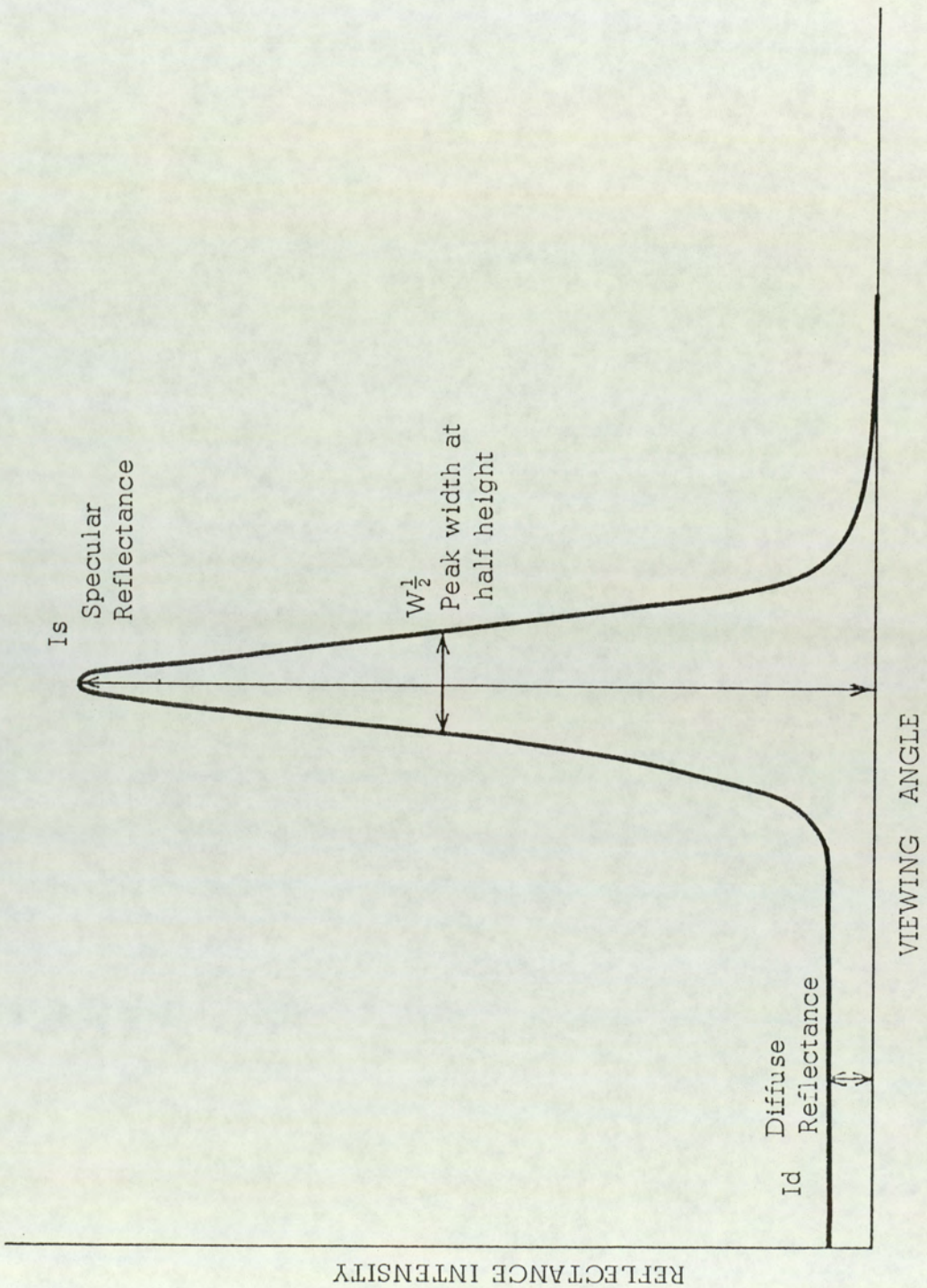
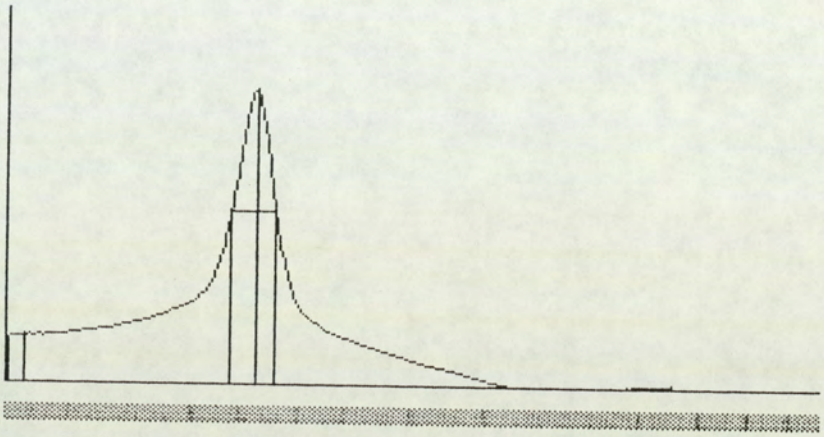


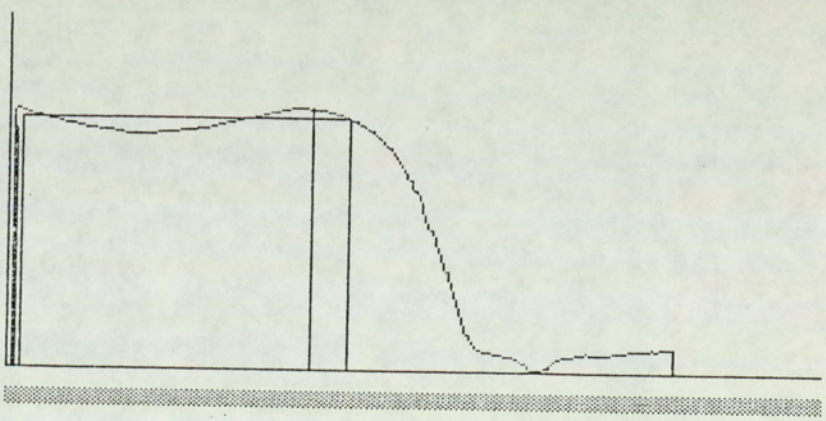
Figure 4.2 Representative goniophotometric spectrum of a partially degraded sample



GONIOPHOTOMETRIC
CURVE -- CODE NO., D300T1

Is = 6.73 UNITS AT 87 DEGREES
Id = 1.11 UNITS AT 131 DEGREES
PEAK HEIGHT = 5.62 UNITS
HALF PEAK WIDTH = 8.5 DEGREES
GLOSS FACTOR = 0.66

Figure 4.3 Representative goniophotometric spectrum of a substantially degraded sample



GONIOPHOTOMETRIC
CURVE -- CODE NO., D30074

Is = 6.13 UNITS AT 76 DEGREES
Id = 5.58 UNITS AT 133 DEGREES
PEAK HEIGHT = 0.55 UNITS
HALF PEAK WIDTH = 68 DEGREES
GLOSS FACTOR = 0.01

4.3.1.2 Results and Discussion

The melt pressed disc form of PHB/PHV was the most suitable for goniophotometric determination of surface changes during the degradation studies. The goniophotometry spectra of various discs were taken concurrently with the hydrolysis studies outlined in chapter three. The goniophotometric parameters derived from the spectra were calculated using a Sharp MZ-80K computer.

Summarised in Tables 4.1 and 4.2 are the goniophotometric parameters of discs at various stages of degradation, together with an indication of the weight loss and degradation conditions. From the tabulated data it can be seen (for example the 300PHB 20% PHV discs degraded in a pH 7.4 buffer at 37°C) that the I_s of the discs increases from its original pre-degradation value to a maximum before decreasing as hydrolysis becomes more pronounced. A corresponding small increase in the I_d value occurs and the peak width broadens, indicating a roughening of the surface. There is a small difference between the different copolymers used with the lower valerate sample showing marginally higher I_d values for the pH 2.3 aqueous buffer degradation conditions at 37°C.

A contrast between the various hydrolytic degradation conditions can be made. This is illustrated by the changes in the goniophotometric parameters that occur when a 300PHB (20% PHV) melt pressed disc is degraded for approximately 3000 hours in pH 2.3, 7.4 and 10.6 buffers @ 37°C. The low pH and neutral buffers

Table 4.1 Summary of Goniophotometric data for a hydrolytically degraded 300PHB (20% PHV) melt pressed disc

DEGRADATION PARAMETERS			GONIOPHOTOMETRY PARAMETERS				
Weight loss @ x Hrs	pH	TEMP /°C	I_s	I_d	Peak Height	$W_{1/2}$ /°	Gloss Factor
---	N/A		54.7	0.79	54	4	13.48
---*	N/A		10.8	0.91	9.9	4	2.48
0.77% @ 608	B.P	37	14.5	1.01	13.5	8.5	1.58
~0.6% @ 3737	2.3	37	16.1	0.91	15.2	5.5	2.76
~0.6% @ 4076	2.3	37	20.5	0.99	19.5	7	2.79
0.66% @ 4315	2.3	37	7.14	1.16	5.98	9	0.66
0.12% @ 240	7.4	37	12.5	1.01	11.5	4.5	2.56
0.14% @ 377	7.4	37	25.8	0.99	24.8	5.5	4.51
0.17% @ 610	7.4	37	45.2	0.79	44.4	4.5	9.87
0.91% @ 3737	7.4	37	42.0	0.79	41.2	3.5	11.78
0.92% @ 4210	7.4	37	29.8	1.18	28.6	5	5.72
0.94% @ 4450	7.4	37	8.14	1.26	6.88	5.5	1.25
10.5% @ 2419	10.6	37	2.01	1.36	0.65	30	0.02
20% @ 3310	10.6	37	3.07	2.96	0.11	>30	~0
4.43% @ 169	10.6	70	2.06	1.56	0.5	24	0.02
9.46% @ 377	10.6	70	1.76	1.66	0.1	>24	~0

* = Pretreated with an oxygen plasma; B.P. = Blood Plasma.

Table 4.2 Summary of Goniophotometric data for a hydrolytically degraded 350PHB (12% PHV) melt pressed disc

DEGRADATION PARAMETERS			GONIOPHOTOMETRY PARAMETERS				
Weight loss @ z Hrs	pH	TEMP /°C	I_s	I_d	Peak Height	$W_{1/2}$ /°	Gloss Factor
---	N/A		31.1	1.21	29.9	4.5	6.65
---*	N/A		11.0	1.31	9.7	4	2.43
0.78%* @ 370	B.P	37	10.6	1.52	9.1	7.5	1.21
~0.8% @ 3737	2.3	37	41.6	1.59	40.0	6.5	6.16
~0.8% @ 4076	2.3	37	31.3	1.58	29.8	8	3.72
0.88% @ 4315	2.3	37	12.0	1.31	10.7	9	1.19

*= Pretreated with an oxygen plasma; B.P. = Blood Plasma.

are approximately the same, but the alkali buffer produces a matt surface ($I_d \sim I_s$ and G.F approximately equal to zero) after 3300 hours.

The low temperature pH 7.4 treated 300PHB (20% PHV) discs exhibit surface polishing by buffer hydrolysis as shown by an initial increase in I_s and GF up to hydrolysis times of three weeks. After more than twenty weeks of degradation however, these values fall consistently up to and including the goniophotometric spectra taken after 4450 hours of buffer hydrolysis.

Elevated degradation temperatures (70°C) produce matt surfaces relatively quickly, with the gloss factor tending towards

zero after only one week of degradation in a pH 10.6 buffer (corresponding matrix weight loss = 4.4%). The less severe hydrolysis study in a pH 7.4 buffer at the same temperature also produces a matt surface. The degradation time however being some 15 times longer (2500 hours).

Pre-treatment of the polymer discs with an oxygen plasma modifies the surface features with a decrease in I_s together with a slight increase in I_d occurring (c.f. Table 4.1) indicating a large increase in surface imperfection size. Similarly, blood plasma degradation broadens the observed goniophotometric peak relative to the undegraded disc data. The series of degradative treatments on 350PHB (12% PHV) samples ranging from untreated to oxygen plasma pre-treated to oxygen plasma plus blood plasma degradation show a cumulative effect on the gloss factor which decreases from 6.57 to 2.43 and to 1.21 respectively. These profiles are shown in Figures 4.4 to 4.6.

Initial hydrolytic degradation (300PHB 20% PHV, pH 7.4 at 37^{o}C) produces a decrease in the observed I_s indicating changes in surface defects less than the wavelength of light. This is followed at latter times by peak broadening which is a better indication of the extent of matrix degradation than the associated (very small) weight loss. Graph 4.1 illustrates the changes of the goniophotometric parameters with degradation time for a 350PHB (12%PHV) disc degraded in a pH 2.3 buffer at 37^{o}C .

GRAPH 4.1 CHANGES OF GONIOPHOTOMETRIC PARAMETERS AS A FUNCTION OF MATRIX DEGRADATION TIME FOR A 350PHB (12%PHV) MELT PRESSED DISC DEGRADED IN A pH 2.3 BUFFER AT 37°C

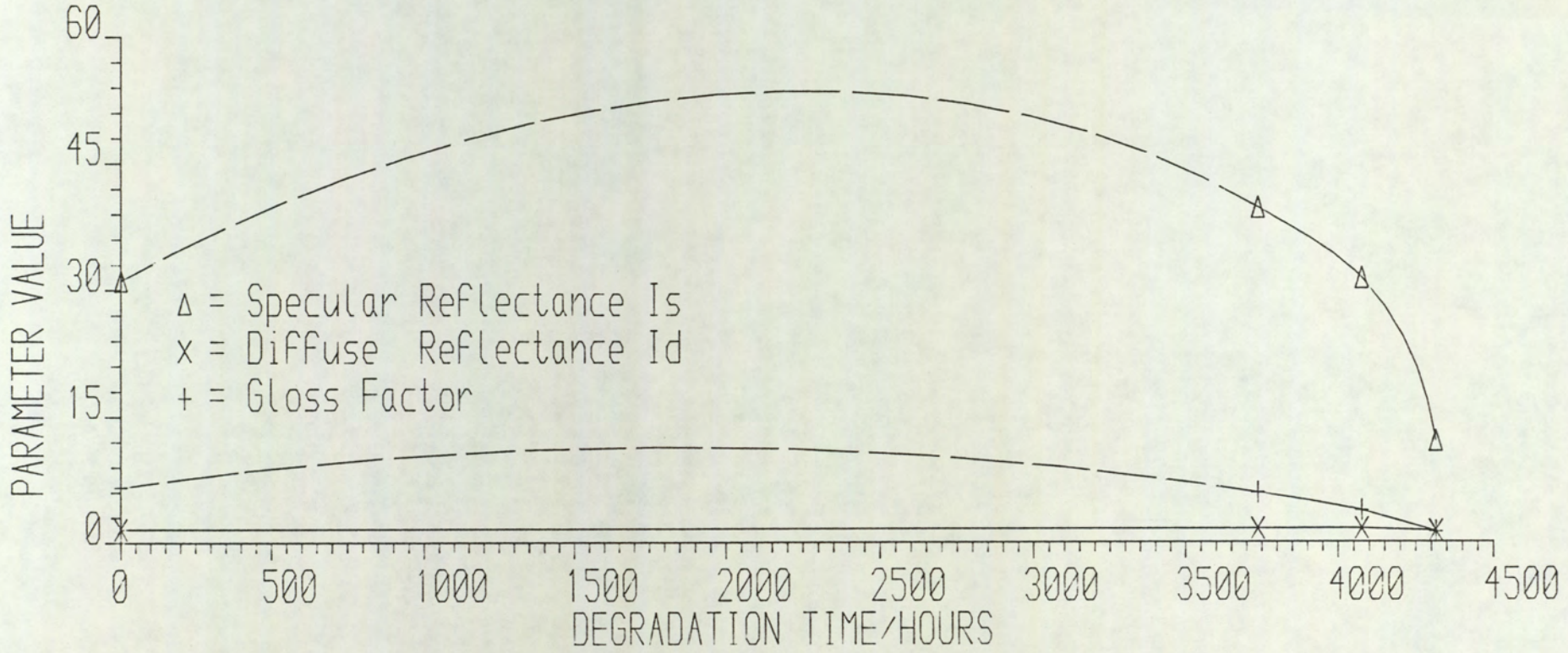
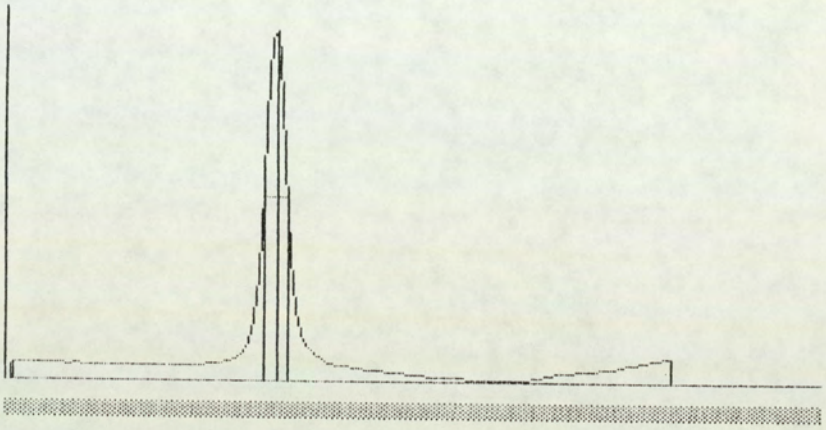


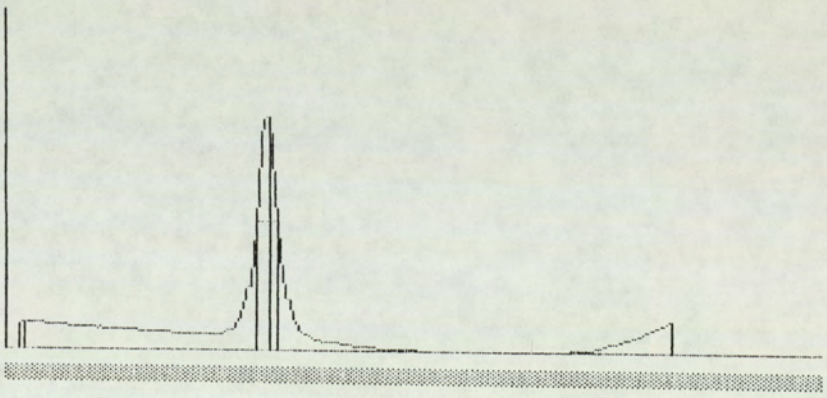
Figure 4.4 Goniophotometric spectrum of an undegraded 350PHB (12% PHV) Disc



G O N I O P H O T O M E T R I C
C U R V E -- C O D E N O., D350UN

Is = 31.14 UNITS AT 82.5 DEGREES
Id = 1.58 UNITS AT 1,38 DEGREES
PEAK HEIGHT = 29.56 UNITS
HALF PEAK WIDTH = 4.5 DEGREES
GLOSS FACTOR = 6.57

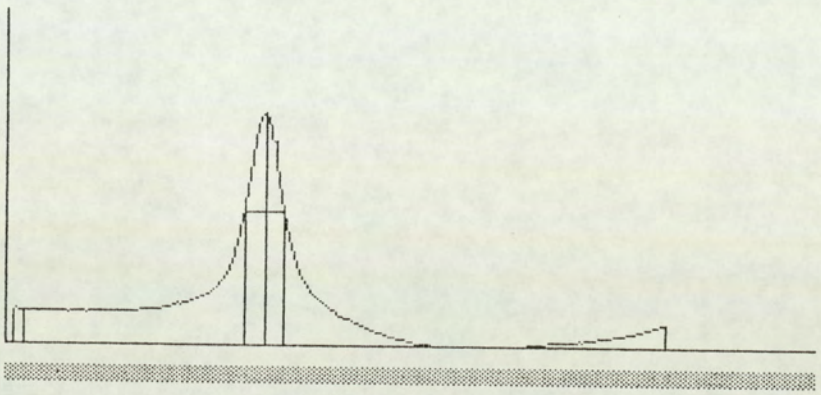
Figure 4.5 Goniophotometric spectrum of an undegraded 350PHB (12% PHV) Disc pre-treated with an oxygen plasma



G O N I O P H O T O M E T R I C
C U R V E -- C O D E N O., DISCT

Is = 11.82 UNITS AT 84 DEGREES
Id = 1.81 UNITS AT 131 DEGREES
PEAK HEIGHT = 9.71 UNITS
HALF PEAK WIDTH = 4 DEGREES
GLOSS FACTOR = 2.43

Figure 4.6 Goniophotometric spectrum of a 350PHB (12% PHV) Disc pre-treated with an oxygen plasma and degraded in blood plasma for 608 hours at 37°C



The gloss factor as defined in the introduction is not a very accurate measurement unless the samples are mounted reproducibly. It was found that small changes in the parameters occured if the samples were reorientated in the vertical plane i.e. rotated in the sample holder.

The best indication of surface changes is the profile of the goniophotometric spectra which alters as degradation of the matrix surface proceeds. For a typical disc in this study the profile is intially a sharp symmetrical peak which deceases in intensity as the degradation proceeds. As the degradation proceeds further an asymmetrical peak is formed which eventually reverts to a matt

surface profile. These stages have been outlined in Figures 4.1 to 4.3.

Errors arising from the electronics of the equipment are low with a possible scaling down of initial intensity from 1000 units to one unit with the same degree of accuracy i.e. the almost matt samples produced by alkali buffer hydrolysis have their surfaces evaluated with the same degree of accuracy as the intially 'glossy' melt pressed discs.

It appears that this technique is more accurate than sample weight loss for determining degradation of polymer matrices during the initial stages of hydrolysis, but it is limited in the case of PHB/PHV disc matrices by the production of a matt surface at relatively low weight losses. However, quantitative surface measurements are achievable with accurate sample mounting.

4.3.2 SURFACE ENERGY MEASUREMENT

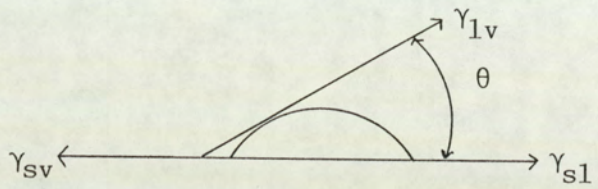
The second method for characterising the polymer matrix surfaces was the measurement of the surface free energy of the various samples throughout the degradation studies.

4.3.2.1 Introduction

A liquid drop in contact with a surface under normal atmospheric conditions can be considered as having three forces acting on it,[193] one at each of the interfaces that occur, these

being liquid/gas, solid/gas and solid/liquid - these are defined in Figure 4.7.

Figure 4.7 Forces acting upon a liquid drop in contact with a solid surface



Where

θ = The Contact Angle

γ_{lv} = The interfacial tension between the liquid and gas phases

γ_{sv} = The interfacial tension between the solid and gas phases

γ_{sl} = The interfacial tension between the solid and liquid phases

The interfacial tension of a surface can also be defined in terms of two components - polar and dispersive (Equation 4.2).

$$\gamma_t = \gamma_p + \gamma_d \qquad \text{..... 4.2}$$

Where:

γ_t = The total surface energy

γ_p = The polar component of the surface energy

γ_d = The dispersive component of the surface energy

Owens and Wendt[191] have related these components in terms of contact angles and surface energy components. If the liquids used for the surface energy determination have known and fixed values for the polar and dispersive contributions then the solid surface energy parameters can be determined from the contact angle measurements using the Owens and Wendt expression (Equation 4.3).

$$1 + \text{Cos}\,\theta = \frac{2}{\gamma_{lv}} \left[(\gamma_s^d \gamma_l^d)^{1/2} + (\gamma_s^p \gamma_l^p)^{1/2} \right] \qquad \ldots\ldots\ldots\ldots \text{4.3}$$

Where the subscripts s and l refer to the surface and liquid phases and the superscripts d and p refer to the dispersive and polar components. γ_{lv} is the liquid / surface tension and θ is the contact angle as defined in section 2.3.3.3.

The two most common liquids used are water and methylene iodide. These have large differences in their polar and dispersive component values and Table 4.3 lists the component values used for water and methylene iodide for the polymer matrix surface energy determinations.[193]

This experimental method reflects increases in the amount of carboxyl and hydroxyl groups on the characterised surface. This would be expected to occur during the hydrolytic degradation of a polyester matrix and is shown in the surface energy data by an increase in the polar component as the degradation proceeds.

4.3.2.2 Results and Discussion

The polar, dispersive and total surface energy components were measured for a series of PHB/PHV films and discs, with additional studies on the effect of matrix pre-treatment with an

Table 4.3 Surface energy parameters of the liquids used for contact angle measurements

LIQUID	POLAR COMPONENT / Dynes cm^{-1}	DISPERSIVE COMPONENT / Dynes cm^{-1}	TOTAL Dynes cm^{-1}
Water	51	21.8	72.8
Methylene Iodide	1.3	49.5	50.8

oxygen plasma and disc degradation by blood plasma. The effect of buffer hydrolysis was also studied.

Summarised in Table 4.4 are the free energy components with the measured contact angles for the solvent cast films studied, and Tables 4.5 and 4.6 list the surface energy data for the melt pressed disc buffer hydrolysis studies.

The contact angles were difficult to determine at enhanced stages at degradation because the discs became very porous which together with the associated increase in surface rugosity made accurate measurements impossible. The various observed trends from the tabulated data are now discussed.

The solvent cast film data shows little variation in polar and total surface energy values, but there is a decrease in the measured contact angle of methylene iodide resulting in an increase in the dispersive component of the surface energy as the valerate content in the copolymers decreases and the molecular weight increases. The film polar values however, are generally less than those of the melt pressed discs which in turn have higher total surface energy values.

Table 4.4 Surface energy parameters for PHB/PHV films

SAMPLE				SURFACE ENERGY COMPONENTS		
Molecular Weight/10^{-5}	% PHV	CA Water	CA MeI	POLAR /Dynes cm^{-1}	DISPERSIVE /Dynes cm^{-1}	TOTAL /Dynes cm^{-1}
3.0	20	74	43	7.4	34	41.4
3.5	12	73	49	9	30	39
4.0	7	73	32	6	39.3	45.3
7.5	10	70	33	8	38	46
8.0	0	75.5	38	6	37	43

CA = Average contact angle / degrees

Hydrolytic degradation by buffer solutions greatly increases the value of the polar component of the surface energy whilst the dispersive values fall slightly. This is shown by contrasting the undegraded 300PHB (20% PHV) disc polar value of 7.45 Dynes cm^{-1}

Table 4.5 Surface energy parameters for 300PHB (20% PHB) disc

DEGRADATION PARAMETERS					SURFACE ENERGY PARAMETERS		
WEIGHT LOSS % @ x Hours	pH	TEMP / ^{o}C	CA Water	CA Mel	POLAR /Dynes cm^{-1}	DISPERSIVE /Dynes cm^{-1}	TOTAL /Dynes cm^{-1}
---		N/A	69	26	7.45	41.1	48.5
---*		N/A	62	30	12	38.15	50.1
0.77 @ 608	BP	37	65	39	11.7	34.4	46.1
0.8 @ 3737	2.3	37	57	39.5	17	32.75	50
0.56 @ 4075	2.3	37	60	30	13.14	37.8	51
0.66 @ 4315	2.3	37	59.5	30.5	13.38	37.55	50.9
0.12 @ 240	7.4	37	58	34	15	35.7	50.7
0.14 @ 377	7.4	37	58	31	14.74	37	51.7
0.17 @ 610	7.4	37	59	30	13.77	37.64	51.4
1.05 @ 3737	7.4	37	6.5	27.5	40.75	33.4	74.1
10.5 @ 2450	10.6	37	16	35	40.7	30.6	72
25.6 @ 2500	7.4	70	9	13.5	37.5	37.6	75
4.43 @ 170	10.6	70	40.5	32	26.6	34.05	60.7
9.46 @ 377	10.6	70	<10	<20	---	---	---

CA = Average contact Angle / degrees, BP = Blood plasma

and * = Oxygen Plasma pre-treated

Table 4.6 Surface energy parameters for 350PHB (12% PHB) disc

DEGRADATION PARAMETERS					SURFACE ENERGY PARAMETERS		
WEIGHT LOSS % @ x Hours	pH	TEMP / ^{o}C	CA Water	CA Mel	POLAR /Dynes cm^{-1}	DISPERSIVE /Dynes cm^{-1}	TOTAL /Dynes cm^{-1}
---		N/A	65	29	10	39.1	49
---*		N/A	62	29.5	12	38.3	50.3
0.69* @ 608	BP	37	55	38.5	18.2	33	51.2
0.8 @ 3737	2.3	37	60.5	37	14	34.8	48.8
0.81 @ 4076	2.3	37	54	32	17.4	36.05	53.5
0.88 @ 4315	2.3	37	54	35	18.05	34.56	52.6
0.85 @ 3000	7.4	37	55	40.5	18.7	32	50.7
9.83 @ 3737	10.6	37	14	25	38.6	34.4	73
11.4* @ 3737	10.6	37	6.5	27.5	40.75	33.4	74

CA = Average contact angle / degrees, BP = Blood plasma

and * = Oxygen plasma pre-treated

with a similar disc treated at 37^{o}C for 4075 hours in a pH 2.3 buffer which has an increased polar component of 13 Dynes cm^{-1} (the corresponding dispersive values are 41.1 and 38 Dynes cm^{-1}respectively for the undegraded and pH 2.3 buffer hydrolysed samples). The difference in the rates of degradation by the various buffer used is examplified by the group of discs degraded at 37^{o}C in acidic, neutral and alkaline buffers. From Table 4.6 the polar values for a 350PHB (12% PHB) disc degraded for

approximately 3750 hours are 14, 18.5 and 38.5 Dynes cm^{-1} respectively for the aforementioned buffers. However, the pH 7.4 buffer treated disc values are for a sample degraded for 750 hours less than the others, thus emphasising the trend of increased polar components with enhanced degradation.

As already indicated the polar component would be expected to increase with hydrolysis time and this can be seen even during early degradation times with a two fold increase in this parameter evident for a 300PHB (20% PHV) disc degraded in a pH 7.4 buffer for only 240 hours. This trend is also seen in the slowly degrading pH 2.3 37^{o}C conditions with an increase in polar component from 14 to 17.5 to 18 Dynes cm^{-1} for a 350PHB (12% PHV) disc degraded for 3740, 4075 and 4315 hours respectively. Faster degradation of the polymer matrices at elevated temperatures is reflected in the surface energy measurements by a large increase in polar component corresponding to a huge decrease in the (apparent) contact angle of water on the matrix surface. The difference between the copolymer compositions used is once again demonstrated by comparing the calculated polar components of the 12 and 20% valerate PHB/PHV copolymers with molecular weights approximately 300,000. The effect of the relatively quickly degrading pH 10.6 aqueous buffer conditions at 37^{o}C on the polar components of the two different copolymers shows a greater increase for the higher valerate copolymer. For example after 3750 hours of buffer hydrolysis under the above conditions the 12%

valerate sample has a polar component of 38.5 Dynes $cm.^{-1}$ This can be compared to a value of 40.7 Dynes cm^{-1} for the higher valerate melt pressed disc matrix which measured some 1300 hours earlier.

Oxygen plasma pre-treatment manifests itself in an increased polar and decreased dispersive component value. For example the 300PHB (20% PHV) disc shows an alteration of the polar and dispersive values from 7.45 to approximately 12 Dynes cm^{-1} and 41 to 38 Dynes cm^{-1} respectively after plasma treatment. Blood plasma degradation also increases the polar value but to a lesser extent than the oxygen plasma treatment. 300PHB (20%PHV) discs exposed to blood plasma for 600 hours show smaller increases in the measured polar component compared to the samples degraded in the similar degradation conditions of a pH 7.4 buffer @ $37^{o}C$. The polar values increase from 7.5 Dynes cm^{-1} for the untreated disc to 12 and 14 Dynes cm^{-1} repectively for blood plasma and buffer treated discs.

However as already stated, polar component values greater than 20 Dynes cm^{-1} are not very accurate due to disc porosity, hence quantitative comparisons are only valid for values which are less than 20 Dynes cm^{-1} a value surpassed (in some cases) when disc weight loss is less than 5%.

4.3.3 SCANNING ELECTRON MICROSCOPY

The third surface technique used to study film and disc hydrolysis was scanning electron microscopy (SEM). This is a very accurate qualitative technique but has the major disadvantage that

samples cannot be used for further hydrolysis studies after SEM preparation.

4.3.3.1 Results and Discussion

Plates 4.1 to 4.3 show a substantially degraded melt pressed 350PHB (12% PHV) disc (pH 10.6, 70°C for 1385 hours, with 60% weight loss) at three magnifications. Illustrated well at high resolution is the crystalline morphology of the sample, the amorphous regions of the disc presumably having been removed by ester hydrolysis - as is the case with other polyester degradation.[82] For comparison, plates 4.4 and 4.5 show undegraded disc micrographs. Plate 4.6 shows an undegraded solvent cast film (300PHB 20% PHV). This can be contrasted with a hydrolytically degraded 750PHB (10% PHV) film in plate 4.7 (pH 7.4 buffer for 2760 hours at 70°C, weight loss 66%), which shows layers of film that have been eroded. These layers presumably form as the polymer is cast from solution with the higher molecular weight fractions depositing first. Plate 4.8 shows the problematic calcium phosphate deposits. This was confirmed by energy dispersive analysis which showed the deposits were composed of 40.4% Calcium and 49.5% Phosphorous with the majority of the rest being Potassium, which presumably came from the buffer solution used to degrade the sample.

Disappointingly, SEM shows only a small difference between the undegraded and blood plasma degraded 300PHB (20% PHV) discs,

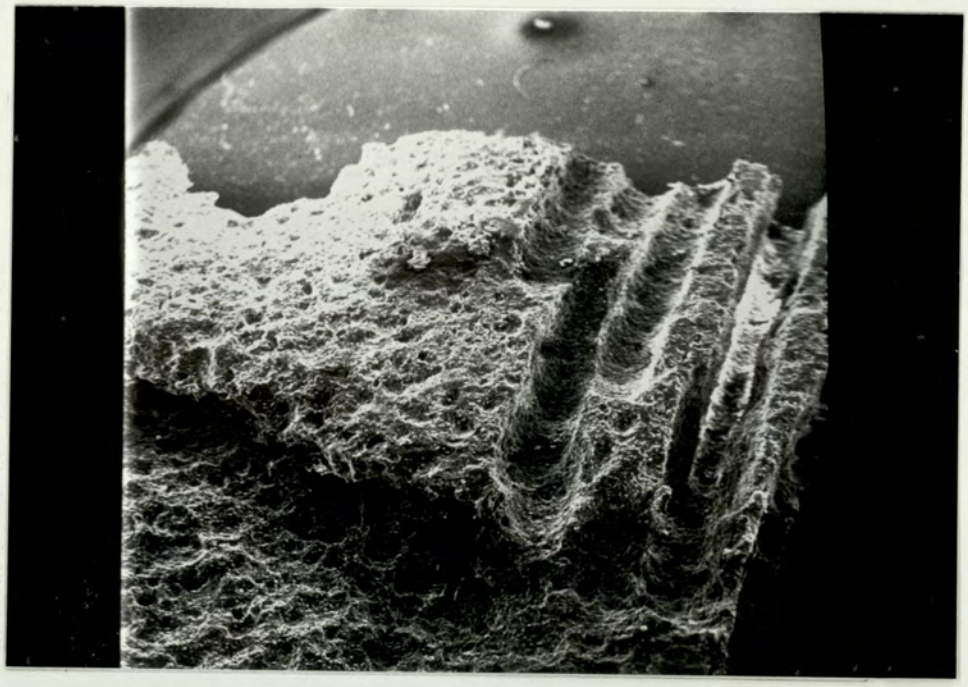


Plate 4.1 SEM (X 22) of a degraded 350PHB (12%PHV) Melt Pressed Disc (pH 10.6 buffer at 70°C for 1385 Hours, 60% Weight Loss)

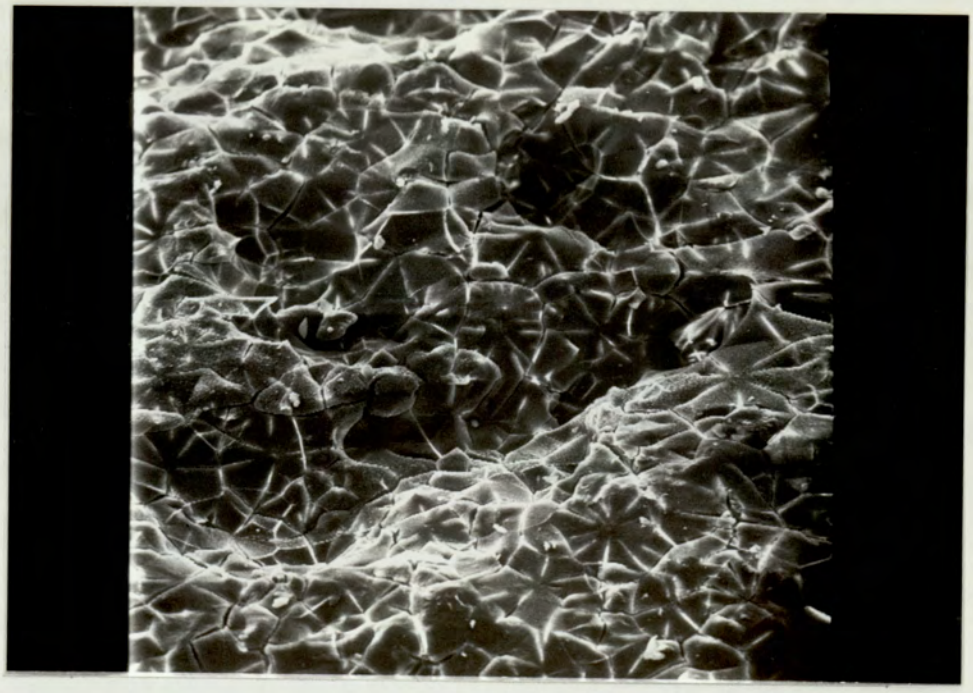


Plate 4.2 SEM (X 222) of a degraded 350PHB (12%PHV) Melt Pressed Disc (pH 10.6 buffer at 70°C for 1385 Hours, 60% Weight Loss)

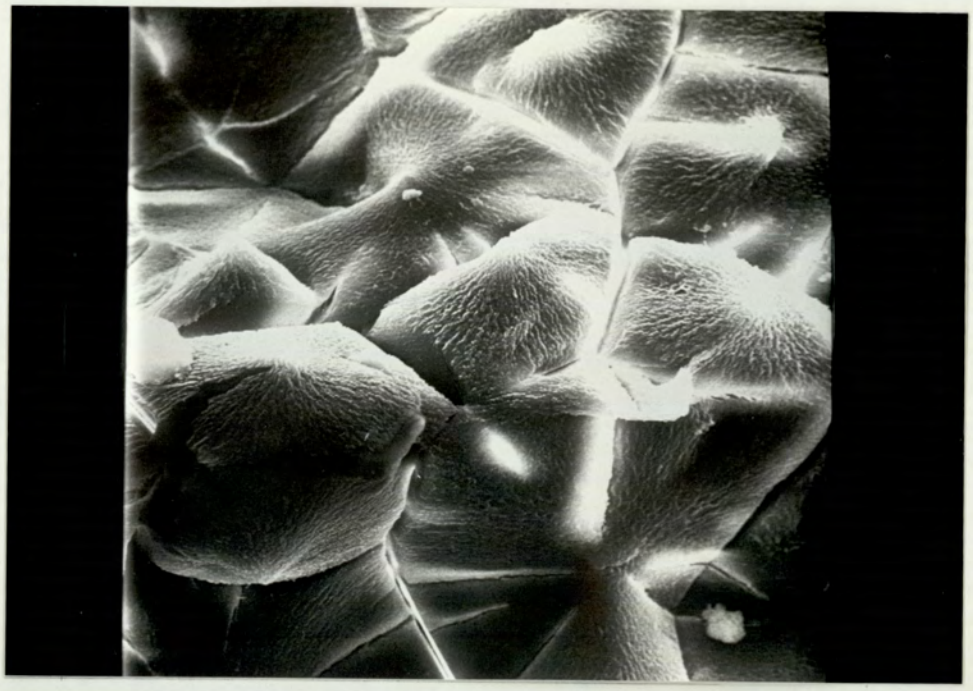


Plate 4.3 SEM (X 1120) of a degraded 350PHB (12%PHV) Melt Pressed Disc (pH 10.6 buffer at 70°C for 1385 Hours, 60% Weight Loss)

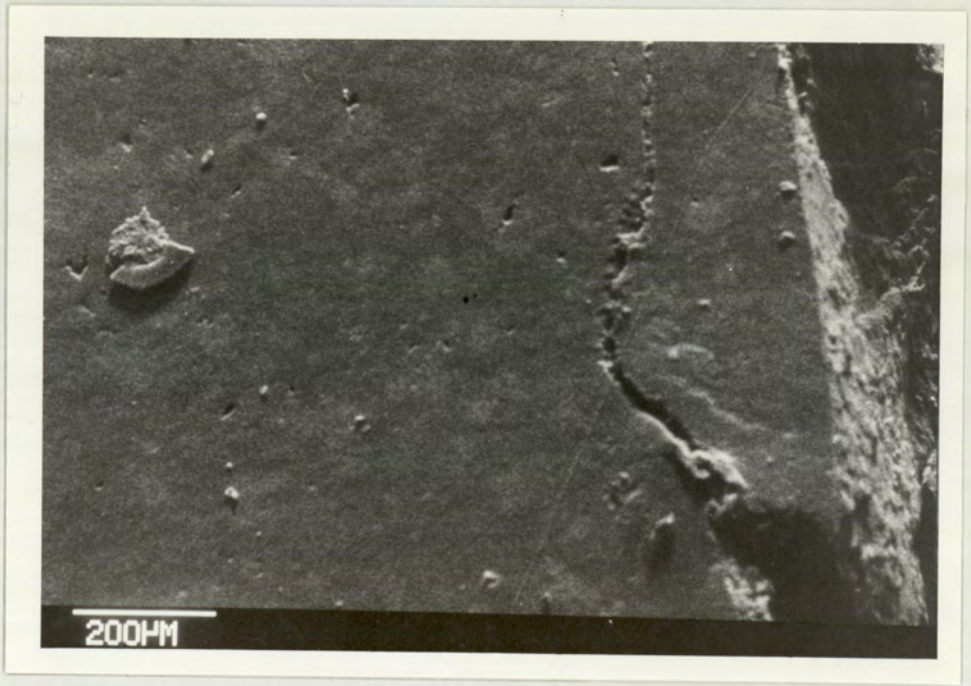


Plate 4.4 SEM (X 100) of an Undegraded 300PHB (20%PHV) Melt Pressed Disc

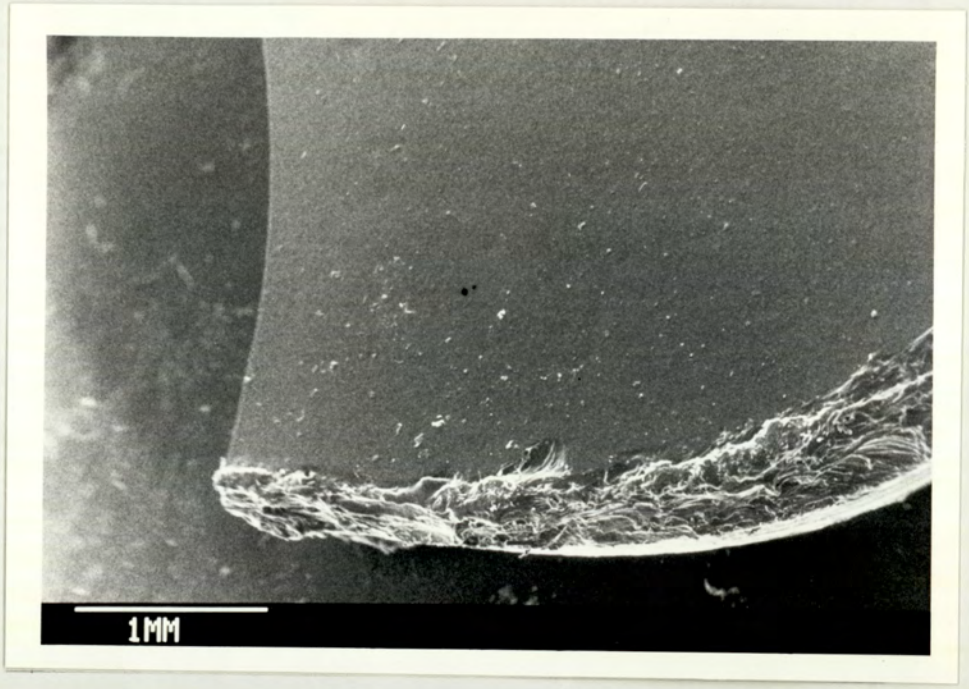


Plate 4.5 SEM (X 20) of an Undegraded 350PHB (12%PHV) Melt Pressed Disc

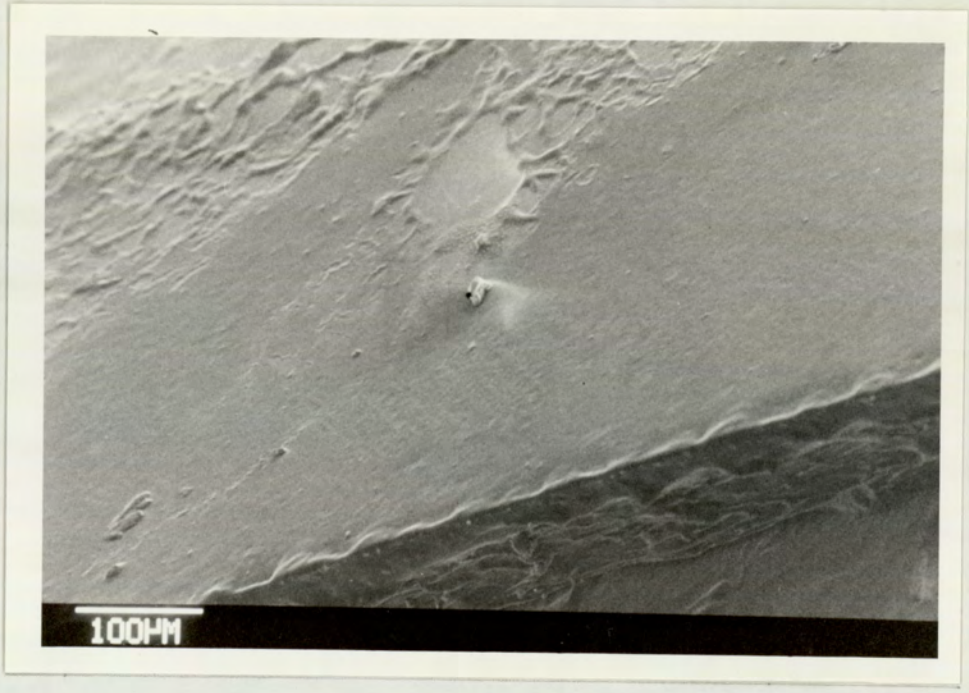


Plate 4.6 SEM (X 200) of an Undegraded 300PHB (20%PHV) Solvent Cast Film

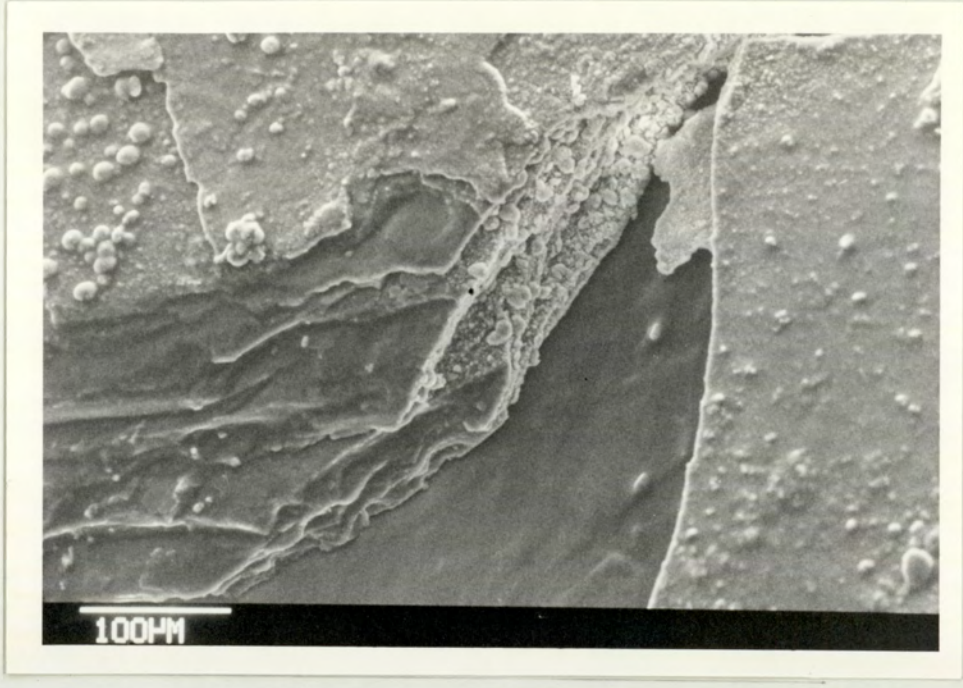


Plate 4.7 SEM (X 200) of a degraded 750PHB (10%PHV) Solvent Cast Film (pH 7.4 buffer at 70°C for 2760 Hours, 66% Weight Loss)

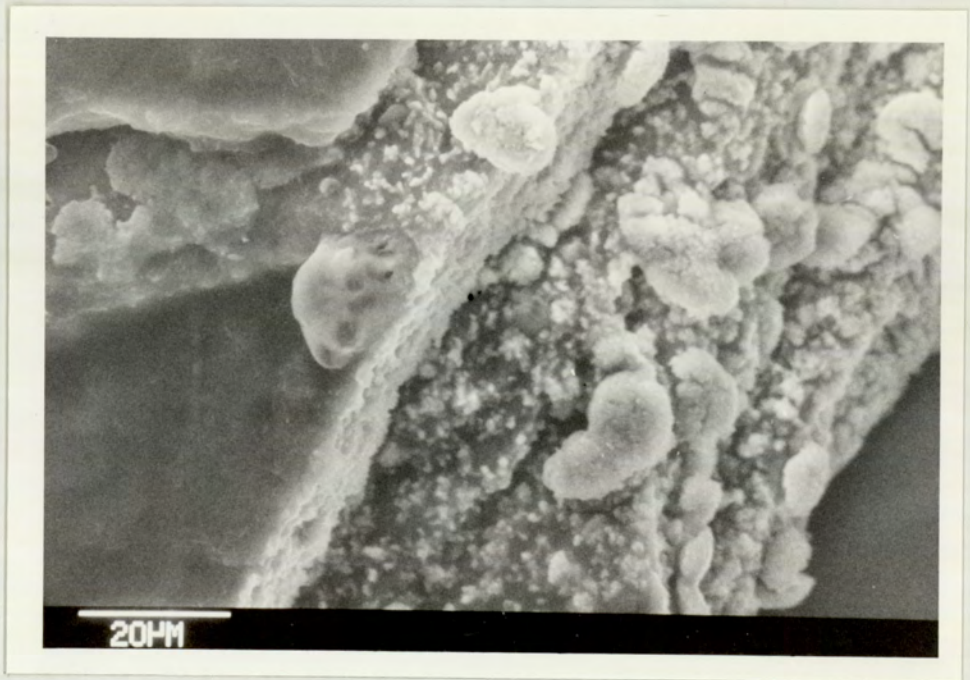


Plate 4.8 SEM (X 1000) of a degraded 750PHB (10%PHV) Solvent Cast Film (pH 7.4 buffer at 70°C for 2760 Hours, 66% Weight Loss) Showing the calcium phosphate surface deposits

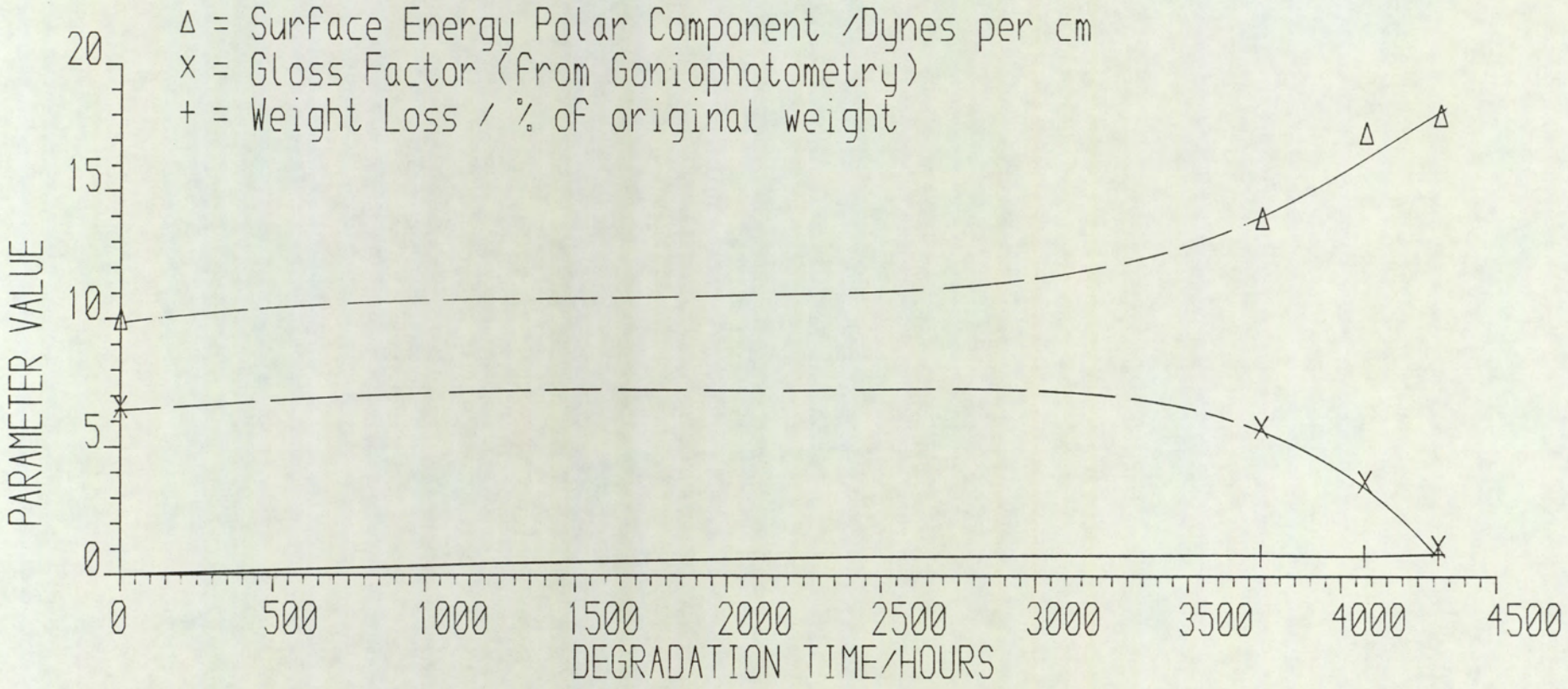
and it can be concluded that the micrographs show that fine resolution of samples is possible, but in the intial stages of degradation little change in the surface is observed (at magnifications below 2000). Additional SEM pictures of cold compressed tablet matrices were taken and these are discussed in chapter 6.

4.4 SURFACE TECHNIQUE DISCUSSION

All three methods illustrate the extent of PHB/PHV matrix degradation more graphically than the simple weight loss studies. SEM pictures (Plates 4.1 to 4.3) show the detailed morphological changes at enhanced stages of degradation, but as already stated this technique has the drawback of being destructive. SEM, however is better than goniophotometry for detecting surface features presumably due to the very small sample sizes and the high vacuum conditions together with the good mounting reproducibility of this technique.

A simple way of comparing the various attributes of the two non-destructive techniques used - surface energy and goniophotometry, is to consider one representative parameter with the associated weight loss. The polar component of the calculated surface energy and the gloss factor from goniophotometric spectra are used, and Graph 4.2 compares these parameters for a 350PHB (12% PHV) disc degraded in a pH 2.3 buffer over a period of 4500 hours. It is obvious from this graph that very small changes in

GRAPH 4.2 COMPARISON OF DEGRADATION PARAMETERS
FROM SURFACE AND BULK MATRIX HYDROLYSIS
MONITORING TECHNIQUES FOR A 350PHB (12%PHB)
DISC DEGRADED IN A pH 2.3 BUFFER AT 37°C



weight loss are reflected by considerable alterations to the surface measured parameters, which implies that small changes within the bulk of the polymer matrices are magnified in surface changes.

Goniophotometry appears to be a very good technique for following the first few percent of matrix degradation, its main limitation being the stage when the surface becomes matt in appearance since this can occur at relatively early stages of (bulk) degradation. An example of this is the decrease of gloss factor from over 13 to almost zero in only 170 hours for a 300PHB (20% PHV) disc degraded in the extreme conditions of pH 10.6, 70^{o}C. Likewise surface energy is a good method for evaluating the early hydrolysis of the polymer matrices used in these studies, the limiting extent of degradation being approximately one tenth of total hydrolysis. Both methods illustrate the effects of pH, temperature, blood plasma and oxygen plasma pre-treatment on the degradation rate of PHB/PHV samples, with similar trends to the weight loss studies observed.

There appears to be a difference between buffer and blood plasma degradation of PHB/PHV melt pressed discs, with goniophotometry indicating a 'self-polishing' effect by the buffer as opposed to a decrease in gloss observed in the plasma studies. Surface energy measurements also indicate a difference between the two degradative methods, with a larger deviation of the polar and

dispersive components from the undegraded sample data for the pH 7.4 / 37^{o}C buffer hydrolysis studies.

During the early stages of degradation surface techniques indicate the extent of bulk degradation up to greater than two orders of magnitude better than the weight loss studies. An example of this vast difference is from the studies on 300PHB (20% PHV) discs hydrolysed in a pH 7.4 buffer at 37^{o}C. After 240 hours there is a weight loss of 0.12%, whereas the observed gloss factor falls from 13.5 (undegraded) to 2.5 an 80% change which is some 600 times greater than the weight loss difference. In addition to surface energy and goniophotometry, SEM is a good technique for illustrating fine detail changes especially at enhanced degradation stages. It appears that when these techniques are used in conjunction with the weight loss data from chapter three a full characterisation of the degradative hydrolysis process of the PHB/PHV copolymer series can be described.

From the degradation information contained in this and the previous chapter, three premium grades of the PHB/PHV copolymer series were chosen for film release studies which are decribed in the next chapter. Other release studies from cold compressed PHB/PHV tablets are outlined in chapter six.

CHAPTER FIVE

POLYMER FILM RELEASE STUDIES

A discussion of previous work in chapter one led to several potential polymers for release studies. This chapter describes the release of dyes from two of these polymers - PCL and PHB/PHV. Three grades of PHB/PHV copolymer were chosen according to the ease of film formation and from the *in vitro* degradation behaviour of the PHB/PHV copolymers presented in chapters three and four. The copolymers used were 300PHB (20%PHV), 350PHB (12%PHV) and 750PHB (10%PHV). It should be noted that in this and the subsequent chapters the term 'dissolution' refers to the release of dye/drug from a polymer matrix as opposed to matrix breakdown.

5.1 INTRODUCTION

The first series of release experiments were on polymer solvent cast films of PCL and fluorescein dye, primarily because of the previous work on low molecular weight marker release from this polymer reported by Pitt et al.[96,110] Later release work was based on the series of macromolecular FITC dextrans from PHB/PHV matrices with comparative low molecular weight studies on fluorescein release from PHB/PHV polymers.

Polymer / dye films were prepared by the solvent casting technique described in section 2.3.1.1. The inherent difficulty of

mixing the PHB/PHV polymers which are halogenated solvent soluble, with the dyes which are water soluble was overcome by considering the solvent solubility parameters.[211] The multisolvent system used to combine polymer and dye solutions was a compromise between the extreme solvent solubility parameter (δ) values of chloroform (δ= 9.3 and is poorly hydrogen bonded) and water (δ= 23.4 with strong hydrogen bonding). The intermediate solvents used were THF and ethanol with the mixing procedure w.r.t. solvent solubility parameters summarised in Table 5.1

Table 5.1 Solvent mixture scheme for preparation of FITC dextran / PHB/PHV films

Phase:	POLYMER	'MIXTURE'		DYE
Solvent:	Chloroform	THF	Ethanol	Water
δ / $(cal/ml)^{-0.5}$	9.3	9.1	12.7	23.4
'Polarity:'	P	M	S	S

Where: P = poorly hydrogen bonded, M = intermediate strength of hydrogen bonding and S = strong hydrogen bonding.

5.2 INITIAL RELEASE STUDIES

The release of fluorescein dye from the PCL matrices was measured by spectrophotometrically determining 5 ml samples of the dissolution medium taken after 1, 4.5, 7, 24, 48, 96 and 170 hours from the start of the 'static' experiment. Further aliquots were taken every few days until no further release occured. The method

of calculating the amount of dyes released is given in the following section.

5.2.1 Calculations

The calibration of fluorescein absorbance against concentration (section 2.3.3.2) on a Perkin Elmer UV137 spectrophotometer was accurate enough to determine concentrations as low as 1×10^{-4}% w/v, the extinction coefficient being 416 ± 10.

The weight of dye released between sampling times t_0 and t_1 ($t_1 > t_0$) was determined by the following procedure: From Equation 2.1 the concentration difference between sampling times is equal to the absorbance difference divided by the product of the extinction coefficient and the cell path length (=1cm). Conversion of this quantity to weight released (in grams) is by multiplying this value by the volume (/mls), and dividing by 100%. In the case of the PCL/fluorescein films this quantity was equal to 75/100 = 0.75. This final weight release expression is given in Equation 5.1

$$\text{Weight of dye released} = \frac{A_1 - A_0 \times V}{{}^{1}_{1}E \times l \quad 100\%} \quad \ldots\ldots\ldots\ldots 5.1$$

Where: A_1 = Absorbance at time t_1; A_0 = Absorbance at time t_0; V = the dissolution volume; ${}^{1}_{1}E$ = the extinction coefficient and l = the cell path length.

Equation 5.1 for the PCL / fluorescein system can be condensed to the expression given in Equation 5.2.

$$\text{Weight of dye released} = A_1 - A_0 \times 1.803\times10^{-3} \quad \ldots\ldots\ldots \quad 5.2$$

(Note: The absorbance at time t_0 must be corrected for dilution if a constant volume system is used. For the PCL / fluorescein calculations the correction factor was equal to (75-5) / 75 which equals 0.933)

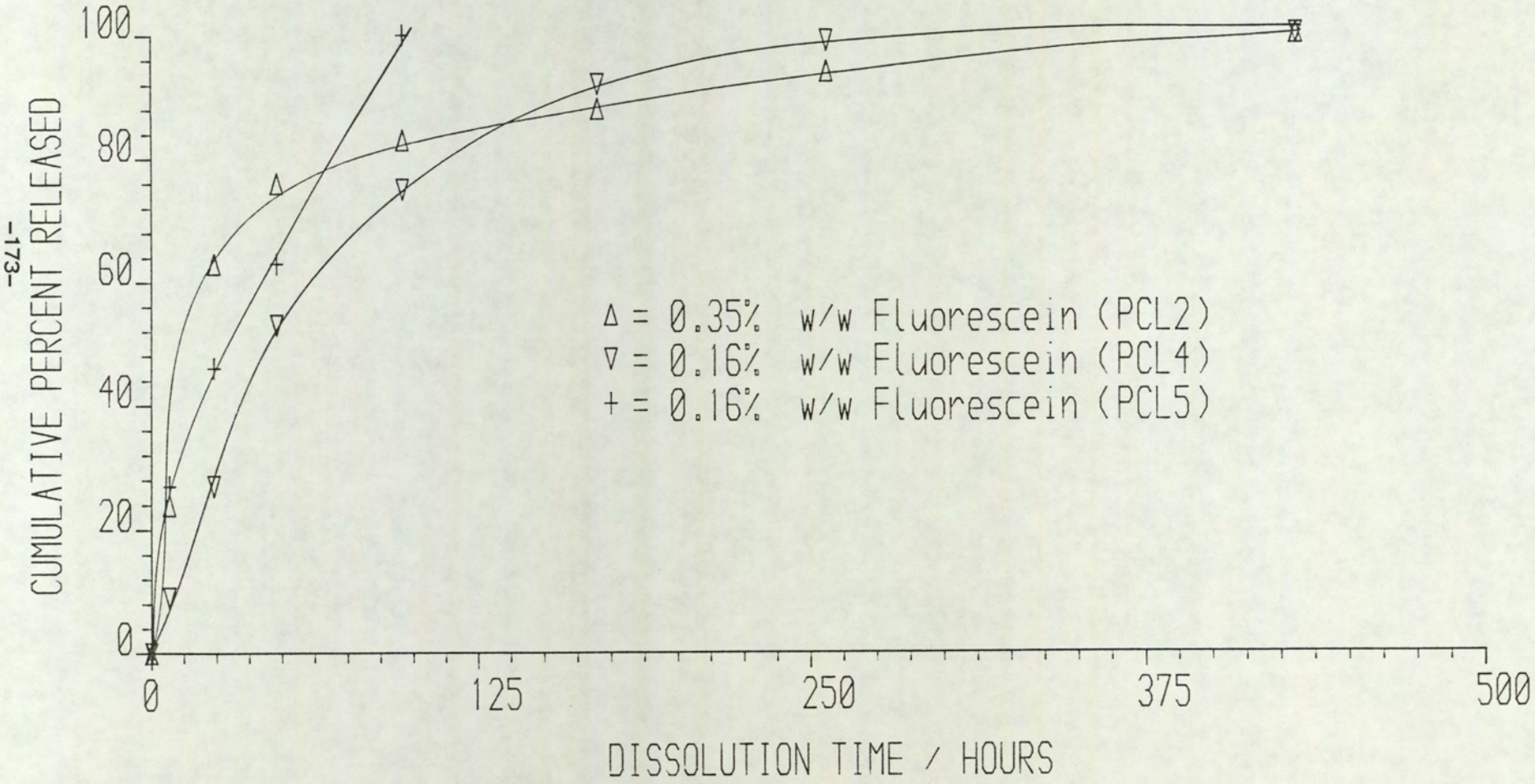
Average release rates between sampling times were calculated by dividing the weight released by the product of the surface area and the elapsed time. The surface area of the film was taken to be the sum of the two faces of the film with the assumption that there were no contributions from the film edges.

5.2.2 Results and Discussion

Graphs 5.1 and 5.2 show the cumulative release profiles of fluorescein from the various matrices. The individual matrix data indicating the co-solvent used in the formation of the films, the matrix thickness and theoretical dye load is given in Table 5.2

The graphs show that release of fluorescein from the poly caprolactone matrices is complete by 430 hours of dissolution, with most films exhausted of dye in the first few days. The release rate is very rapid with diffusion of dye out of the matrix

GRAPH 5.1 Static Dissolution of Fluorescein
From a Polycaprolactone Film



GRAPH 5.2 Static Dissolution of Fluorescein
From a Polycaprolactone Film

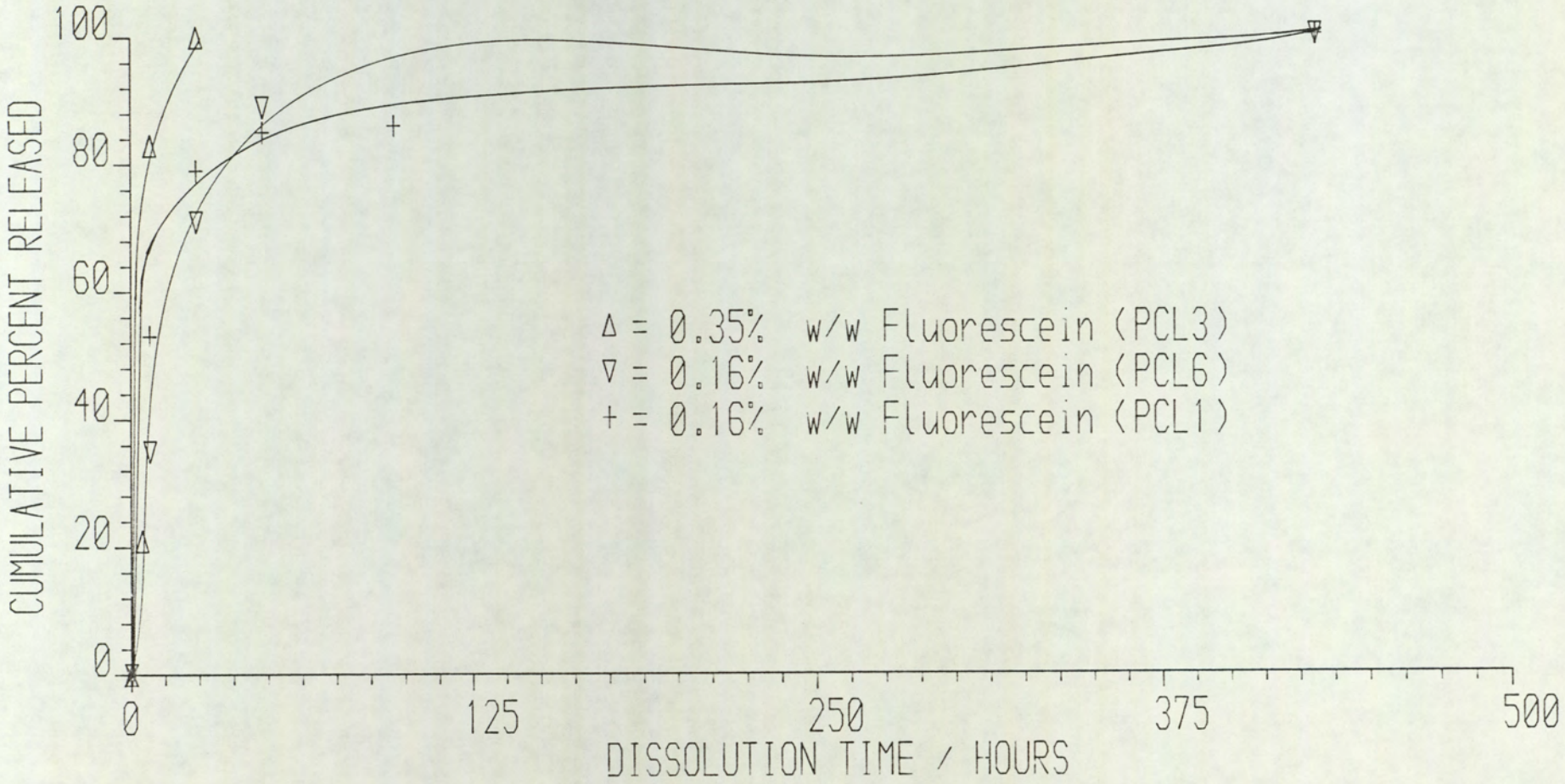


Table 5.2 PCL / Fluorescein film matrix data

SAMPLE Number	CO-SOLVENT	SURFACE AREA/cm^{-2}	THICKNESS / 10^{-3} cm	THEORETICAL LOAD / 10^{-4} g
PCL 1	CYPE	5.67	7.66	0.354
PCL 2	CYHX	8	6.54	0.864
PCL 3	CYHX	8	5.06	0.723
PCL 4	CYHP	8	14.95	1.058
PCL 5	CYHP	8	9.18	0.611
PCL 6	CYHP	5.67	6.08	0.287

Where: CYPE = Cyclopentane; CYHX = Cyclohexane and CYHP = cycloheptane.

being the most likely release mechanism since matrix degradation would play an insignificant part over the time scale of the experiment,[92] and a low molecular weight dye would be expected to diffuse out of a PCL matrix as has previously been reported by Pitt and Schindler et al.[93,96]

Two loadings of dye were used 0.16% and 0.345% and as can be seen in Graphs 5.1. and 5.2 release increases with load, although the rate is not proportional to the dye loading probably due to the difficulty in incorporating the higher amounts of fluorescein

into the polymer matrix which arises from the limit of dye solubility in the polymer solution.

The difference between the solvent cast matrices formed from matrices using cyclohexane or cycloheptane is small. The use of cyclopentane as a cosolvent however, produces films which were depleted of dye after less than 48 hours the other films releasing dye long after this.

Matrix thickness is a better indicator of dye release rate. Graph 5.3 shows the linear increase in release rate with matrix thickness over the range of 0.6 to 1.5 x 10^{-2}cm. However, there is insufficient data to determine a quantitative relationship and the line drawn between the experimental points on the graph is only approximate.

The average release rates of fluorescein from the PCL films after the initial burst of dye from the surface are summarised in Table 5.3. Also included in this table are the derived release rates of a hormone (Norgestrel) from a capsular PCL matrix reported by Pitt et al.[96] The release rates displayed in Figure 6 of this paper[96] have been altered from (µg/day/cm of tubiar matrix) to (µg/hour/cm^2 of exposed matrix).

The release rates for the Pitt studies are generally higher, but the rate would be expected to be higher for the films used in this study as these films were prepared as a homogeneous matrix

GRAPH 5.3 Initial rate of Fluorescein Release From Polycaprolactone Films as a Function of Matrix Thickness

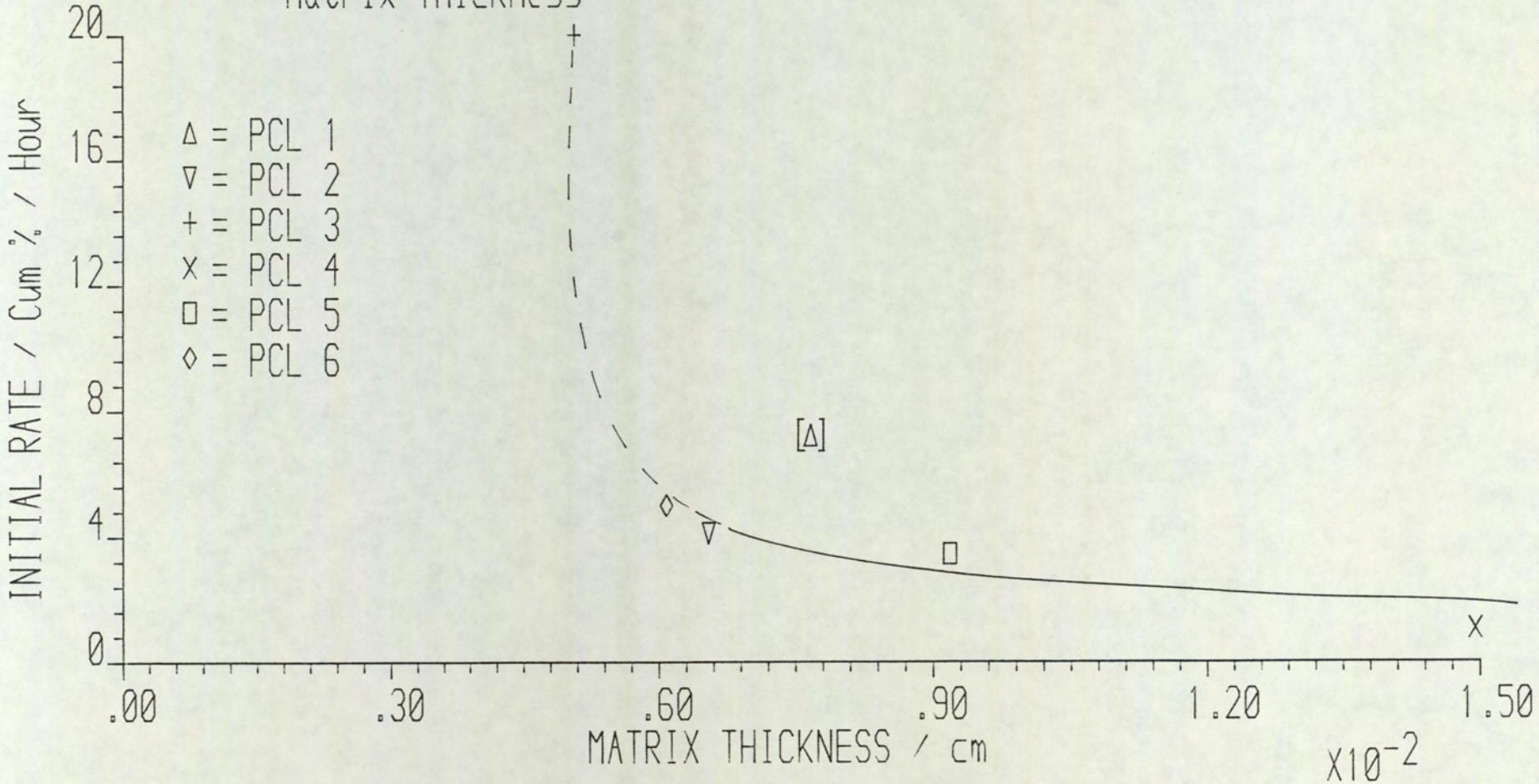


Table 5.3 Comparison of low molecular weight 'active' release from PCL matrices

SAMPLE	TIME SCALE / Hours	RELEASE RATE /ug/cm^2/hour
PCL1	7 to 24	0.065
PCL2	48 to 170	0.033
PCL3	0 to 24	1.021
PCL4	4 to 96	0.0203
PCL5	24 to 96	0.075
PCL6	7 to 48	0.0826
PCL / Drug[96]	0 to 20	0.22 - 0.36
PCL / Drug[96]	20 to 90	0.22 - 0.18

(as opposed to the reservoir type matrix used by Pitt) which on dissolution of dye became porous hence aiding any further release. However, the dye loadings used in the Pitt studies were several times greater than the polyer/dye films in this work. Therefore true comparisons are difficult to make because of differences between the dyes used in these studies and the drugs used by Pitt.

5.3 PHB/PHV - FLUORESCEIN DYE RELEASE STUDIES

The release of a low molecular weight dye - fluorescein, from polyhydoxybutyrate-co-valerate matrices was followed by dissolution of films prepared by solvent casting as described in

section 2.3.1.1. Two dissolution methods were used: Static and the standard United States Pharmacopoeia (USP) method. These are described in section 2.4.4.

5.3.1 Introduction

Two grades of PHB/PHV copolymer were used (300PHB 20% PHV and 350PHB 12% PHV) to form the polymer / fluorescein matrices, with two dye loadings (2.68 and 5.3% w/w). The higher loading approached the limit of dye loading by the single solvent casting technique.

Square sections of the films were dissoluted into a pH 7.4 buffer at 37^{o}C using either of the dissolution methods mentioned above. Dye release was measured by spectrophotometric analysis of the dissolution medium via either a sampling 'loop' every 15 minutes up to 50 hours for the USP dissolution experiments, or by taking 5 ml samples of the dissolution medium (maintaining constant volume) at regular intervals (every hour for the first 10 hours, then twice daily upto one week, thereafter every two to four days up to 720 hours.

5.3.2 Calculations

The amount of dye released was calculated using the same method given in section 5.2.1. The dissolution volumes were 50 mls for the static experiments and 900 mls for the USP experiments.

5.3.3 Static Dissolution

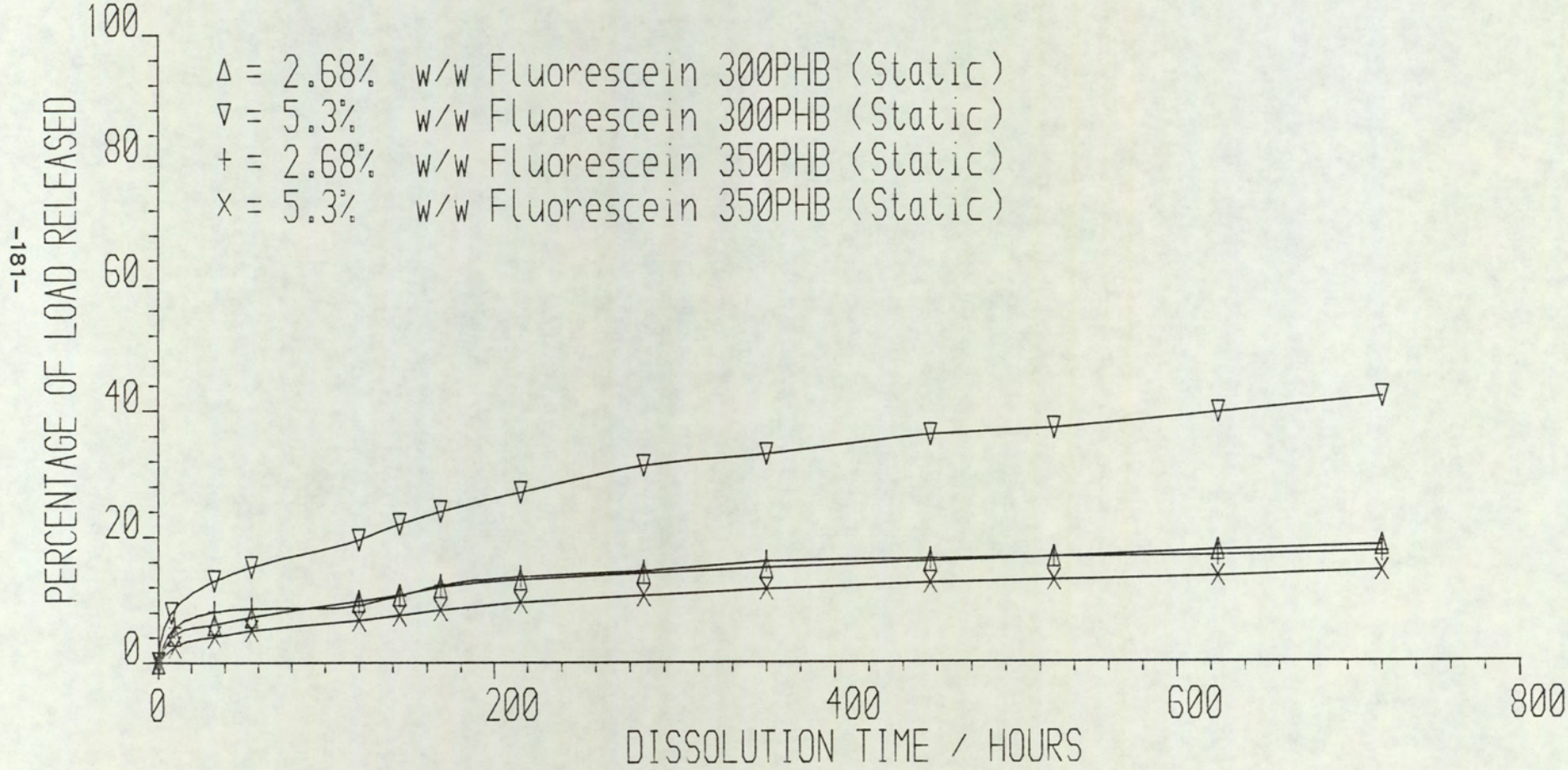
Graph 5.4 illustrates the cumulative release of two fluorescein loadings from the two grades of polymer for both dissolution techniques. A summary of the various release parameters for the two dissolution methods is given in Table 5.4. The terms $t_5\%$, and $t_{10}\%$ and refer to the times for the release of 5 and 10% of the theoretical load.

Table 5.4 Release parameters for PHB-PHV - flourescein films

POLYMER GRADE	DYE LOADING / % w/w	DISSOLUTION METHOD	$t_5\%$ / Hours	$t_{10}\%$ / Hours
300PHB (20% PHV)	2.68	Static	10	150
300PHB (20% PHV)	5.3	Static	7	~22
350PHB (12% PHV)	2.68	Static	9	150
350PHB (12% PHV)	5.3	Static	50	280
350PHB (12% PHV)	2.68	USP	41	---
350PHB (12% PHV)	5.3	USP	30	---

The films dissoluted under static conditions have profiles which are characterised by a fast 'burst' release (of surface dye) followed by a gradual tailing-off of the release rate. This is consistant with a diffusional release mechanism, which would be expected for the release of a low molecular weight species.[212]

GRAPH 5.4 Dissolution of Fluorescein From
300PHB (20% PHV) and 350PHB (12% PHV) Films



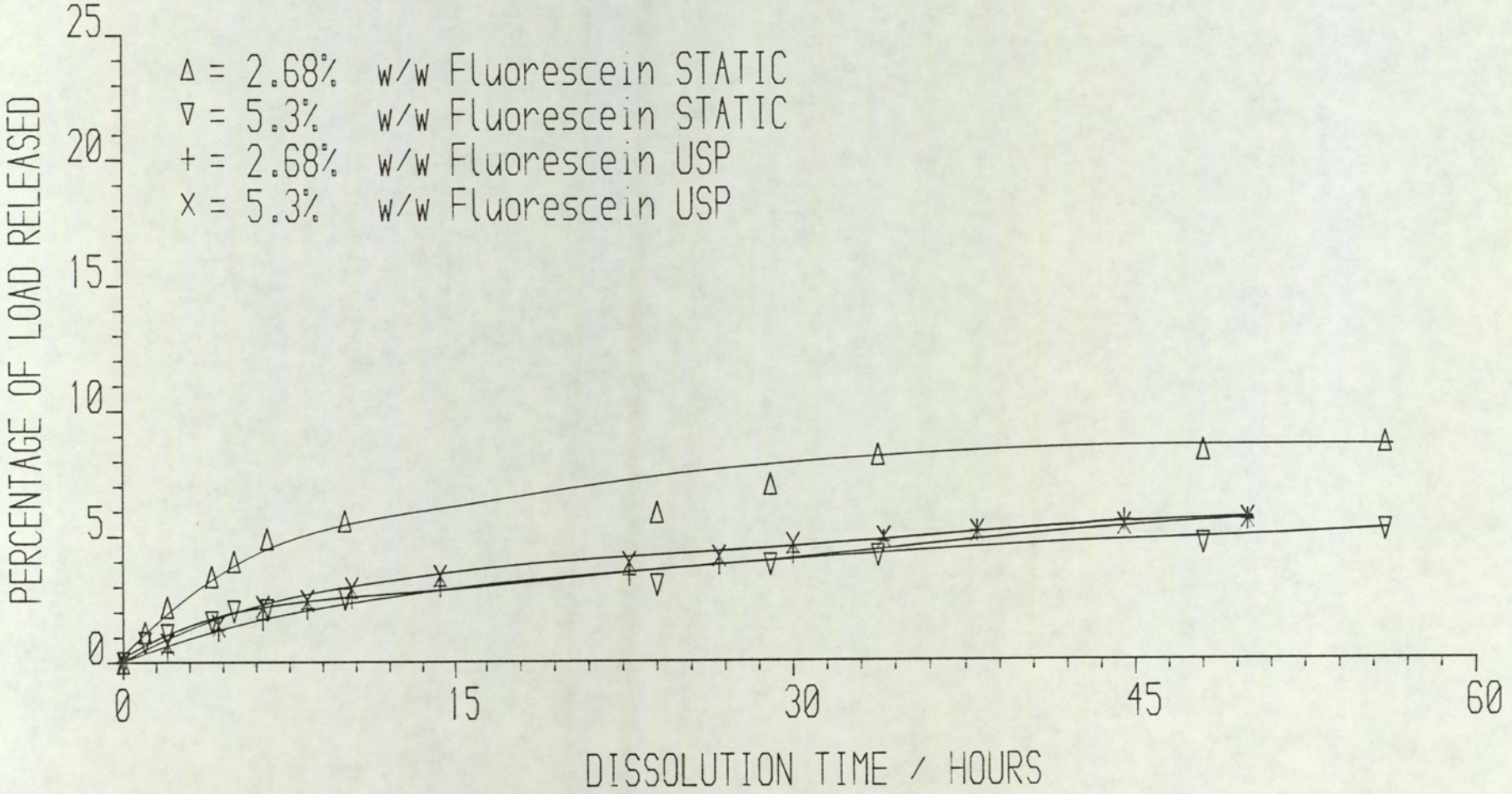
Doubling the dye load increases but does not double the amount of dye released from the 350PHB (12%) polymer grade. This suggests that release of flourescein from this type of polymer matrix is dependant on the rate of fluid influx. The 300PHB (20% PHV) grade of polymer however, shows a large increase in the release rate for the higher loading, as is reflected in the times for five and ten percent release presented in Table 5.4. This is presumably due to poor mixing of the dye and polymer in the film casting process, which results in a fast dissolution of the dye out of the matrix.

At the lower dye loading there is little difference between the polymer grades, but release from the 300PHB (20% PHV) polymer matrix containing 5.3% w/w fluorescein is faster than the corresponding lower valerate copolymer, presumably due to differences in the physical properties of the polymers.

5.3.4 USP Dissolution

The release profiles of fluorescein from 350PHB (12%PHV) film matrices under USP ('sink') conditions are contained in Graph 5.5 together with the equivalent static release profiles over the same time scale. A similar release profile to that obtained for the static experiments is evident. From Table 5.4 it can be seen that increasing the dye load two fold almost exactly doubles the amount of dye released (in weight terms), indicating an apparently linear relationship between loading and release over the range studied.

GRAPH 5.5 Dissolution of Fluorescein From 350PHB (12% PHV) Films



5.3.5 Discussion

There appears to be a difference between the release rates of fluorescein from the two dissolution methods. The profiles indicate a slight increase in the dissolution rate of the dye for the static method compared with USP dissolution. This unexpected trend (normally the near sink conditions would provide a faster dissolution rate), however, can be explained by a consideration of the experimental errors: The manually assayed static method on a Perkin Elmer UV 137 spectrophotometer is less accurate than the computer controlled Uvikon spectrophotometer used in the USP dissolution studies. Other errors arise from the maintenance of constant volume conditions in the static experiments, together with the use of only two matrices for each static experiment as opposed to three for each USP dissolution study. The overall errors contained in the static data are significant enough to allow for a possibly slower static release, however the calculated results prove inconclusive.

5.4 PHB/PHV - FITC DEXTRAN RELEASE STUDIES

The feasibility of controlled macromolecular release from polyhydroxybutyrate-co-valerate matrices was followed by incorporating a series of FITC dextran dyes as described in section 2.3.1.1.

5.4.1 Introduction

The 750PHB (10% PHV) grade of polymer was used and films were made with two loadings (3.5% and 6.3% w/w) of macromolecular dyes (molecular weights 4,000 to 150,000). The Higher drug loading approached the limit of the amount of dye that could be incorporated without separation of the dye and polymer solutions on mixing during film preparation.

The various films were dissoluted using the 'static' method into varying buffer conditions at 37^{o}C, with 5 ml samples taken for spectrophotometric analysis at dissolution times of 1, 4, and 24 hours; then every day up to and including a sample at 431 hours, after which there was no significant release detected.

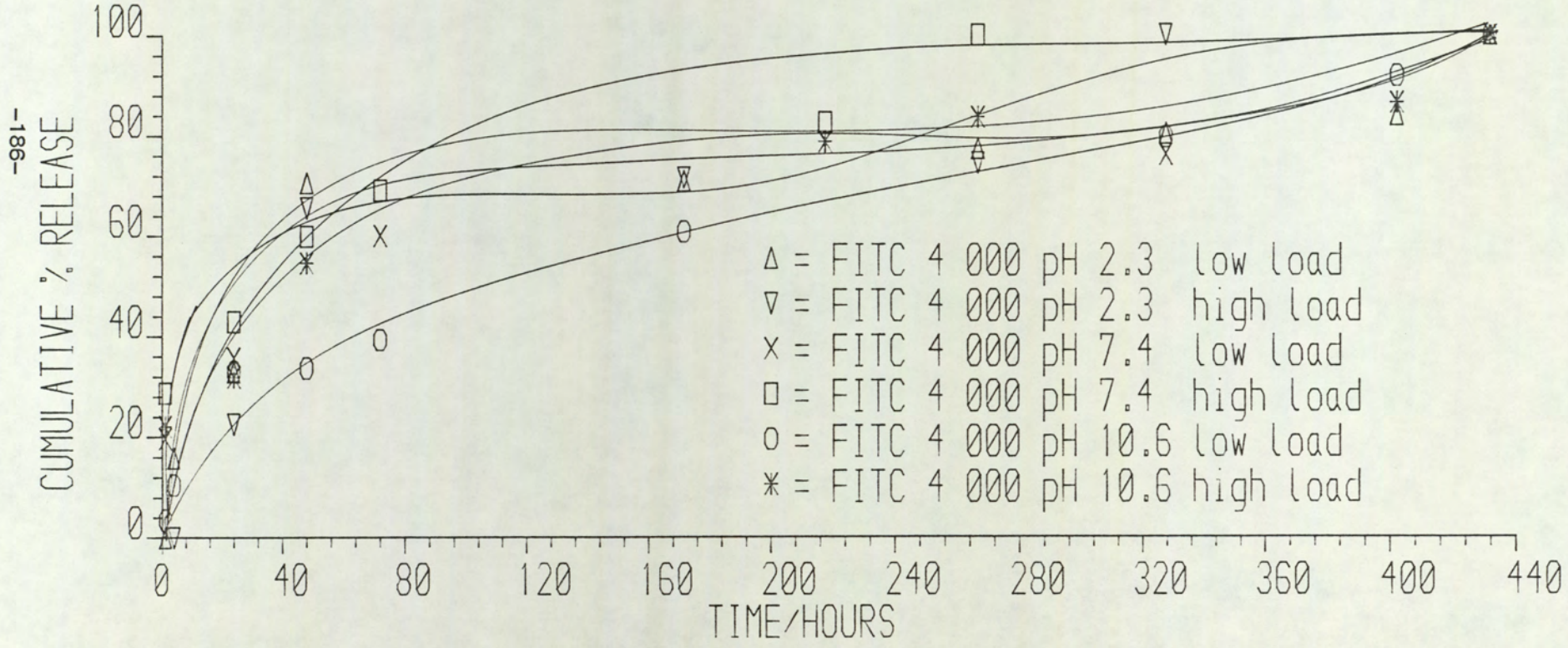
5.4.2 Calculations

The amount of dye released was calculated by the same method outlined in section 5.2.1, with alteration of a few of the experimental parameters (dissolution volume = 25 mls, differing extinction coefficients - see section 2.3.3.2). The release rates were calculated as before.

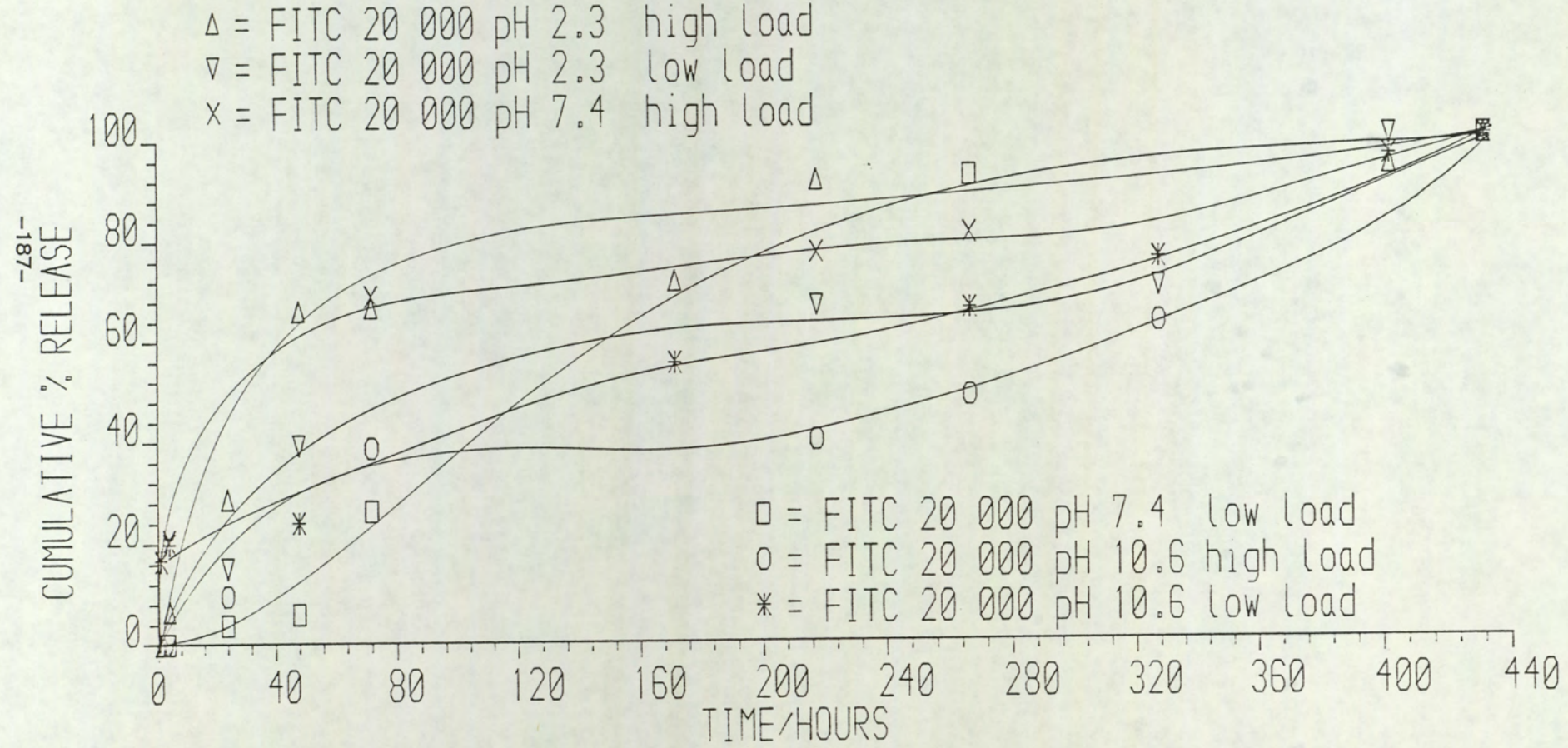
5.4.3 Results and Discussion

Graphs 5.6 to 5.10 show the cumulative release profiles of fluorescein against time. The total amount of dye detected throughout the release experiment was used as the 100% value. This occured as early as 217 hours but more typically was after 430

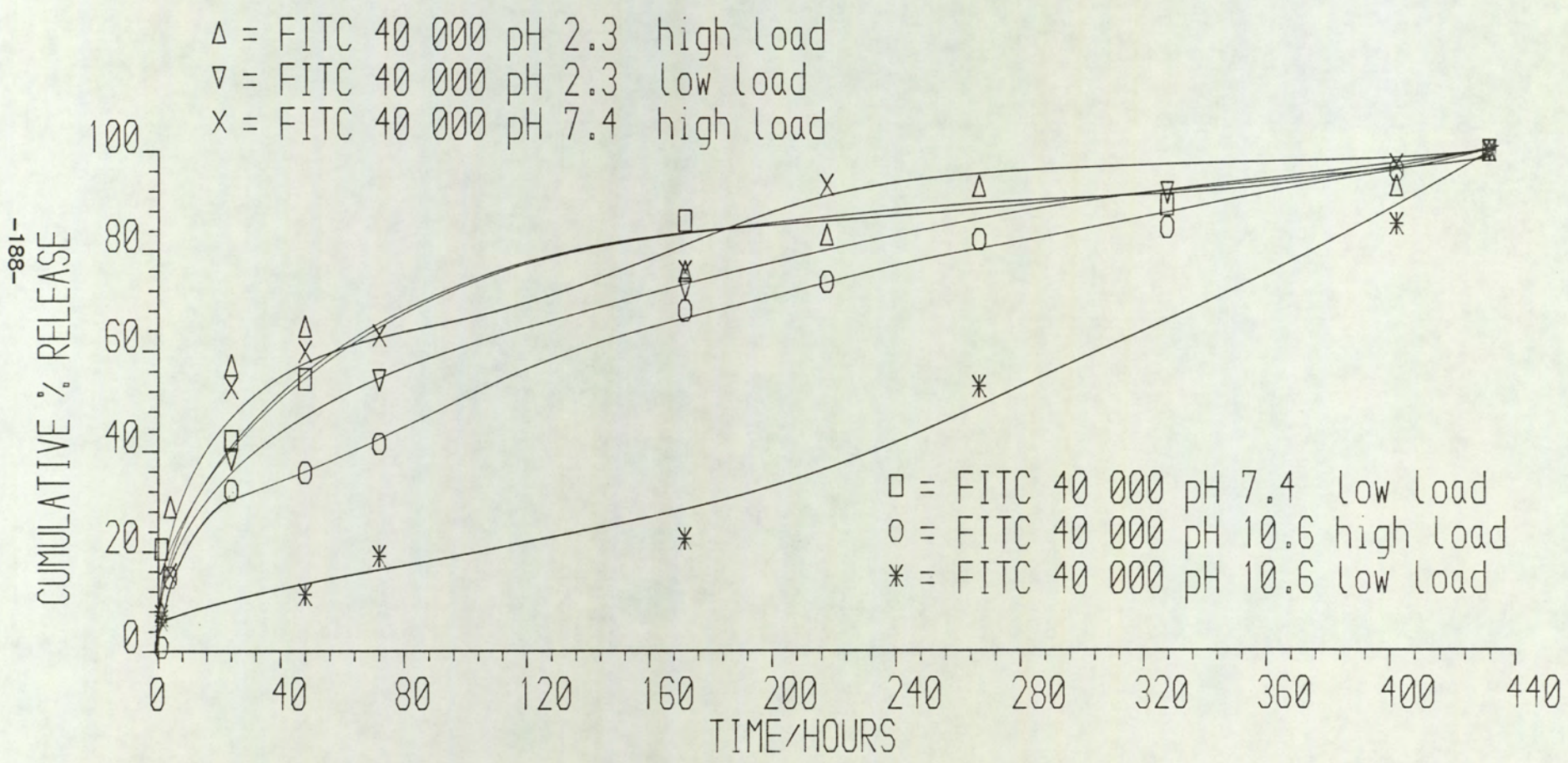
GRAPH 5.6 Static Release Profiles of FITC4 Dye
From 750PHB (10%PHV) Films



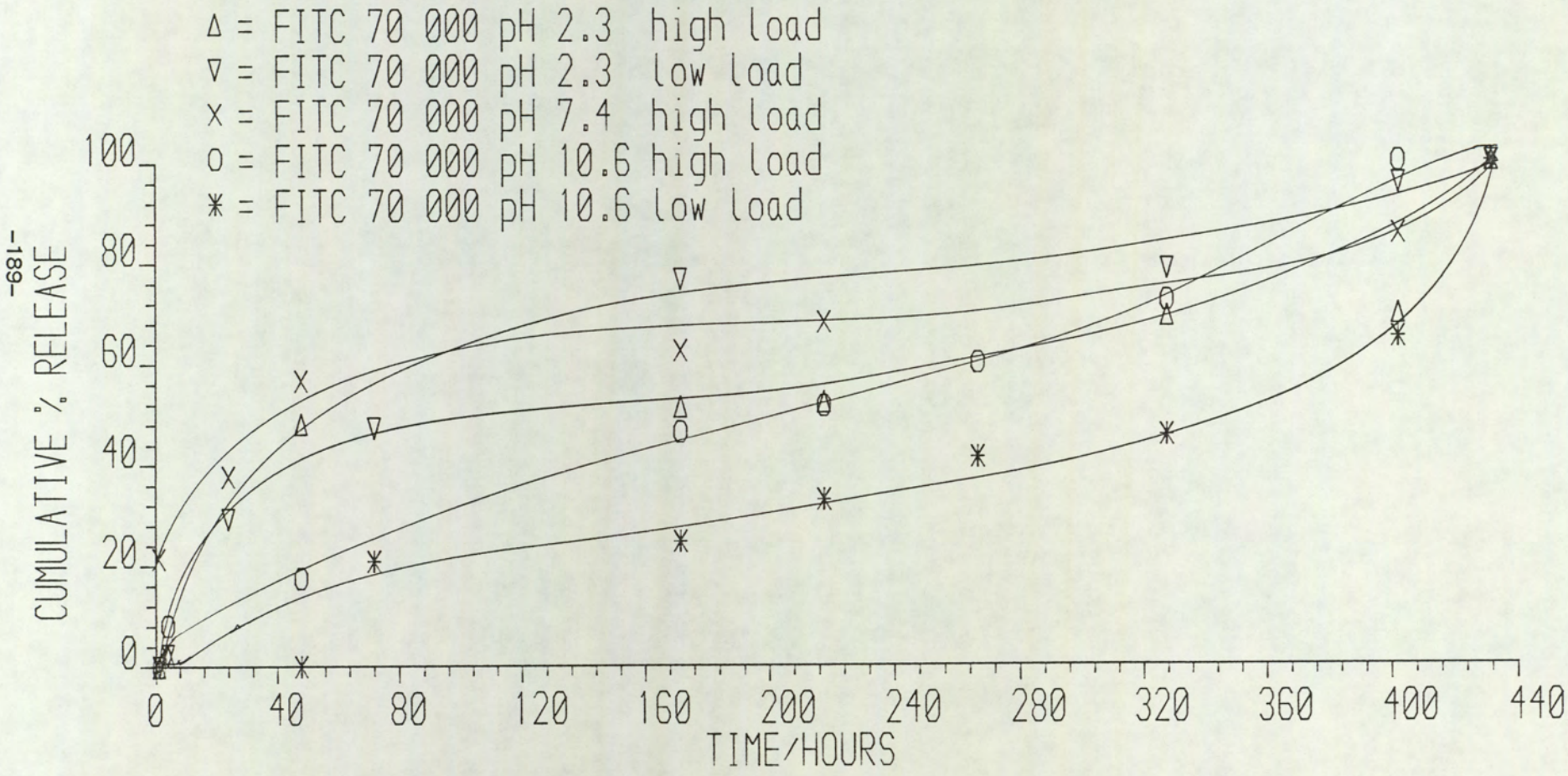
GRAPH 5.7 Static Release Profiles of FITC20 Dye
From 750PHB (10%PHV) Films



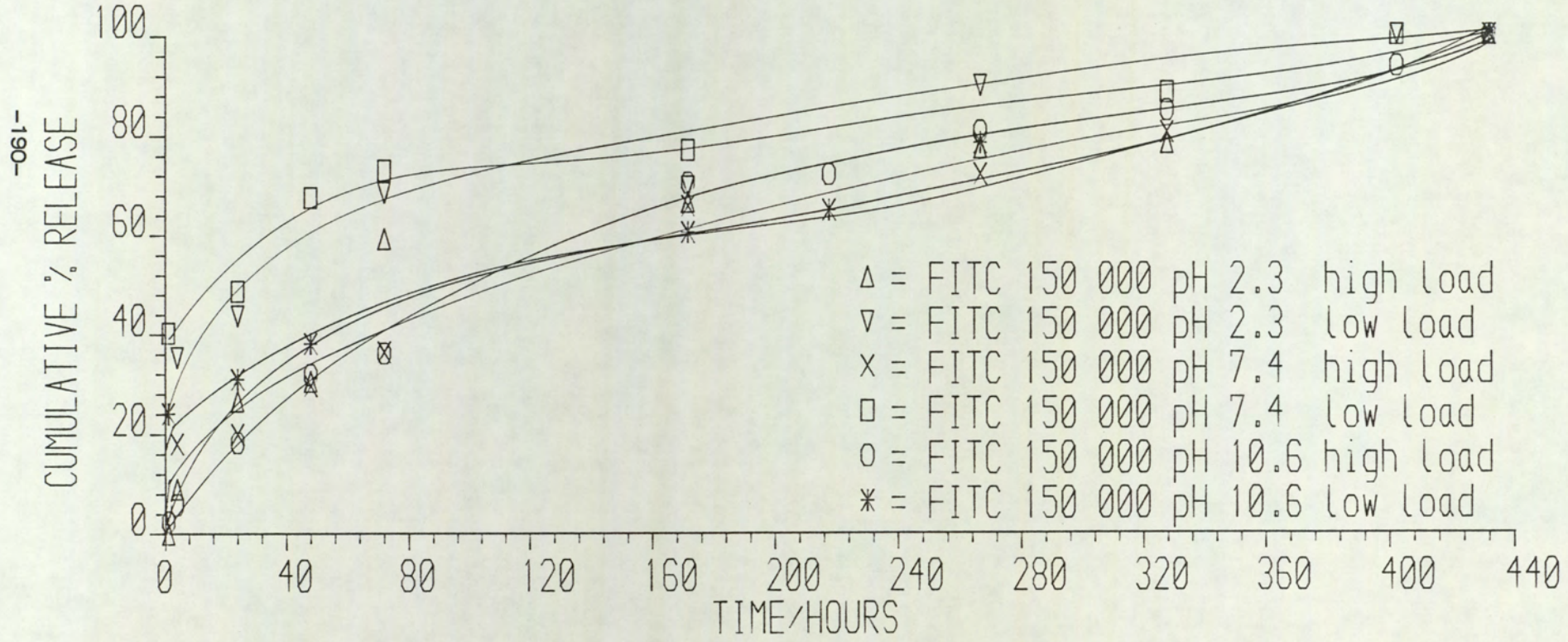
GRAPH 5.8 Static Release Profiles of FITC40 Dye
From 750PHB (10%PHV) Films



GRAPH 5.9 Static Release Profiles of FITC70 Dye
From 750PHB (10%PHV) Films



GRAPH 5.10 Static Release Profiles of FITC150
Dye From 750PHB (10%PHV) Films



hours. Physical matrix data of film thickness, surface area, degradation conditions and total amount of dye released as a percentage of the theoretical load (assuming perfect homogeneity) is given in Table 5.5. The actual amount of dye detected during dissolution ranged from a very small 0.91% of the theoretical load for the FITC 20 film 'high' loading / pH 7.4 buffer, to 59% for the FITC 4 film pH 2.3 'low' loading.

On inspection of the graphs it can be seen that release of the dextran dyes from the polymer films is fast with the initial release during the first 24 to 48 hours comprising the majority of the observed release profile. This is termed the 'burst effect' which arises from the dissolution of matrix surface dye. The initial release rates expressed as a percentage of the cumulative release per hour (for the first 48 hours) for the high and low release profiles observed for each grade of dye (together with an average polymer/dye film) are listed in Table 5.6.

The fastest and average release rates show little correlation with molecular weight, the only general difference between the dyes being the relatively narrow spread of the high molecular weight profiles and the general increase in rates as molecular weight increases. This trend is contrary to that predicted by Fick's laws of diffusion[212] which predicts a decrease in diffusive release as the molecular weight of the diffusing species increases. The most consistent release observed is that of the

Table 5.5 Matrix data for the FITC Dextran / PHB films

DYE MOLECULAR WEIGHT	THICKNESS / $x10^{-2}$cm	SURFACE AREA/cm^{-2}	BUFFER pH	LOAD / % w/w	TOTAL AMOUNT OF DYE DETECTED
4,000	7.4	2	2.3	3.16	59% @ 431 Hours
4,000	7.9	8	2.3	6.19	11% @ 327 Hours
4,000	6.5	4	7.4	3.16	14% @ 431 Hours
4,000	7.6	8	7.4	6.19	2% @ 266 Hours
4,000	6.4	4	10.6	3.16	16% @ 431 Hours
4,000	7.5	8	10.6	6.19	5% @ 431 Hours
20,000	8.45	8	2.3	3.25	7% @ 402 Hours
20,000	7.1	7.8	2.3	6.23	4% @ 431 Hours
20,000	7.4	8	7.4	3.25	3% @ 431 Hours
20,000	6.9	7.8	7.4	6.23	1% @ 431 Hours
20,000	8.6	8	10.6	3.25	2% @ 431 Hours
20,000	7.5	7.8	10.6	6.23	1% @ 431 Hours
40,000	7	7.56	2.3	3.31	28% @ 431 Hours
40,000	11.2	4.5	2.3	6.23	57% @ 431 Hours
40,000	6.7	7.84	7.4	3.31	3% @ 431 Hours
40,000	6.85	4.5	7.4	6.27	19% @ 431 Hours
40,000	6.8	7.3	10.6	3.31	11% @ 431 Hours
40,000	6.7	4.5	10.6	6.27	41% @ 431 Hours

Table 5.5 (Cont) Matrix data for the FITC Dextran / PHB films

DYE MOLECULAR WEIGHT	THICKNESS / $x10^{-2}$cm	SURFACE AREA/cm^{-2}	BUFFER pH	LOAD / % w/w	TOTAL AMOUNT OF DYE DETECTED
70,000	7.6	7	2.3	3.27	27% @ 431 Hours
70,000	6.8	7.4	2.3	6.1	22% @ 431 Hours
70,000	6.5	7	7.4	3.27	20% @ 171 Hours
70,000	7.5	8	7.4	6.1	5% @ 431 Hours
70,000	6.8	8.1	10.6	3.27	6% @ 431 Hours
70,000	6.6	8	10.6	6.1	28% @ 402 Hours
150,000	7.25	6.5	2.3	3.38	37% @ 402 Hours
150,000	7.0	6.72	2.3	6.1	16% @ 431 Hours
150,000	7.05	6.5	7.4	3.38	7% @ 402 Hours
150,000	7.5	6.72	7.4	6.1	7% @ 431 Hours
150,000	6.75	6	10.6	3.38	10% @ 431 Hours
150,000	6.9	6.72	10.6	6.1	23% @ 431 Hours

FITC 20 dye, which for the 'average' profile only releases 38% of the observed total after 48 hours which is less than for most of the other dyes. This shows that the 20,000 molecular weight dye is contained in a more homogeneous polymer matrix than the other (especially the high molecular weight) dyes.

The release studies into an acidic buffer tend to show a higher release rate than the neutral or alkaline media experiments, with the pH 10.6 release studies tending to be the

Table 5.6 Initial Release Rates of FITC Dextran dyes from PHB/PHV films

DYE MOLECULAR WEIGHT		BUFFER pH	LOAD / % w/w	INITIAL RELEASE RATE OVER 48 HOURS Cumulative % / hour	ug / hour / cm^{-2}
4,000	H	2.3	3.16	1.5	3.63
4,000	Av	7.4	6.19	1.25	0.177
4,000	L	10.6	3.16	0.69	0.407
20,000	H	2.3	6.23	1.38	0.308
20,000	Av	2.3	3.25	0.79	0.252
20,000	L	7.4	3.25	0.125	0.125
40,000	H	2.3	6.27	1.35	0.786
40,000	Av	7.4	3.25	1.125	0.024
40,000	L	10.6	3.31	0.23	0.029
70,000	H	7.4	6.1	1.19	0.319
70,000	Av	2.3	6.1	1.0	1.22
70,000	L	10.6	6.1	0.35	0.514
150,000	H	7.4	3.38	1.46	0.396
150,000	Av	10.6	3.38	0.79	0.306
150,000	L	7.4	6.1	0.625	0.343

H = High release profile; Av = Average release profile and L = Low release profile

slowest. However differences in dye solubility in non-neutral pH conditions have a significant effect on the detected release rate. Differences between the two dye loadings used are small when the normalised release profiles are considered.

The overall conclusions of the high molecular weight studies are that release is via diffusion and dissolution of the dye through imperfections in the films rather than by degradation of the film matrix. The fast initial diffusional release exhibited by nearly all the films show that the FITC dyes are less than perfectly homogeneous. The best example of consistent release observed was from the FITC 20 dye matrices, with the higher molecular weight dyes showing faster release rates than the FITC 4 or FITC 20 polymer dye samples.

5.5 FILM RELEASE DISCUSSION

The polymer / dye release studies contained within this chapter cover a wide range of dye molecular weights (ranging from 332 for fluorescein to approximately 150,000 for the highest member of the FITC dextran dyes tested) from two different polymers - poly caprolactone and the PHB/PHV copolymer series. In addition two methods of polymer matrix dye incorporation were used, with a single solvent system for the low molecular weight fluorescein / polymer films and a multi-solvent system for the high molecular weight PHB/PHV - FITC dextran films (since a one solvent system could not be found due to polymer / dye

incompatability - c.f. section 5.1). Hence the observed differences in dye dissolution rates from the various matrices are not unexpected.

The release of dye from the films was characterised by an intial 'burst' release due to surface dye dissolution. In some cases this formed a considerable part of the release. Further observed release showed fairly consistent release rates until complete dye dissolution was reached.

The PCL / fluorescein films showed relatively high release rates compared to the equivalent PHB/PHV films. This is presumably due to the inherent differences in the physical characteristics of the two polymers, for example polymer crystallinity and molecular weight. The expected change in the dye hydrodynamic boundary layer around the film matrix which is observed when matrices are dissoluted by two differing methods (namely USP and static) was not seen, with little change in the rate of release of fluorescein from 350PHB (12% PHV) films between the two dissolution systems. As already mentioned this was probably due to the limitations of the static release experiment which made a true comparison between this and the USP method difficult.

Release from the PHB/PHV films is essentially correlated to dye molecular weight with the larger dyes being released relatively quickly contrary to the classic prediction by Fick's law of diffusion. This is due to differences in the degree of incorporation of dye within the matrix. The higher molecular

weight dyes form less homogeneous matrices and therefore are released more quickly. There appears to be only a small difference between the observed rates of dye release in non neutral dissolution media.

From the hydrolysis data in chapter three a weight loss of less than 0.5% over 720 hours for any of the three grades of PHB/PHV copolymer used in the film release studies would occur in a pH 7.4 buffer at 37^{o}C. Hence the effect on the release rate of dye from the polymeric matrices would be expected to be insignificant over the time scale of the dissolution experiments.

There have been various models of diffusional release from solid (non degrading) matrices: Langer and Peppas[8] propose that release can be classified into four catagories for the first 40% of release. Cases one and three apply to matrices in which: a) the drug is molecularly dissolved with release by diffusion via a solution-diffusion mechanism, and b) to matrices in which the drug is dissolved in the polymer matrix with release by diffusion through water filled pores in the matrix, respectively. These expressions are given in Equations 5.4 and 5.5, and appear to be the most suitable for the polymer / dye films in this study. Other similar models have been published by Chien.[2] However, no attempt is made here to fit the data presented to any of these models since certain parameters - for example polymer matrix tortuosity were not determined in the course of these studies.

$$\frac{d\,Mi}{A\,dt} = 2C_d \left[\frac{D_{lm}}{\pi}\right]^{1/2} t^{-1/2} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 5.4$$

(The left hand side of the equation = the release rate of drug per unit area of exposure, C_d = initial drug loading, D_{lm} = drug diffusion coefficient in the polymer matrix and t = time.)

$$\frac{d\,Mi}{A\,dt} = 2C_d \left[\frac{D_{lw}\,\varepsilon}{\pi\tau}\right]^{1/2} t^{-1/2} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 5.5$$

(Where ε = porosity factor; τ = tortuosity factor and D_{lw} = drug diffusion coefficient for the drug in the pore liquid phase. All other symbols as Equation 5.4.)

Overall, the dye release is related to the extent of polymer / matrix homogeneity. Plates 5.1 to 5.3 illustrate the differences between PHB/PHV films prepared using differing incorporation techniques. Plate 5.1 shows a fluorescein / 300PHB (20% PHV) film. The dye appears to be well dispersed within the matrix with no dye heterogeneities evident. By comparison Plate 5.2 shows a picture of a high molecular weight dextran dye (FITC70) / 750PHB (10% PHV) film. The local dye concentrations within the matrix indicate a less well dispersed dye distribution within the matrix than the fluorescein films. This is reflected in the relative release rates which are more prolonged for the fluorescein films than for the rapidly releasing PHB/PHV - FITC dextran films.

Plate 5.1 300PHB (20% PHV) Film loaded with 5.3% w/w Fluorescein (X 100)

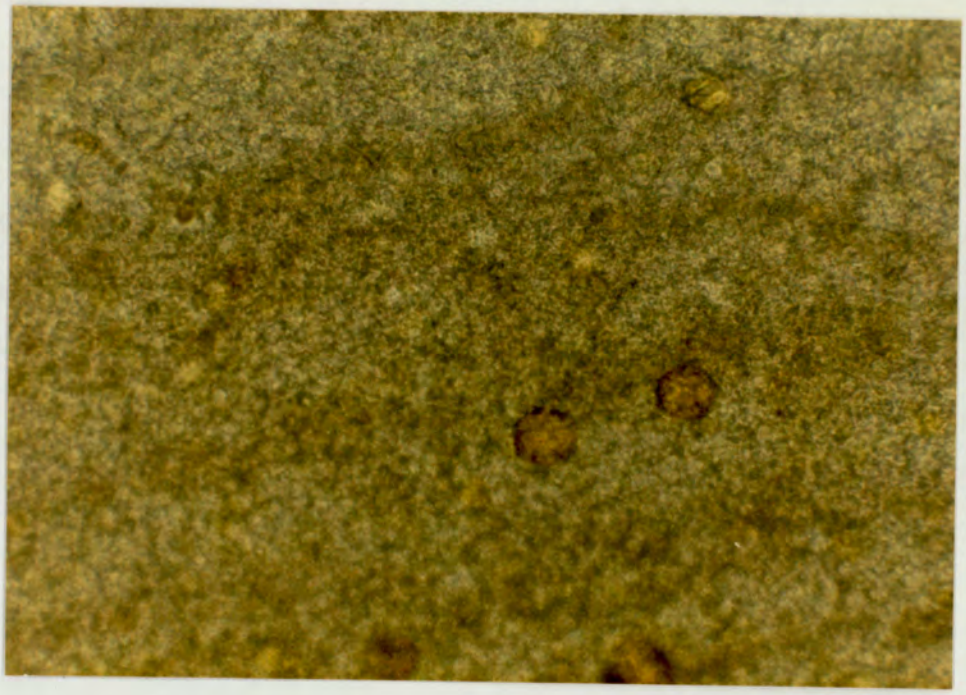


Plate 5.2 750PHB (10% PHV) Film loaded with 6.1% w/w FITC Dextran (X 100)

To illustrate an example of a very poorly dispersed polymer / dye film Plate 5.3 shows a 300PHB (20% PHV) / red perylene dye (Poly R-478, Sigma Chemical Co.) film prepared by simple dispersal

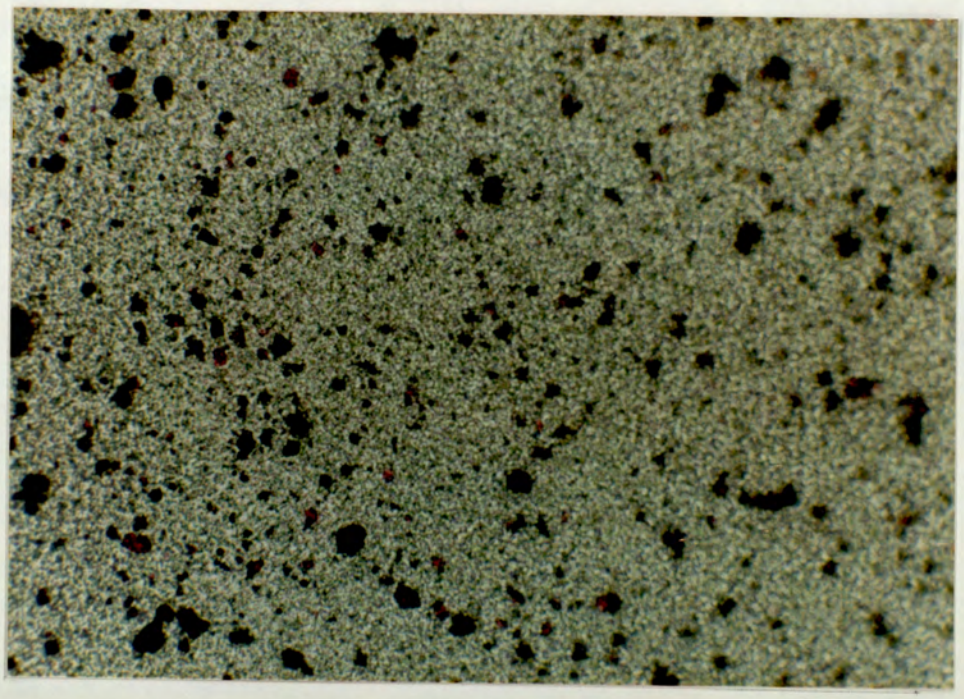


Plate 5.3 Red Perylene dye (1.2% w/w) / 300PHB (10% PHV) Film (X 100)

of the powdered dye into a solution of polymer dissolved in chloroform. Discrete dye particles are evident with very little mixing of the dye within the polymer matrix. Inconsistent release rates would be expected from such a system although Plate 5.3 is only included as a simple example with no dissolution studies having been performed on this type of film.

The polymer dye film matrices produce reasonably constant release profiles after the initial removal of surface dye for the

range of dyes tested in this study. However in some cases (especially the higher FITC dextrans) this burst release period represents the vast majority of the observed release and the diffusional nature of the dye release is no improvement on conventional low molecular weight 'drug' releasing polymer films. However, the PHB/PHV film are potential candidates for (diffusional) macromolecular release systems.

The release of the various dyes tested in the film studies was also determined from physically blended cold compressed tablets of powdered PHB/PHV copolymer. These studies are desribed in the next chapter.

CHAPTER SIX

TABLET RELEASE STUDIES

This chapter describes the preparation and release characteristics of cold compressed tablets made from two grades of PHB/PHV copolymer together with a range of dyes plus a series of excipients usually associated with pharmaceutical tablet formulation.

6.1 INTRODUCTION

Oral administration of drugs from solid dosage forms is well established. The most common matrix types being tablets and capsules. Obvious advantages of tablets include the simple method of preparation and good stability, together with ease of dispensation and accurate rate of drug delivery.

Tablets can generally be divided into two classes depending on their method of preparation - either by compression or by moulding. Compression tablets are made from powdered, usually crystalline materials and frequently contain additives which enhance the tablet quality / method of production. These additional materials include binders, lubricants, diluents and where a fast release rate is desirable, disintegrants.

Moulded tablets or tablet 'triturates' are usually fabricated from moist blends of diluent and / or drug which are extruded to

form a large batch of material. They are usually made when very rapid dissolution of drug is required. The studies in this chapter deal with the former method of tabletting.

Essential requirements of compressed tablet blends include the cohesiveness of the formulation prepared for blending together with ease of lubrication and good free flowing characteristics. In order to impart these qualities on tablet blends addition of excipients is usually required.

There are three methods of tablet preparation - wet and dry granulation and direct compression. The polymer / dye powdered blends evaluated in this work were prepared by direct compression which unlike wet and dry granulation involves no alteration of the physical form of the tablet blend. The granulation methods have been extensively reviewed in the literature.[213]

Direct compression was originally restricted to the compression of single, powdered compounds to form a tablet without addition of any other substance. More recently though the term refers to the formulation of a 'compact' by compression of powdered blends of the drug plus additives. The main advantage of direct compression is its low cost, as this method does not require heat or addition of water contrary to the other preparative techniques.

This study set out to assess the release characteristics of a range of 'actives' (in the form of dyes) from powdered polymer blends. Measurement of the release of the dyes was by dissolution

of the prepared tablets using the standard USP dissolution technique. Different additives were used to change the physical form of the tablet and the following section describes the rationale behind the choice of excipients.

6.2 SYSTEMS STUDIED

Table 6.1 summarises the different excipients used in the preparation of tablet blends with the reasons for choice.

Excipients fall into seven catagories these being fillers (used to enlarge the bulk of the tablet e.g lactose), binders (used in wet granulation e.g. starch - although none were used in this study), lubricants (to prevent the compacts sticking to the tablet tooling e.g. magnesium stearate), disintegrants (to aid tablet breakdown by causing the tablet to imbibe fluid and swell e.g. hydroxypropylmethyl cellulose), glidants (these improve the flow properties of the powdered blends - none used in these studies), Colour additives (to improve tablet appearance - none used) and flavours (which are used to mask any inherent tastes in the tablet formulation). The experimental aim was to fabricate a tablet matrix prepared from PHB/PHV polymers that released dye at a constant rate by alteration of the formulation. Microcrystalline cellulose (MCC (Avicel)) and hydroxypropylmethyl cellulose were

Table 6.1 Excipients used in the preparation of direct compression tablets of PHB/PHV powdered blends

EXCIPIENT	POTENTIAL MERITS
None	Standard reference 'base' blend.
MCC (Avicel)	Commercially used excipient, matrix swells on contact with water.
Lactose	Soluble excipient - produces enhanced release rates.
Poly Acrylic acid (Carbopol)	Creates a microenvironmental acidic media. Forms a gel on hydration, some potential to retard release rate.
Hydroxypropyl--methylcellulose (Methocel)	Forms a gel on hydration which slowly erodes - should decrease release of active.
Cholestramine resin (Amberlite)	Produces local alkaine environment - may enhance rate of polymer matrix breakdown.
Buffer salts	As for Amberlite.

used as excipients because they would be expected to form a gel which would create a diffusion layer around the tablet and decrease the dissolution rate, although not necessarily providing time independent (zero order) release. In addition cholestramine resin (Amberlite), poly acrylic acid (Carbopol) and buffer salts were used to create microenvironmental pH's around the tablet in order to alter the hydrolysis rate of the polymer blend. Lactose a soluble excipient, was used to evaluate the effects of a disintegrant additive.

The tablets (n=3) were dissoluted in a standard USP dissolution system (section 2.3.4.4). Two different buffers were used as dissolution media, one at physiological pH and the other at pH 1.3 to simulate gastric fluid i.e. oral tablet behaviour in the stomach. In addition the dissolution stirring rate was varied and the effect of tablet size, dye loading and the inclusion of various excipients on the dye release rate was investigated. The majority of the excipient loading studies were carried out on blends containing lactose or MCC. The range of additive loadings studied for the two grades of PHB/PHB copolymer used are summarised in Table 6.2.

The rates of release were calculated using an automated computer controlled dissolution system and profiles of drug release were plotted as a percentage of load released against dissolution time. Additional plots of percentage load released

Table 6.2 Tablet blends studied

POLYMER GRADE	DYE / LOAD / % w/w	EXCIPIENT	EXCIPIENT LOADINGS / % w/w
300PHB (20% PHV)	Fluor / 7.5	Base	N/A
350PHB (12% PHV)	Fluor / 7.5	Base	N/A
300PHB (20% PHV)	FITC4 / 7.5	Base	N/A
"" ""	FITC4 / 7.5	Avicel	12.5
"" ""	FITC4 / 7.5	Lactose	12.5, 25, 50
300PHB (20% PHV)	FITC40 / 7.5	Base	N/A
"" ""	FITC40 / 7.5	Avicel	7.5, 25
"" ""	FITC40 / 7.5	Lactose	7.5, 12.5, 25
"" ""	FITC40 / 7.5	HPMC E4M	10
"" ""	FITC40 / 7.5	pH 10.6	3
350PHB (12% PHV)	FITC4 / 7.5	Base	N/A
"" ""	FITC4 / 7.5	Avicel	7.5
"" ""	FITC4 / 7.5	Lactose	7.5, 12.5, 25
"" ""	FITC4 / 7.5	Carb 941	5
"" ""	FITC4 / 7.5	HPMC E15	10
350PHB (12% PHV)	FITC40 / 7.5	Base	N/A
"" ""	FITC40 / 15	Base	N/A
"" ""	FITC40 / 7.5	Avicel	7.5, 12.5

Table 6.2 (Cont)

POLYMER GRADE	DYE / LOAD / % w/w	EXCIPIENT	EXCIPIENT LOADINGS / % w/w
350PHB (12% PHV)	FITC40 / 7.5	Lactose	7.5, 12.5
"" ""	FITC40 / 7.5	Lactose	7.5 (Double size)
"" ""	FITC40 / 15	Lactose	7.5
"" ""	FITC40 / 7.5	Carb 934	5
"" ""	FITC40 / 7.5	Carb 941	5
"" ""	FITC40 / 7.5	HPMC E4M	10
"" ""	FITC40 / 7.5	HPMC E15	10
"" ""	FITC40 / 7.5	pH 10.6	3
"" ""	FITC40 / 7.5	Amberlite	5

Fluor = Fluorescein; Base = no excipients; Carb = Carbopol; HPMC = Hydroxypropylmethyl cellulose; pH 10.6 = Alkaline buffer salts (Na_2CO_3 / $NaHCO_3$)

against square root of dissolution time were drawn in order to assess any correlation of the release rate with the square root of time.

6.3 RESULTS AND DISCUSSION

The dye release profiles from the various tablet blends are divided into two sections depending on the experimental method used for tablet dissolution - either 'static' or USP.

The variations of individual blend release profiles are indicated by a representative error bar. The major deviation from the constructed profiles is from sample data spread, The errors in dye concentration measurement being small for most systems. The FITC 4 dye profiles however, do contain an error from the measurement of the dye concentration. This error stems from the very low extinction coefficient of this dye and has been incorporated into the general error bar drawn on the graphs. The type of dissolution method used also made a contribution to the total error bar with a significant error from sampling technique occurring in the 'static' dissolution technique.

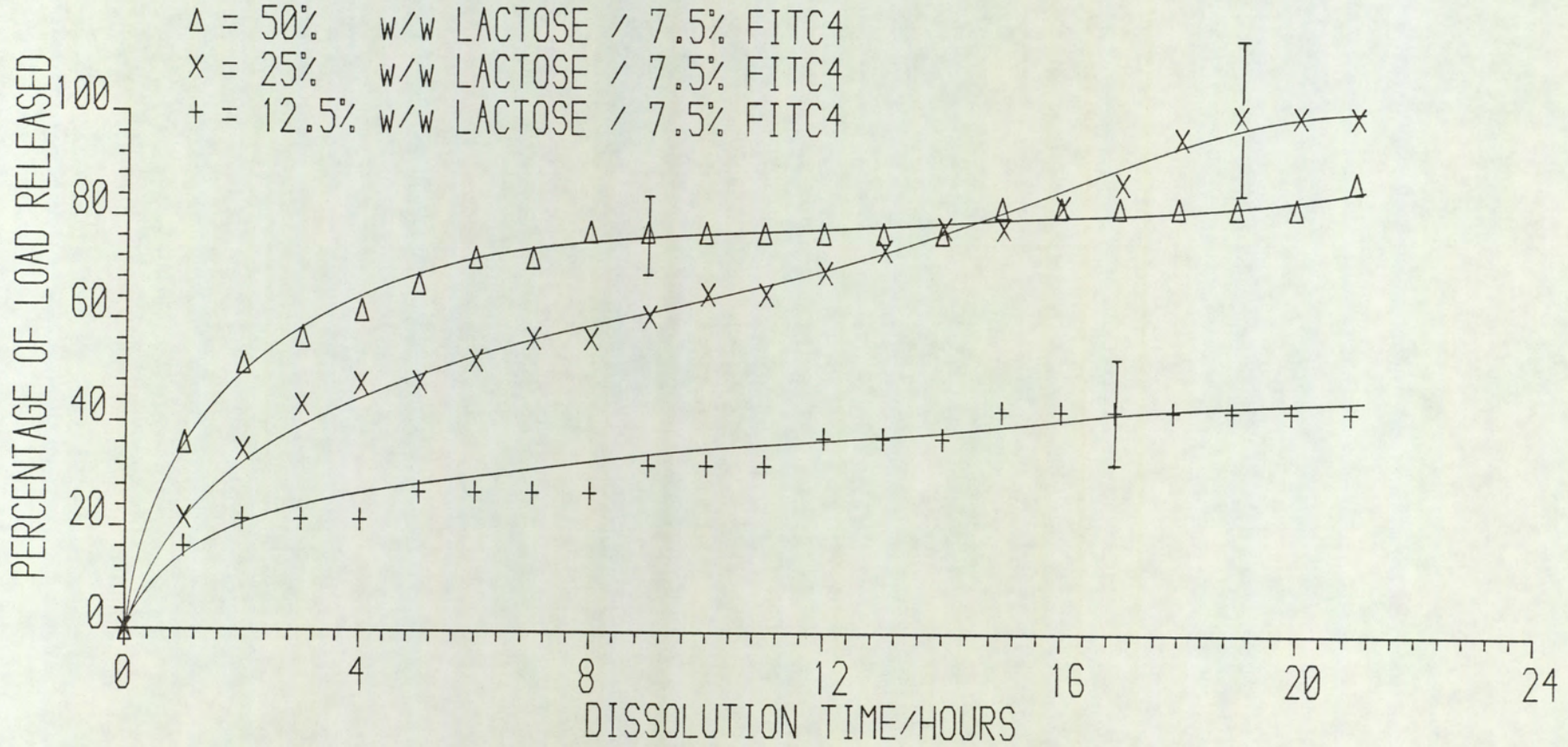
6.3.1 USP DYE DISSOLUTION

The USP release profiles are considered in the following sections according to the incorporated excipient in the tablet blends. A summary of the dye release from the various tablet systems in terms of times for 50% and 90% dye release (t_{50} and t_{90} repectively) is given in Table 6.3.

6.3.1.1 Lactose

The first series of graphs (Graphs 6.1 to 6.3) show the release of the 4,000 and 40,000 FITC dyes from the 300PHB (20% PHV) grade of copolymer. Comparative 'base' (no excipient) profiles are presented in Graphs 6.4 and 6.5. The profiles are characterised by an initially fast release of dye followed by a

GRAPH 6.1 USP DISSOLUTION OF 300PHB (20%PHV) / FITC DYE TABLETS WITH LACTOSE FILLER IN A pH 7.4 BUFFER AT 37°C

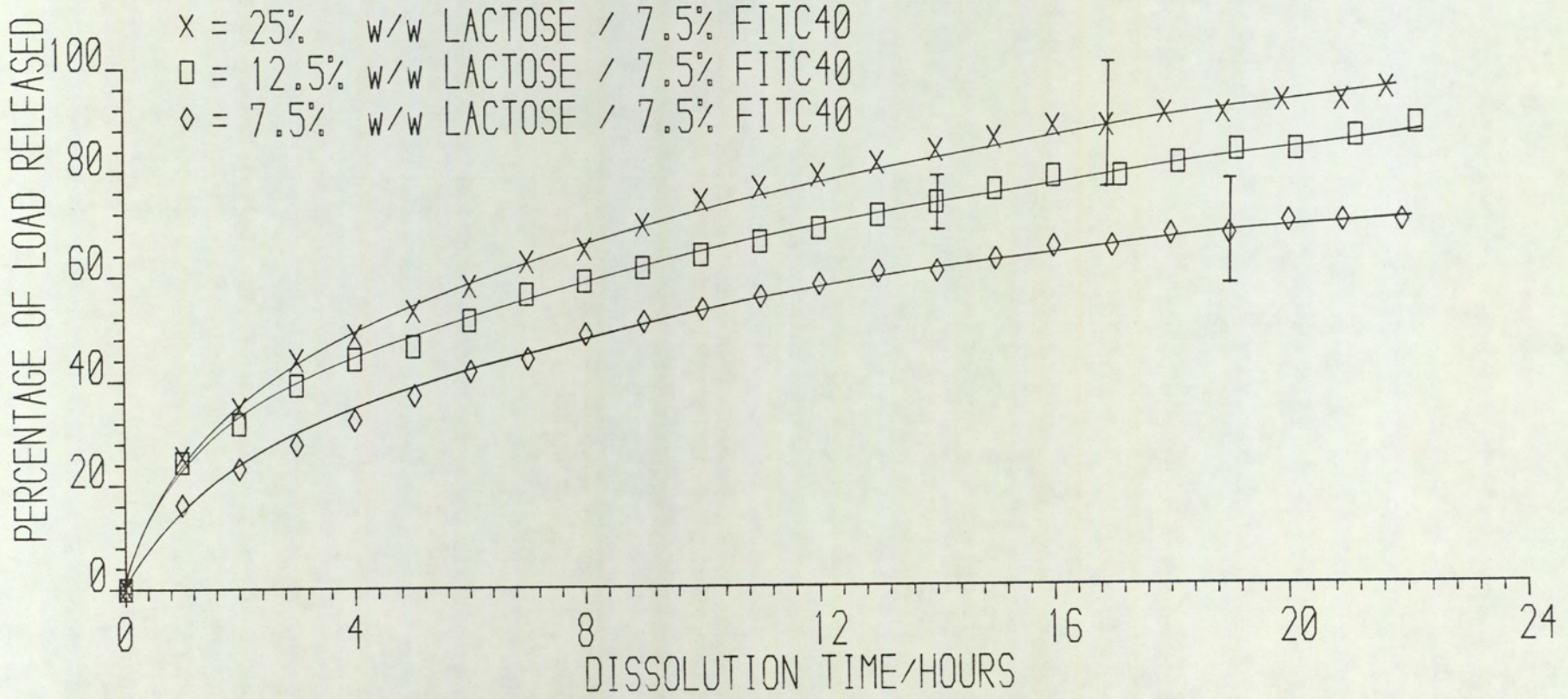


gradual tailing off, indicating a diffusional release mechanism. Release over the time scale of the dissolution experiments varied from 30% to complete dissolution of dye.

The first observable trend is the expected increase in release rate with increasing lactose content. This is shown both by the time for 10% release and the amount of dye released after six hours data from Tables 6.3 to 6.5. Inspection of graphs 6.1 and 6.2 also shows the almost linear increase of release rate with lactose load. A dependence of the release rate on dye molecular weight is clearly illustrated in Graph 6.3, the 7.5% lactose blends, for example, show an increase in the amount of dye released after 6 hours from 33 to 49% as the dye molecular weight increases from 4,000 to 40,000. This was unexpected because as already stated in Chapter 5, the larger dye would be expected to diffuse out of the matrix at a lower rate. This indicates that the higher molecular weight dye tablet blends contain more heterogeneities than the 4,000 FITC dextran dye.

A difference between polymer grades is also observed, with the lower copolymer (350PHB 12% PHV) showing a marginally faster release rate than the higher valerate (300PHB 20%PHV) polymer blends. An example of this difference can be seen in the times for 50% FITC40 release from tablet matrices containing 7.5% lactose. A decrease in the t_{50}% value occurs as polymer valerate content decreases from 20 to 12%.

GRAPH 6.2 USP DISSOLUTION OF 300PHB (20%PHV) / FITC DYE TABLETS WITH LACTOSE FILLER IN A pH 7.4 BUFFER AT 37°C



GRAPH 6.3 USP DISSOLUTION OF 350PHB (12%PHV) / FITC DYE TABLETS WITH LACTOSE FILLER IN A pH 7.4 BUFFER AT 37°C

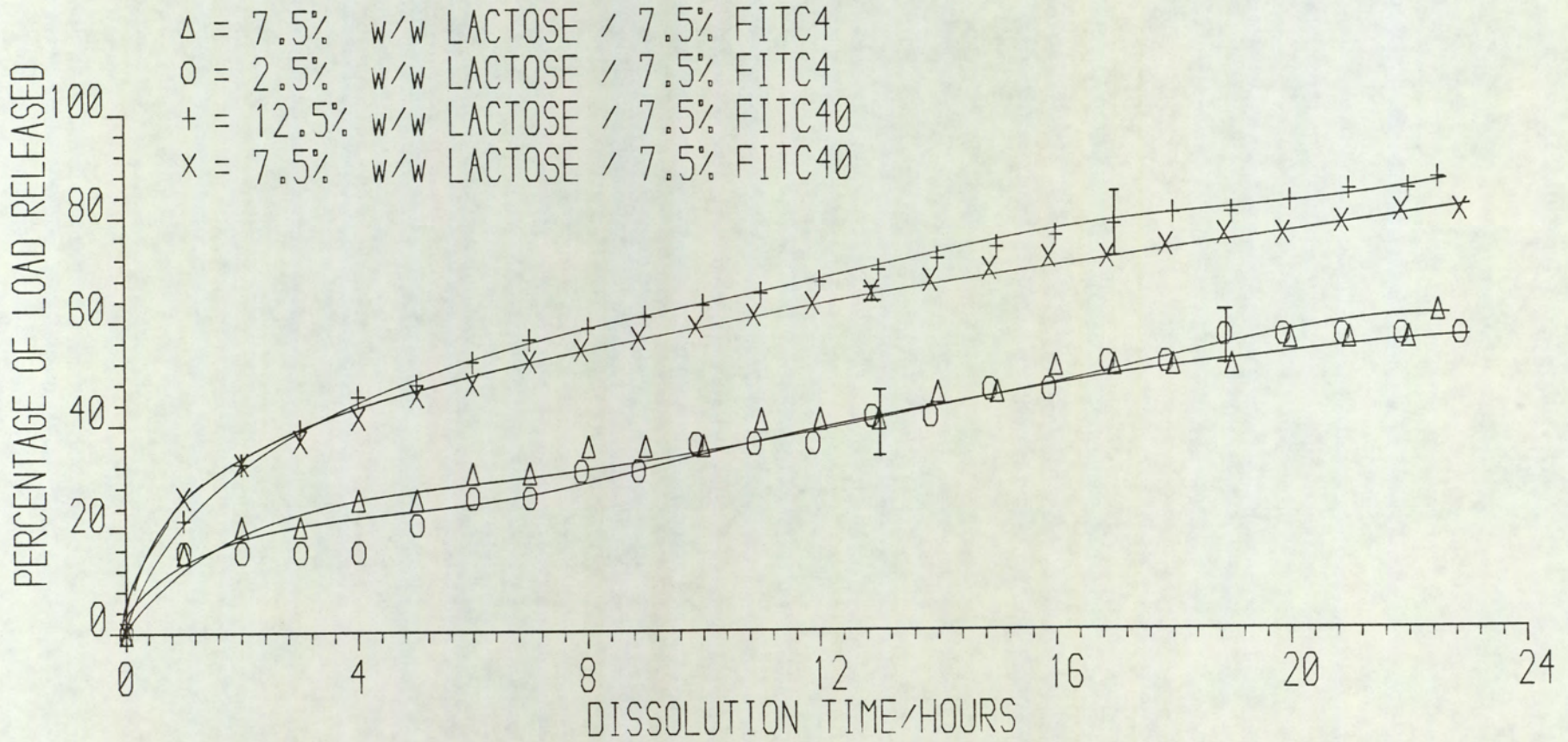


Table 6.3 Dissolution parameters for USP tablet dissolution: Base and Avicel (MCC) studies

TABLET BLEND	%AGE RELEASED @ 6 Hours	t_{50}% / Hours	t_{90}% / Hours	%AGE RELEASED @ End of Diss^{n}
300/ Base /FITC4	18	>24	>24	38 @ 21 Hours
300/ Base /FITC40	33	18	>24	57 @ 22 Hours
350/ Base /FITC4	21	>24	>24	36 @ 23 Hours
350/ Base /FITC40	35	16	>24	61 @ 23 Hours
350/ Base# /FITC40	59	4.1	>24	62 @ 7 Hours
300/ Base / F	5.6	>24	>24	7.5 @ 23 Hours
350/ Base / F	6.2	>24	>24	8.4 @ 23 Hours
300/12.5% A/FITC4	26	>24	>24	35 @ 21 Hours
300/50% A/FITC4	73	2.8	15.1	95 @ 21 Hours
300/7.5% A/FITC40	32	18.5	>24	55 @ 21 Hours
300/12.5% A/FITC40	27	>24	>24	46 @ 21 Hours
350/7.5% A/FITC4	12	>24	>24	24 @ 23 Hours
350/7.5% A/FITC40	35	13.7	>24	65 @ 23 Hours
350/12.5% A/FITC40	37.5	12.6	>24	71 @ 23 Hours

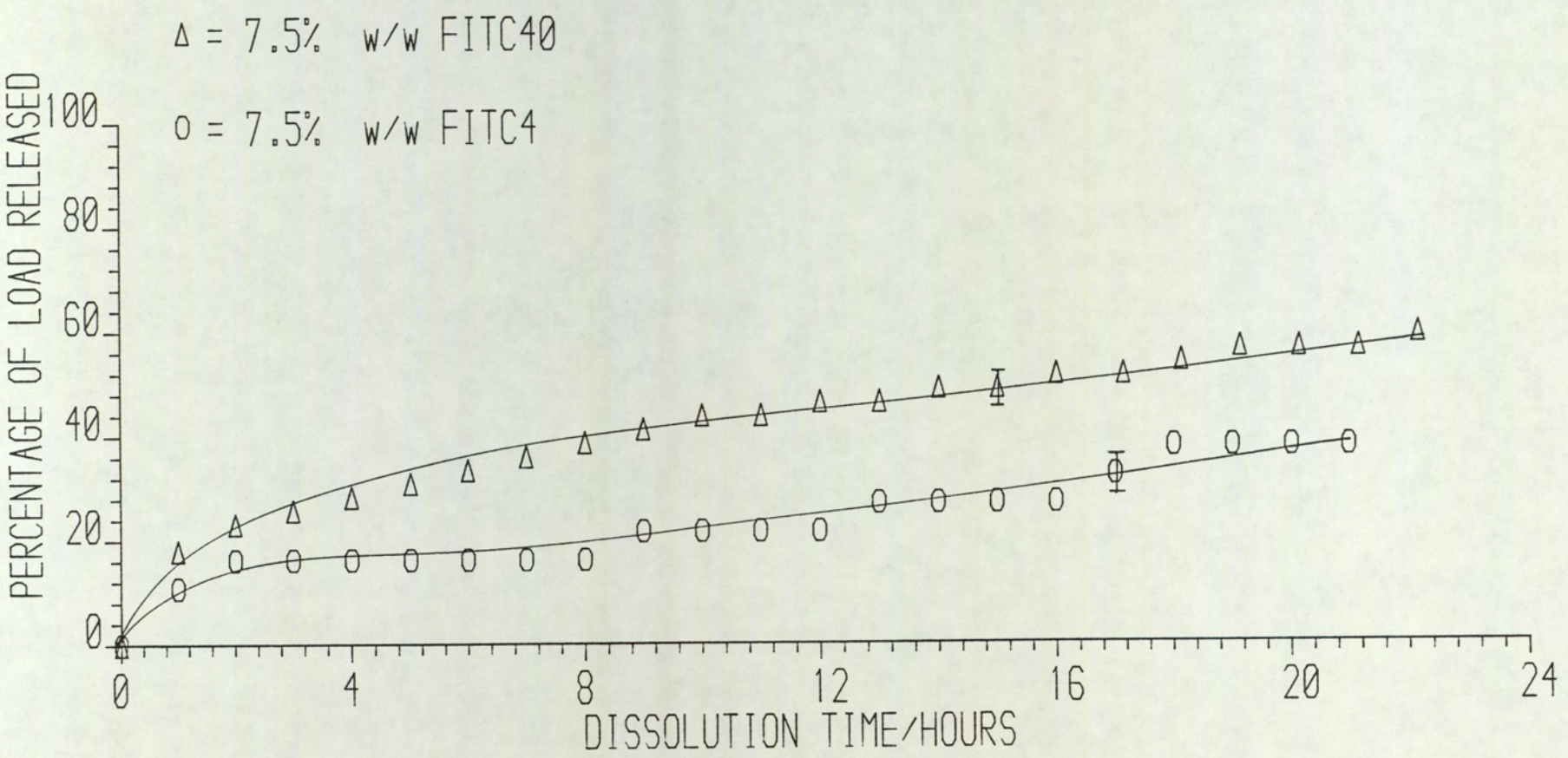
300 and 350 refer to polymer grades 300PHB (20%PHV) and 350PHB (12%PHV); F = Fluorescein; A = Avicel (MCC); Dye loadings = 7.5% w/w (#= 15% w/w).

Table 6.4 Dissolution parameters for USP tablet dissolution:Lactose studies

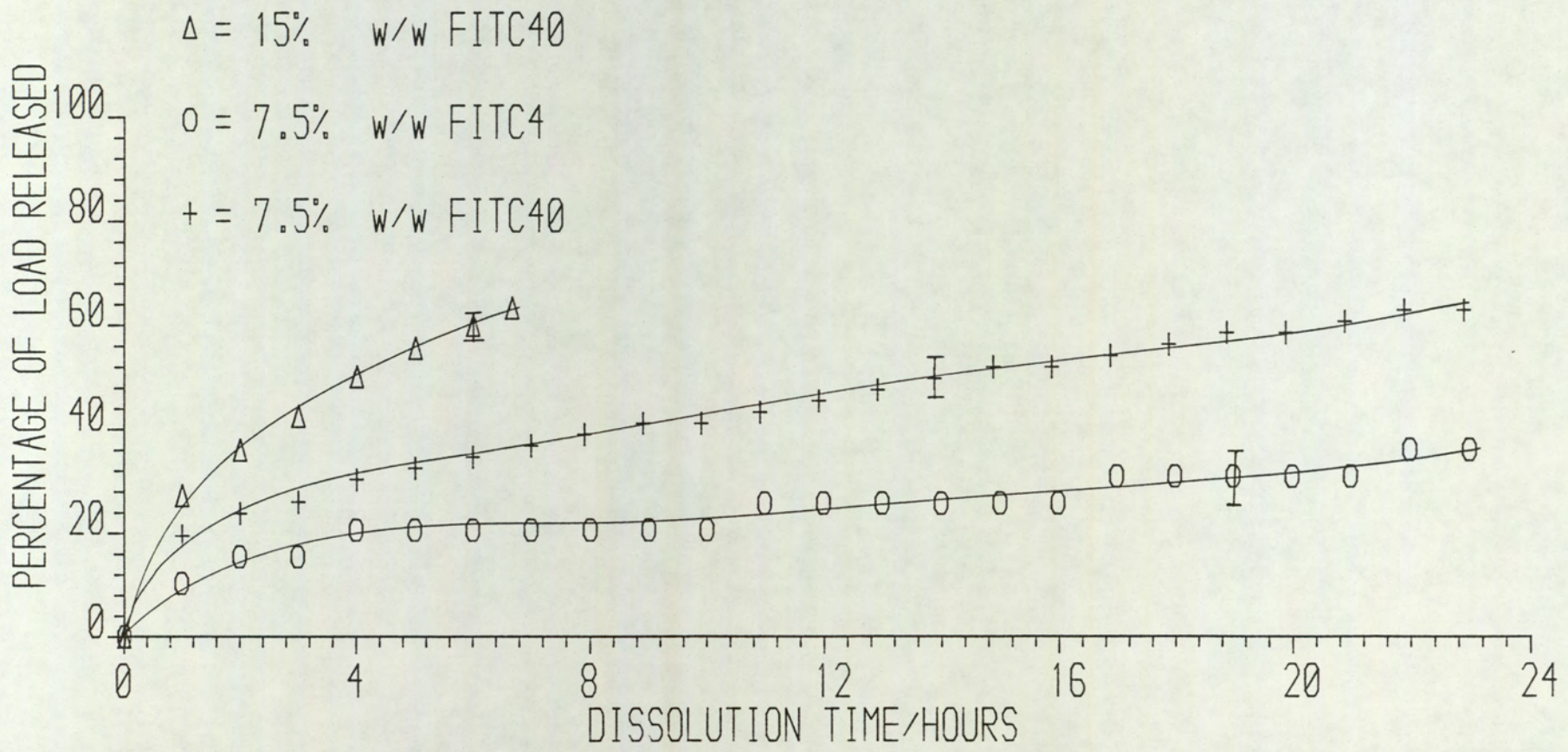
TABLET BLEND	%AGE RELEASED @ 6 Hours	t_{50}% / Hours	t_{90}% / Hours	%AGE RELEASED @ End of Diss^{n}
300/12.5% L/FITC4	27	>24	>24	44 @ 21 Hours
300/25% L/FITC4	54	5.4	>24	86 @ 21 Hours
300/50% L/FITC4	71	2.0	17.5	100 @ 19 Hours
300/7.5% L/FITC40	41	9.3	>24	70 @ 22 Hours
300/12.5% L/FITC40	52	6.0	>24	88 @ 22 Hours
300/25% L/FITC40	58	4.5	20.75	94 @ 22 Hours
350/2.5% L/FITC4	28	16	>24	56 @ 23 Hours
350/7.5% L/FITC4	33	15.5	>24	60 @ 23 Hours
350/7.5% L/FITC40	49	6.6	>24	79 @ 23 Hours
350/12.5% L/FITC40	52	5.7	>24	87 @ 23 Hours

300 and 350 refer to polymer grades 300PHB (20%PHV) and 350PHB (12%PHV). All dye loadings 7.5% w/w.

GRAPH 6.4 USP DISSOLUTION OF FITC4 AND 40
FROM 300PHB (20%PHV) TABLETS (NO EXCIPIENTS)
IN A pH 7.4 BUFFER AT 37°C



GRAPH 6.5 USP DISSOLUTION OF FITC4 AND 40
FROM 350PHB (12%PHB) TABLETS (NO EXCIPIENTS)
IN A pH 7.4 BUFFER AT 37°C



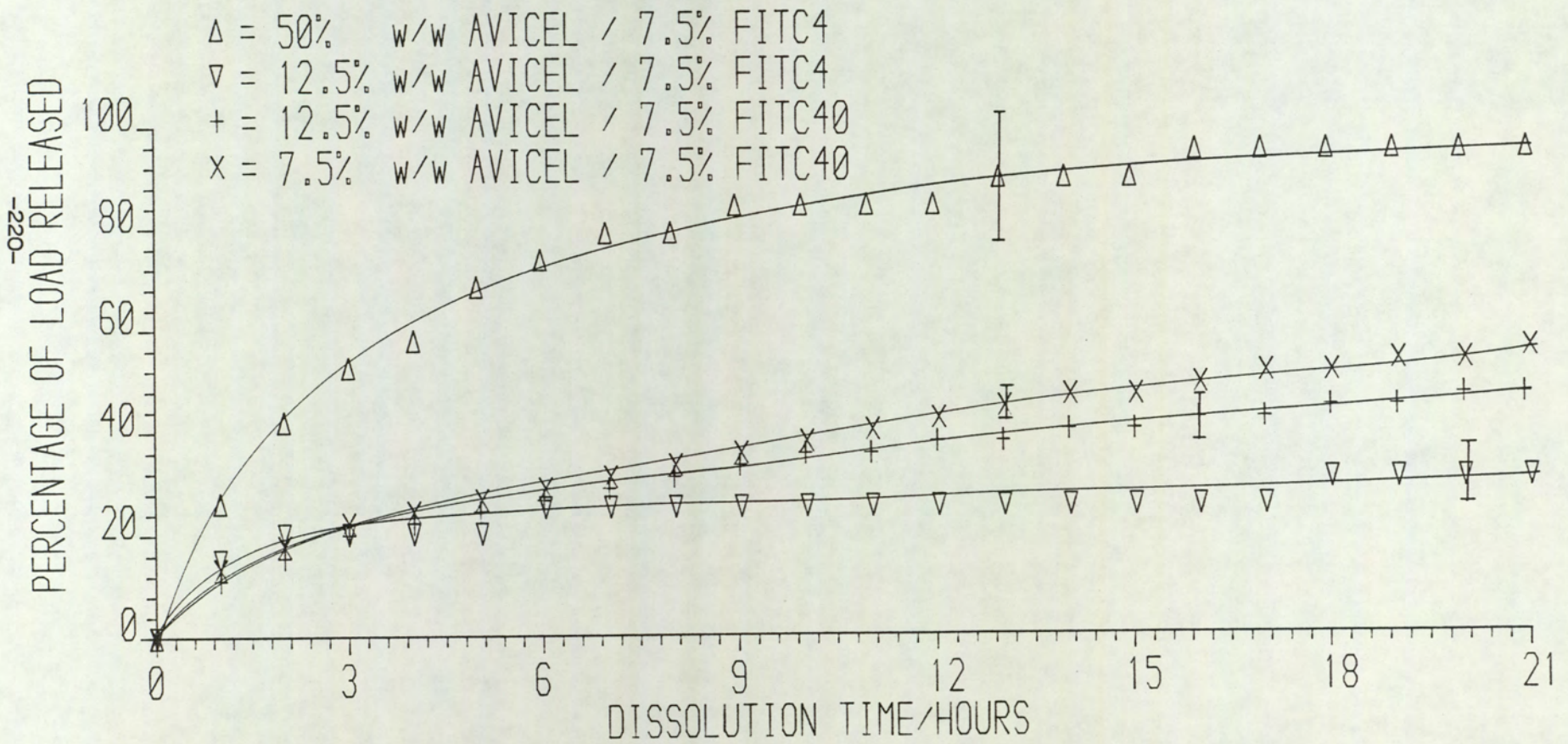
The release profiles show that the lactose aids dye release, with the excipient dissolving out of the matrix at a fast rate. However at low loadings, there is little difference between release rates with dye dissolution from the 7.5% lactose loading (350PHB 12% PHV polymer grade) being approximately the same as the 2.5% loading, and the low loading rates generally approach those of the base tablet matrices.

6.3.1.2 Microcrystalline Cellulose (MCC)

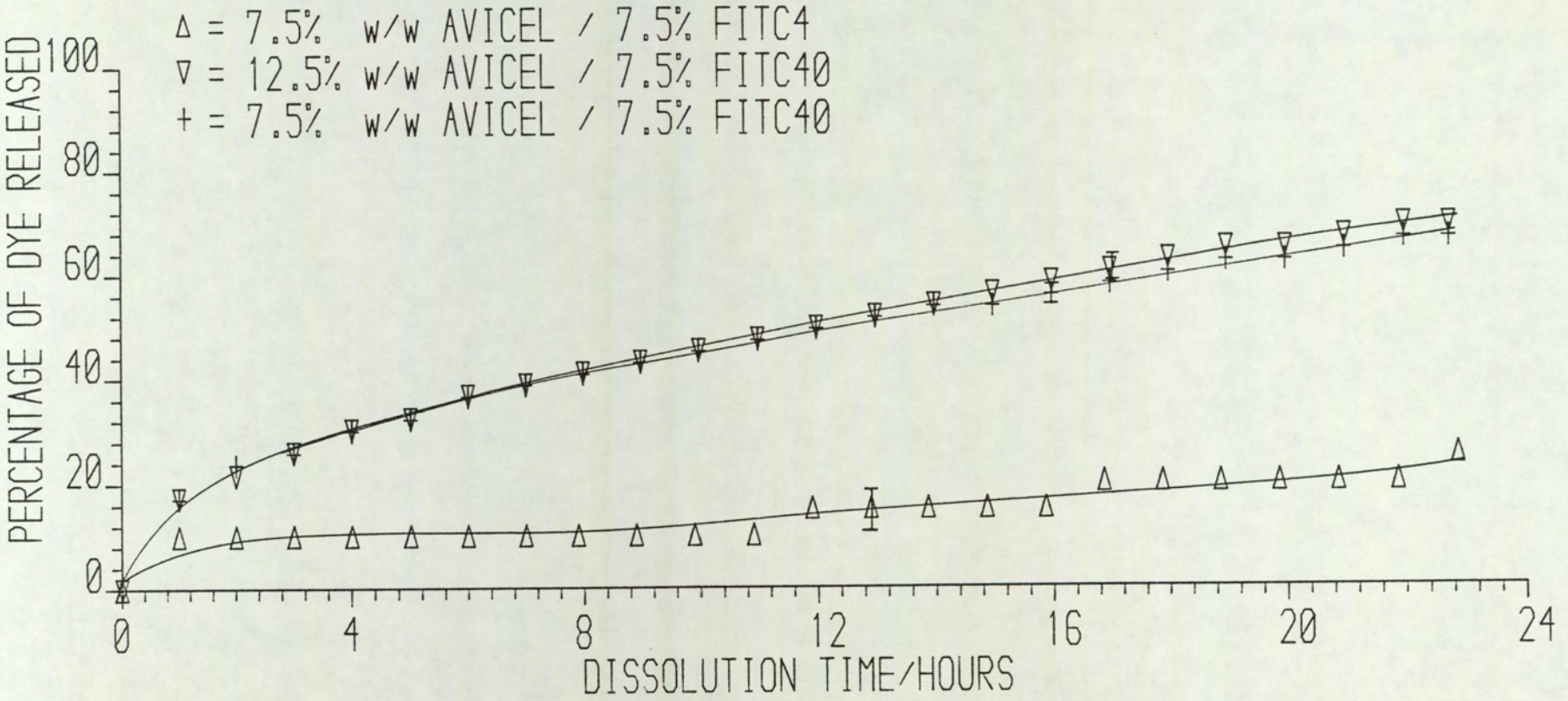
A similar series of release profiles to those obtained from the lactose blends illustrating release of FITC4 and FITC40 from MCC blends are shown in Graphs 6.6 and 6.7. The same almost linear dependence of dye release rate on excipient loading is seen for the 300PHB (20% PHV) grade of polymer. The 350PHB (12% PHB) polymer blends however exhibit little difference between the release rate at moderate excipient loadings with the 7.5% MCC loading releasing FITC40 dye at a similar rate to the 12.5% loading (t_{50}% times 13.7 and 12.6 hours respectively). The associated increase in the dye dissolution rate for the higher molecular weight dyes is again shown by the 12.5% MCC loadings for the 300PHB (20% PHV) grade, which release more of the larger dye (46%) than the FITC4 dye (35%) after 21 hours, with a similar pattern for the other polymer grade.

A difference in dye release rates between the two polymer grades is also seen with this excipient, and is shown by the

GRAPH 6.6 USP DISSOLUTION OF 300PHB (20%PHB) / FITC DYE TABLETS WITH AVICEL FILLER IN A pH 7.4 BUFFER AT 37°C



GRAPH 6.7 USP DISSOLUTION OF 350PHB (12%PHV) / FITC DYE TABLETS WITH AVICEL FILLER IN A pH 7.4 BUFFER AT 37°C



percentage of FITC40 released at the end of the dissolution from tablet matrices containing 12.5% MCC rising from 46 to 71% for the 300PHB and 350PHB copolymer blends respectively. As with the lactose blends, low MCC loadings produce similar profiles to the base blends, with the amount of dye released from MCC matrices being generally less than equivalent lactose matrices.

6.3.1.3 Other excipients

Graphs 6.8 to 6.11 show the release of the two FITC dextran dyes from PHB/PHV tablet matrices containing various excipients other than lactose or MCC. All exhibit very much greater rates of dye release than the corresponding lactose, MCC or base blends.

The HPMC tablets were expected to gel on hydration. This could influence the release rate in two ways: On the one hand a corresponding increase in rate would be expected as the swelling of the matrix would increase matrix porosity and weaken the tablet matrix, and on the other it has been suggested[214,215] that the gelling could suppress the rate of 'active' release. The profiles seen in Graph 6.8 obviously show no such retardation, with the former matrix porosity effect due to swelling being the major effect.

An associated increase in release rate as dye molecular weight increases is again illustrated by the times for 50% dye release from 350PHB (12% PHV) matrices containing 10% of the E15 grade of

GRAPH 6.8 USP DISSOLUTION OF PHB/PHV / FITC DYE TABLETS WITH HPMC FILLER IN A pH 7.4 BUFFER AT 37°C

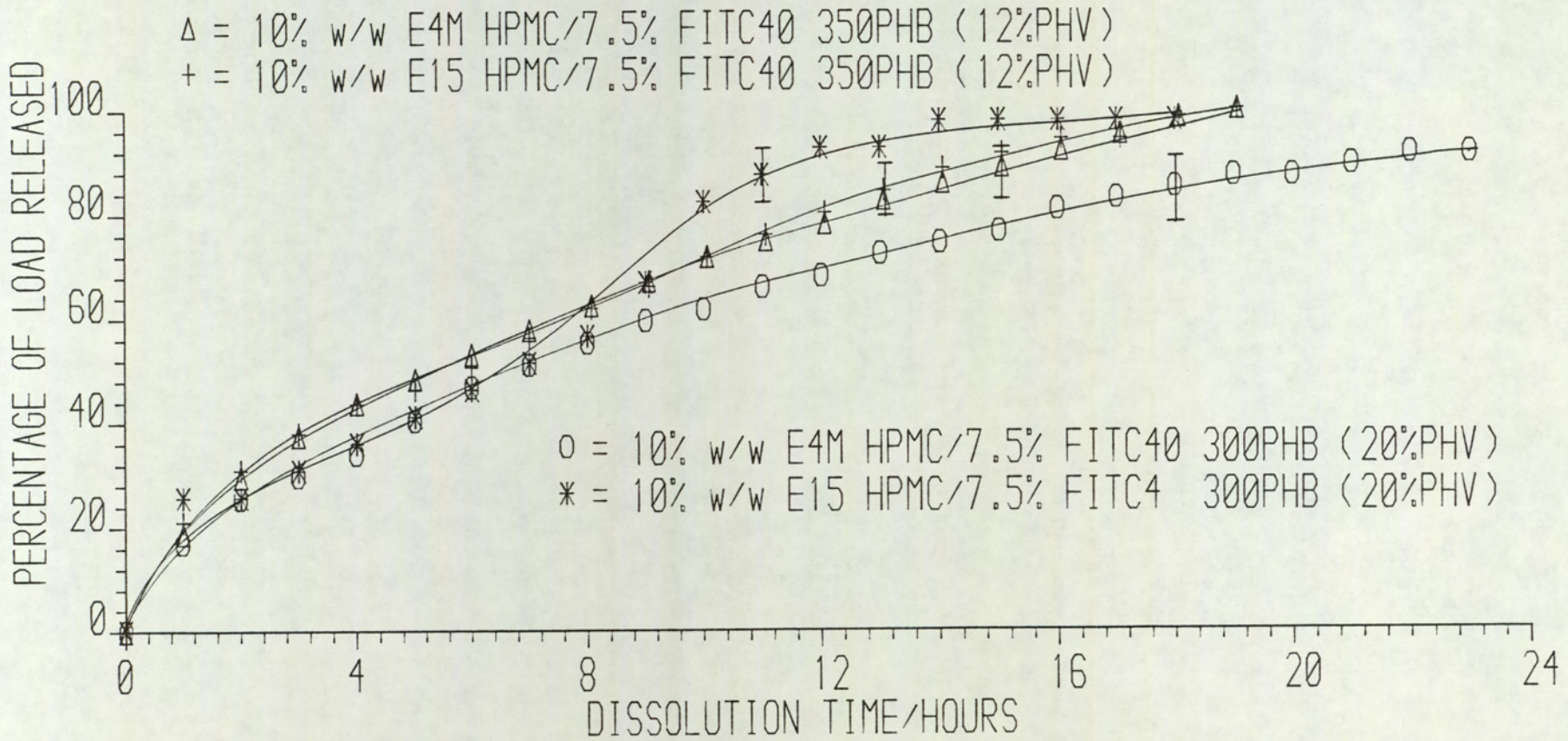


Table 6.5 Dissolution parameters for USP tablet dissolution studies: Various excipients

TABLET BLEND	%AGE RELEASED @ 6 Hours	t_{50}% / Hours	t_{90}% / Hours	%AGE RELEASED @ End of Diss^{n}
350/5% C934/FITC40	47	6.9	18.1	100 @ 23 Hours
350/5% C941/FITC4	34	14.6	>24	56 @ 18 Hours
350/5% C941/FITC40	41	7.8	18.1	100 @ 23 Hours
300/5% Amb /FITC40	38	12.4	>24	63 @ 24 Hours
350/5% Amb /FITC40	41	7.7	14.6	100 @ 23 Hours
300/10% H4M/FITC40	47	6.7	21.4	92 @ 23 Hours
350/10% H4M/FITC40	53	5.5	15.4	100 @ 19 Hours
350/10% H15/FITC4	44	6.7	11.5	100 @ 14 Hours
350/10% H15/FITC40	51	5.6	14.5	100 @ 19 Hours
300/3% 10.6/FITC40	48	6.7	14.4	100 @ 23 Hours
350/3% 10.6/FITC40	60	4.4	13.7	100 @ 19 Hours

300 and 350 refer to polymer grades 300PHB (20%PHV) and 350PHB (12%PHV); C = Carbopol; Amb = Amberlite; H4M = HPMC Grade E4M; H15 = HPMC Grade E15 and 10.6 = Buffer salts. All dye loadings 7.5% w/w.

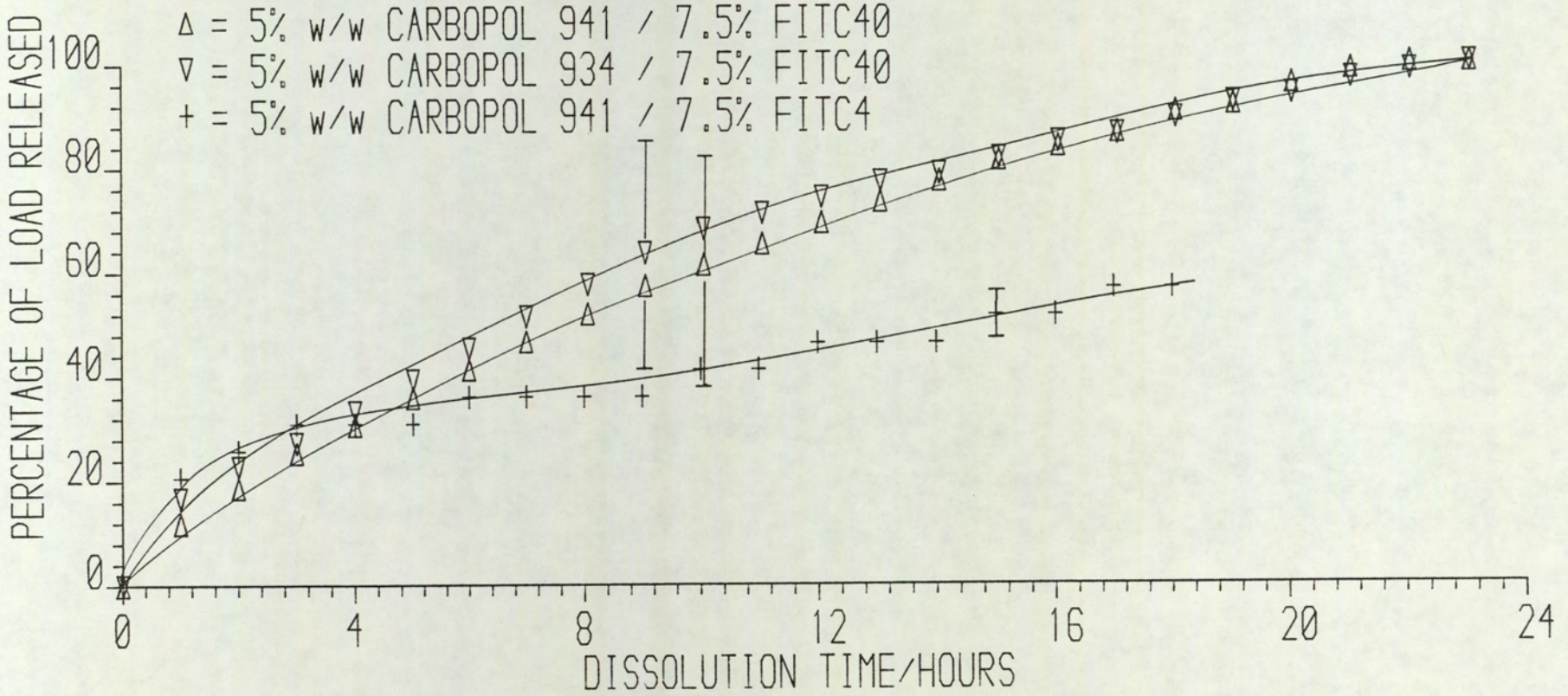
HPMC. The t_{50}% values decrease from 6.7 to 5.6 hours as dye molecular weight increases from 4,000 to 40,000. A small difference between the two polymer grades is also evident but it is less pronounced than that observed in the lactose and MCC systems.

The excipients used to provide a microenvironmental pH around the tablets on hydration were poly acrylic acid (Carbopol) for acidic conditions; with cholestramine resin (Amberlite) and buffer salts (Na_2CO_3 / $NaHCO_3$ - formulated to give a pH 10.6 buffer conditions) providing the the aqueous alkaline conditions. All three exhibited relatively fast release rates and the release profiles are shown in Graphs 6.9 to 6.11.

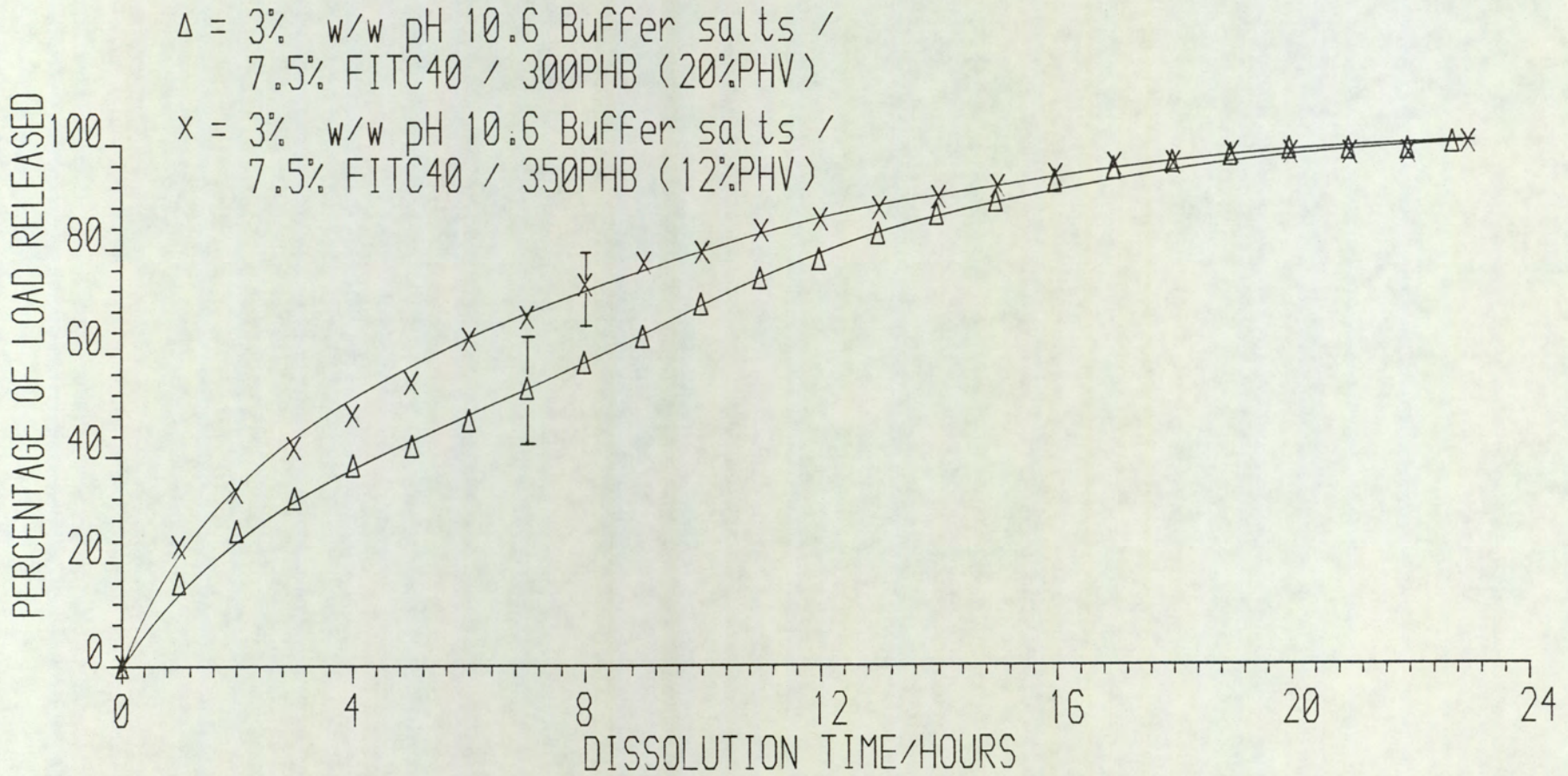
The Carbopol blends show the characteristic dye molecular weight dependence on the release rate, with the buffer salt and cholestramine resin profiles illustrating the large differences between the release rates of the FITC40 from the different polymer grades - as shown by the t_{90}% times for the pH 10.6 buffer salt matrices which decrease from 14.4 to 13.7 hours for the 300PHB and 350PHB polymer grades respectively.

The overall order of release for these three excipients is pH 10.6 buffer salts > Carbopol > Amberlite. The high release rate observed for the buffer salt blends is presumably due to an increase in matrix porosity as the very soluble salts are dissolved. The Carbopol matrices showed a fast rate of release due to the observed matrix disintegration at the end of the study.

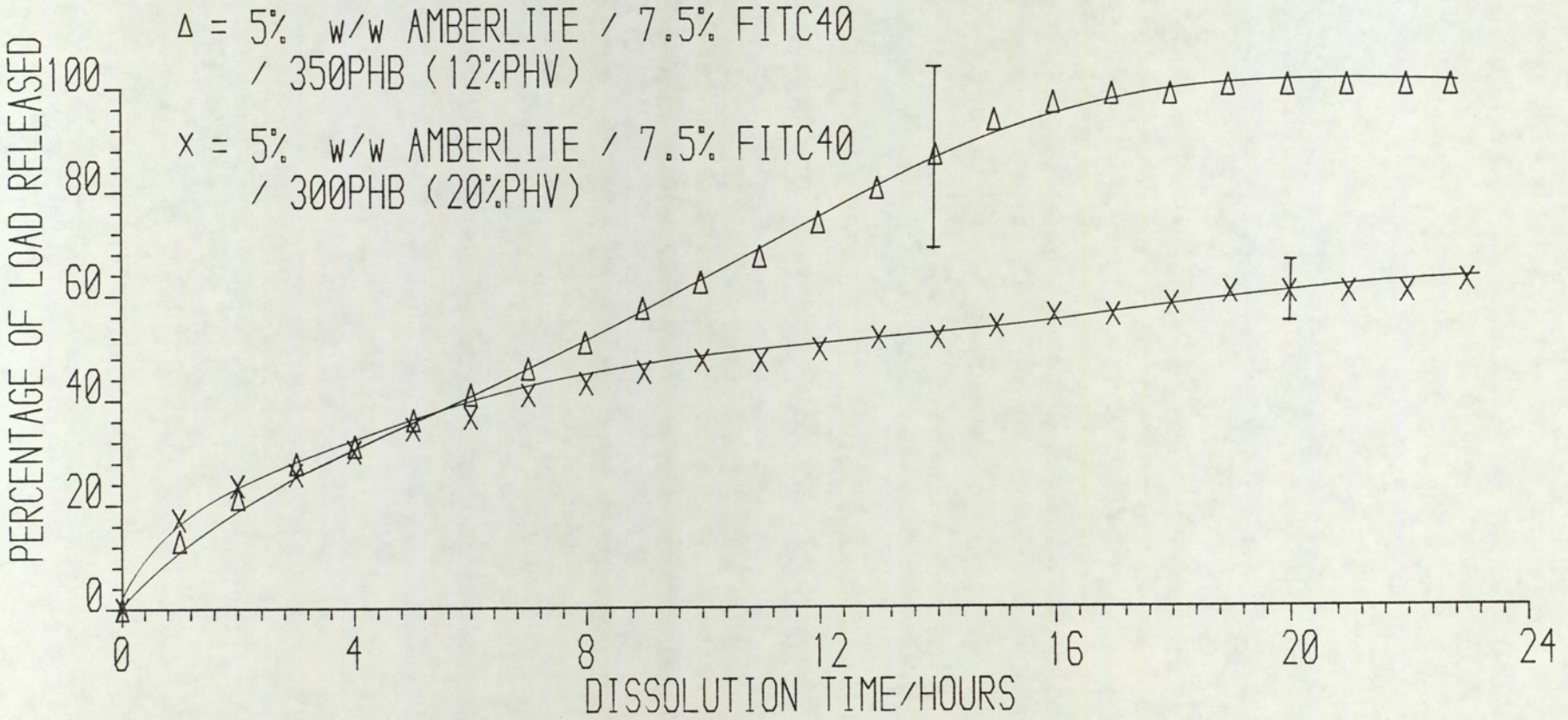
GRAPH 6.9 USP DISSOLUTION OF 350PHB (12%PHV) / FITC DYE TABLETS WITH CARBOPOL FILLER IN A pH 7.4 BUFFER AT 37°C



GRAPH 6.10 USP DISSOLUTION OF PHB/PHV - FITC DYE TABLETS WITH pH 10.6 BUFFER SALTS AS FILLER IN A pH 7.4 BUFFER AT 37°C



GRAPH 6.11 USP DISSOLUTION OF PHB/PHV / FITC DYE TABLETS WITH AMBERLITE FILLER IN A pH 7.4 BUFFER AT 37°C



There was no change in dissolution media pH during the experimental runs for any of the non-neutral excipients tablets.

6.3.1.4 Fluorescein Studies

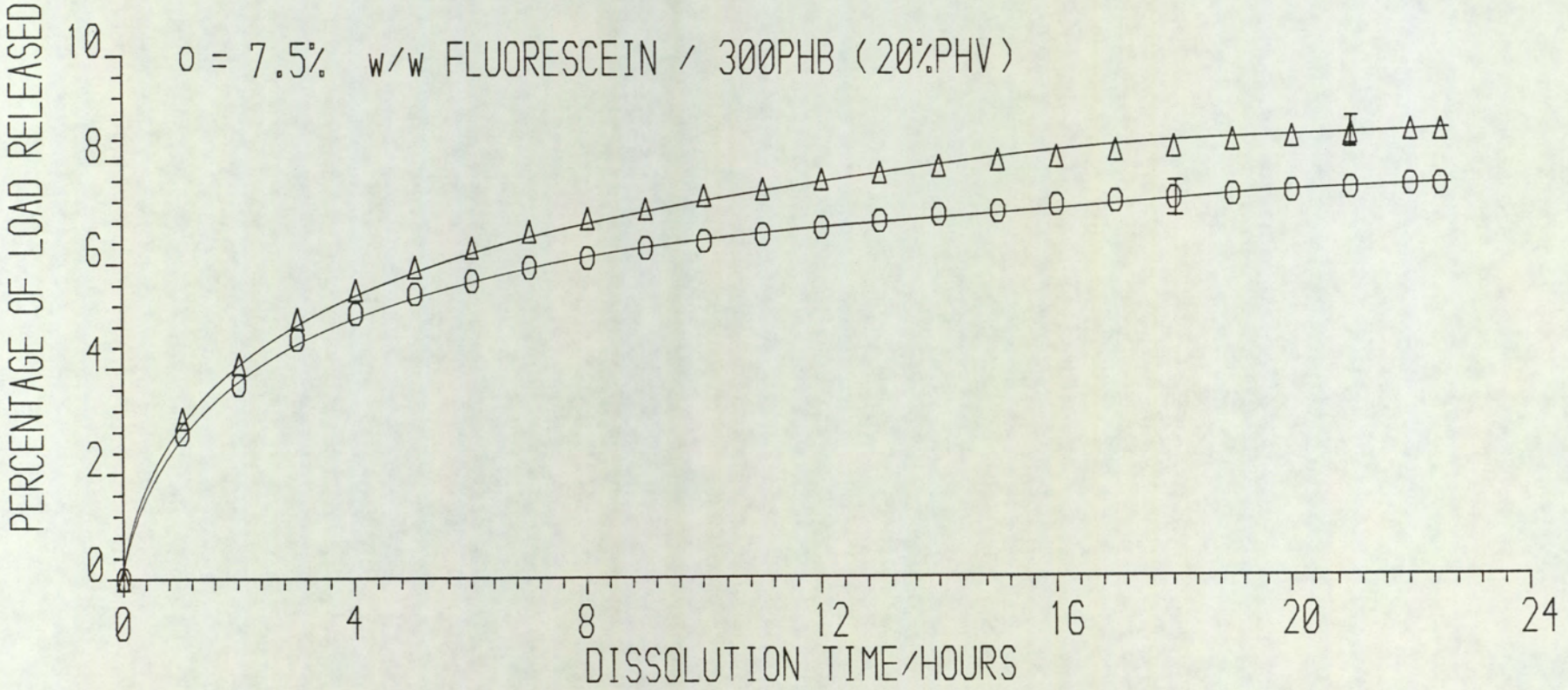
For comparative studies to the macromolecular dye blends the release of fluorescein - a low molecular weight dye from polymer blends (no excipients) was investigated. Graph 6.12 shows the release of this dye from the two grades of polymer (note the 'y' axis scale, which is one tenth of the other tablet ordinate scales). The profiles drawn in Graph 6.12 are very precise. This stems from the high extinction coefficient of the fluorescein dye in neutral buffers. The release rate is an order of magnitude slower than the FITC4 and FITC40 dye / excipient dissolution rates and five times slower than the corresponding FITC dextran dye / polymer base (no excipient) blends. After the initial 'burst' release an essentially constant release rate is maintained up to the end of the dissolution study, after which less than 9% of the total dye load had been released.

The difference between the two polymer blends is again illustrated by a slower release of the 300PHB (20% PHB) grade of polymer - 5.6% of the dye load released as opposed to 6.2% for the lower polymer valerate grade after six hours.

GRAPH 6.12 USP DISSOLUTION OF FLUORESCEIN
FROM PHB/PHV TABLETS (NO EXCIPIENTS)
IN A pH 7.4 BUFFER AT 37°C

Δ = 7.5% w/w FLUORESCEIN / 350PHB (12%PHV)

o = 7.5% w/w FLUORESCEIN / 300PHB (20%PHV)



6.3.2 USP Experimental variations

The standard dissolution test was on 100mg tablets containing 7.5mg of dye with or without excipients dissoluted in a pH 7.4 buffer at 37^{o}C. Alteration of the variables was investigated and the results are discussed in the following sections.

6.3.2.1 Low dissolution rate

As has already been discussed in section 2.4 the thickness of the hydrodynamic boundary layer of a drug when released from a solid matrix depends on the dissolution rate of the dissoluting medium. Studies on representative polymer/dye/excipient tablets were performed at one quarter of the normal dissolution rate for 5.5 hours. The results are plotted in Graph 6.13 which is plotted on the same scale as the other tablet release graphs to enable comparisons. Table 6.6 lists the percentage of dye load released after five hours as a function of dissolution stirring rate.

The amount of dye released at the lower stirring rate varies from a value of approximately 70% of the higher stirring rate for the base FITC4 blend with similar two thirds values for the other FITC4 tablet blends, up to 80 to 90% for the FITC40 dyes indicating the importance of stirring rate for the slowly diffusing low molecular weight dye.

GRAPH 6.13 USP DISSOLUTION OF PHB/PHV TABLETS IN A pH 7.4 BUFFER AT 37°C AT A REDUCED BASKET SPIN SPEED OF 25 rpm (normal speed 100 rpm)

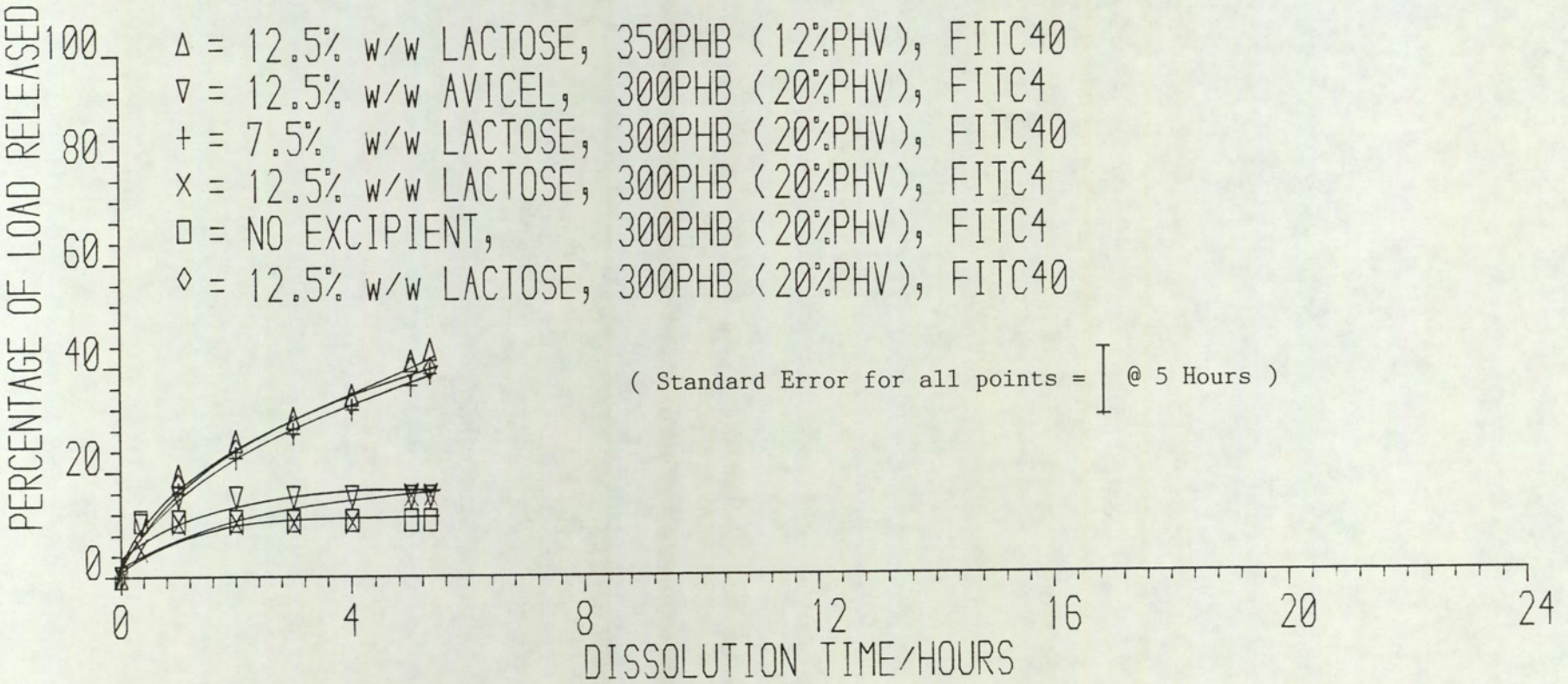


Table 6.6 Amount of dye released from tablets as a function of USP stirring rate

TABLET BLEND	PERCENTAGE OF DYE RELEASED @ 5 HOURS	
	S.R. = 25 r.p.m.	S.R. = 100 r.p.m.
300/ Base /FITC4	11	16
300/12.5% L/FITC4	16	24
300/12.5% A/FITC4	16	25
300/7.5% L/FITC40	36	38
300/12.5% L/FITC40	39.5	48
350/12.5% L/FITC40	41	49

300 and 350 refer to polymer grades 300PHB (20%PHV) and 350PHB (12%PHV); L = Lactose; A = Avicel (MCC) and SR = Stirring rate. All dye loadings 7.5%.

6.3.2.2 Tablet size and dye loading

Typical tablets were prepared using 6mm diameter tooling with a dye content of 7.5% w/w. One tablet blend - 12.5% lactose / FITC40 / 350PHB (12% PHV) was chosen to study the effect on the release rate of doubling the tablet surface area and dye loading. 8.5mm tooling was used to prepare the larger tablets and a 15% w/w dye loading was used for the high load studies.

Graph 6.14 illustrates the dissolution of the dye from these blends (with release normalised to 100% of theoretical loading) together with the 'normal' blend profile. Table 6.7 compares the

GRAPH 6.14 USP DISSOLUTION OF FITC40 FROM 350PHB (12%PHV) TABLETS WITH LACTOSE (12.5%) FILLER IN A pH 7.4 BUFFER AT 37°C SHOWING THE EFFECT OF DYE LOADINGS AND TABLET SIZE

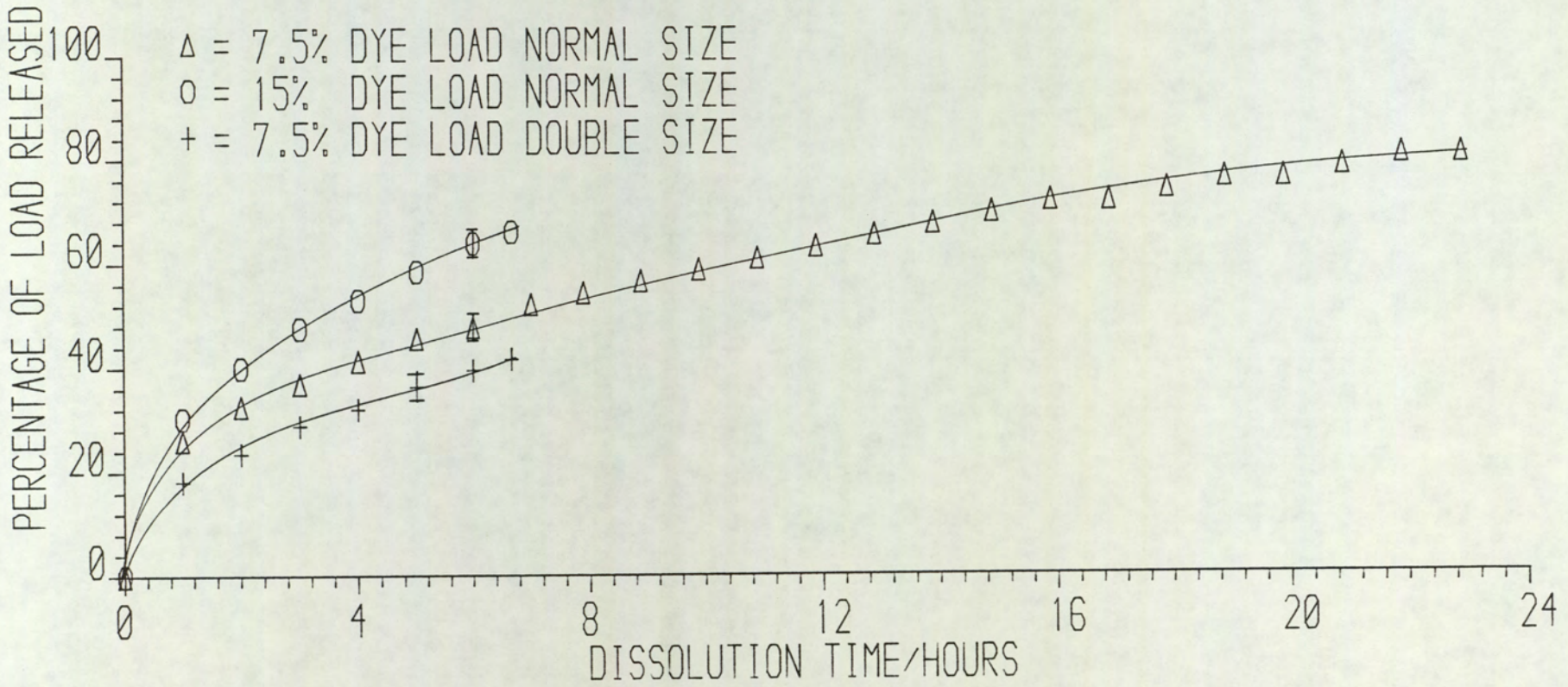


Table 6.7 Amount of dye released from tablets as a function of tablet size and dye loading

TABLET BLEND	DYE LOAD / % w/w	TABLET PUNCH SIZE/mm	PERCENTAGE OF DYE RELEASED @ 6 HOURS
350/12.5% L/FITC40	7.5	6.5	48
350/12.5% L/FITC40	7.5	8.5	38
350/12.5% L/FITC40	15	6.5	63

350 = 350PHB (12%PHV) and L = Lactose.

release after six hours for the tested blends.

Increasing the tablet size two fold results in a fall in (normalised) release by some 20%. This would normally be expected as the surface area to volume ratio decreases as tablet size increases. However the 8.5mm tooling used to produce the larger tablets was chosen to make tablets with twice the weight and twice the surface area of the 6mm tablets. The observed shortfall in release therefore can be attributed to the rate of permeation of the dissolution medium into the tablet matrix and the subsequent dye dissolution i.e water uptake. This would be slower for the larger tablets because of an increase in the internal path length of the pores / imperfections.

Doubling the dye load increases the rate of release by some 30% over and above that of the lower (7.5% w/w) loading. This can be linked to the poorer homogeneity of the tablet blends as dye

load is increased. However, studies on high dye content base blends show a further increase in the relative release rate (see Graph 6.5 and Table 6.3) as dye load is doubled, again showing that high loading of dye in the powdered blends leads to dye heterogeneities within the tablet matrix.

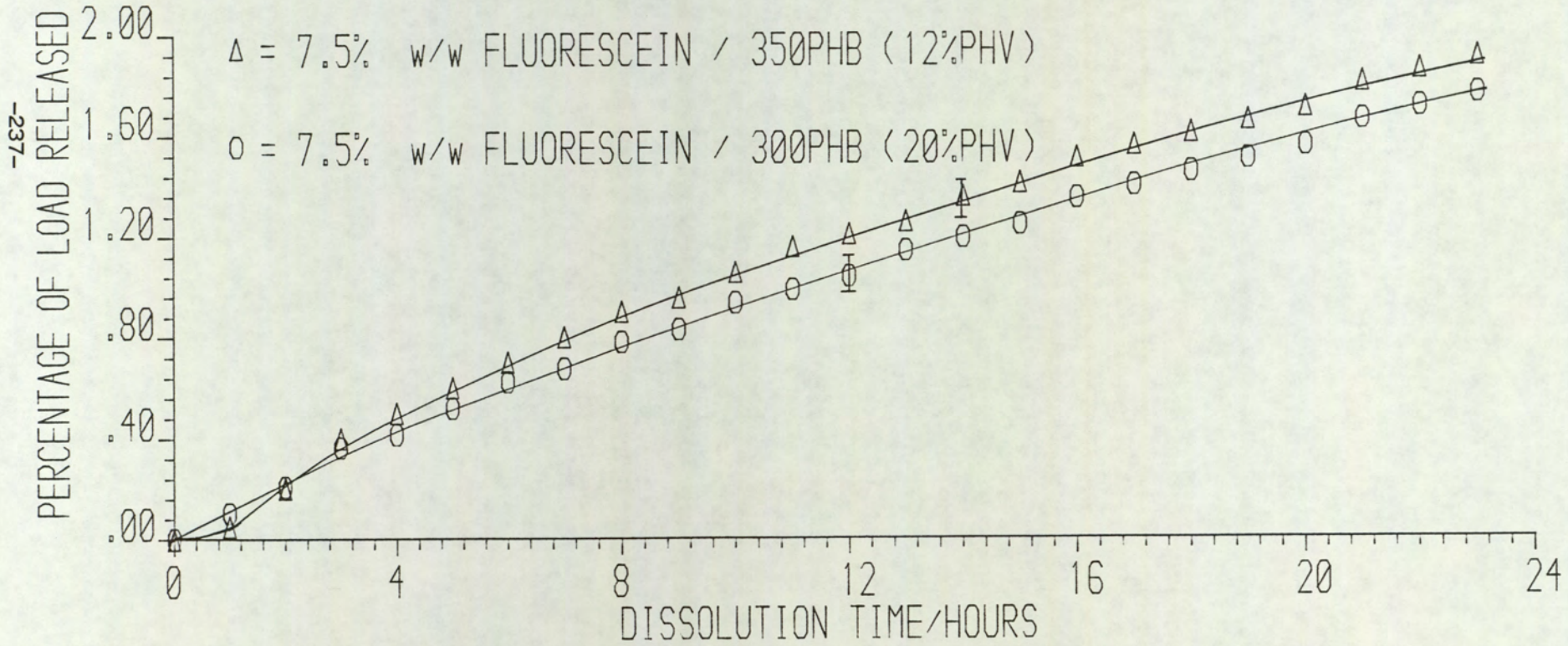
6.3.2.3 Dissolution medium pH

A change in tablet dissolution medium from physiological to simulated gastric fluid (SGN, pH 1.3) led to a decrease in the rate of dye release. Graphs 6.15 to 6.18 show the release profiles for a variety of tablet blends dissoluted in SGN. The amount of dye detected after six hours of dissolution and at the end of the experimental runs for the tablets is summarised in Table 6.8.

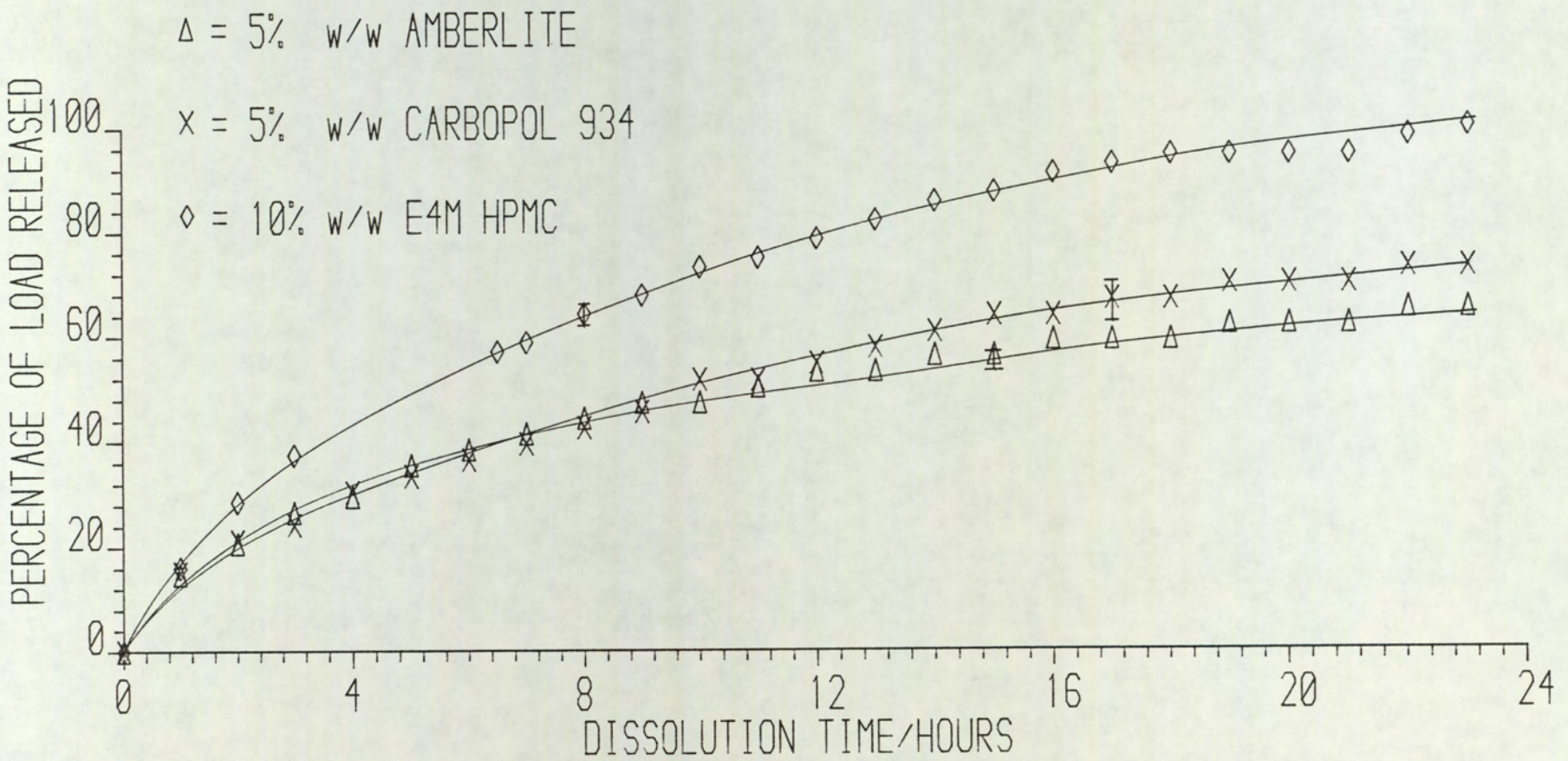
There is a general decrease in the rate of dye release in the acidic conditions, the most marked change being for the fluorescein system, which decreases almost ten fold moving from pH 7.4 to SGN conditions. The Carbopol, Amberlie and MCC tablet blends all show some decrease in the amount of dye released throughout the dissolution with the lactose and base (FITC dextran dye) blends exhibiting little difference between the two conditions.

This decrease is probably due to the lowering of dye solubility which occurs in acidic conditions (especially so for fluorescein). However the MCC, lactose and Amberlite tablets all

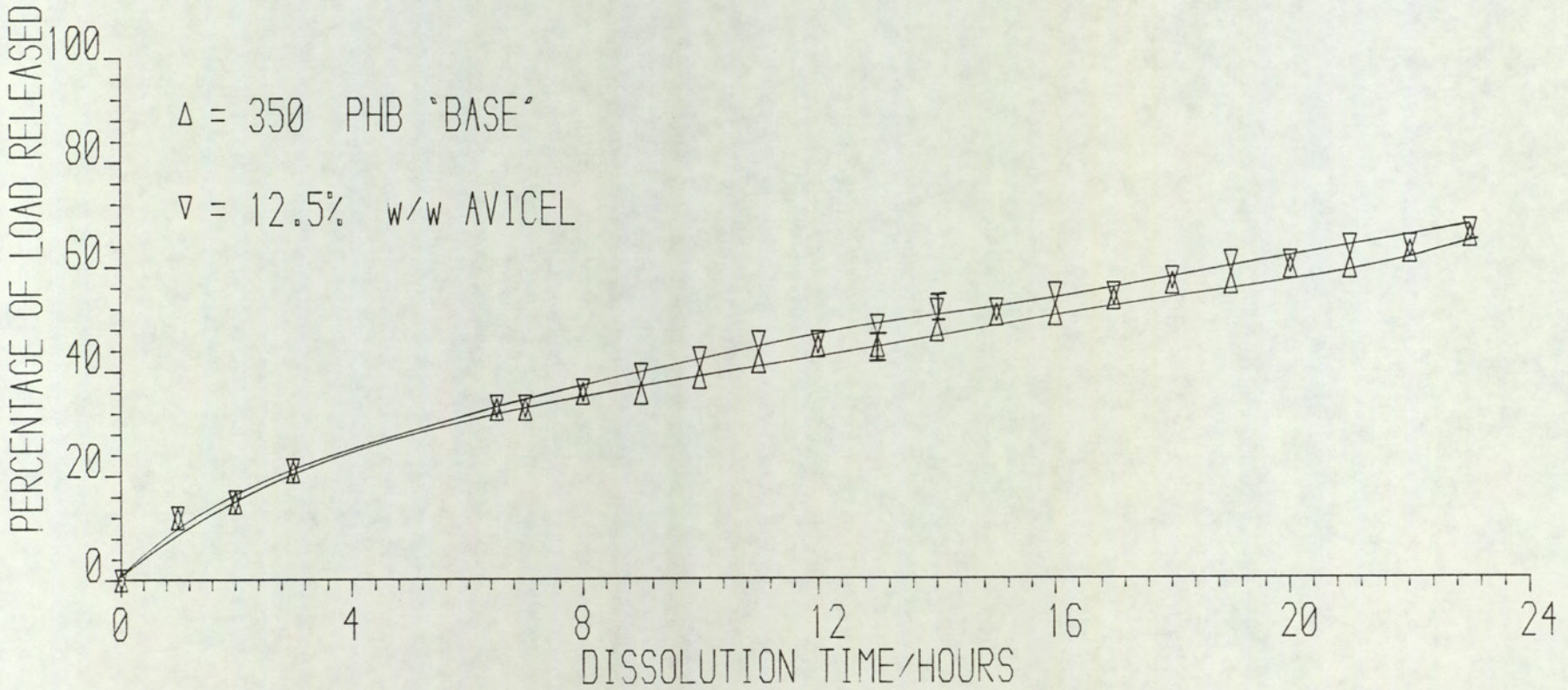
GRAPH 6.15 USP DISSOLUTION OF FLUORESCEIN
FROM PHB/PHV TABLETS (NO EXCIPIENTS)
IN A pH 1.3 BUFFER AT 37°C



GRAPH 6.16 USP DISSOLUTION OF 350PHB (12%PHV) / FITC40 DYE TABLETS WITH VARIOUS EXCIPIENTS IN A pH 1.3 BUFFER AT 37°C



GRAPH 6.17 USP DISSOLUTION OF VARIOUS
350PHB (12%PHV) / FITC40 TABLETS
IN A pH 1.3 BUFFER AT 37°C



GRAPH 6.18 USP DISSOLUTION OF 350PHB (12%PHV) / FITC4 DYE TABLETS WITH LACTOSE FILLER IN A pH 1.3 BUFFER AT 37°C

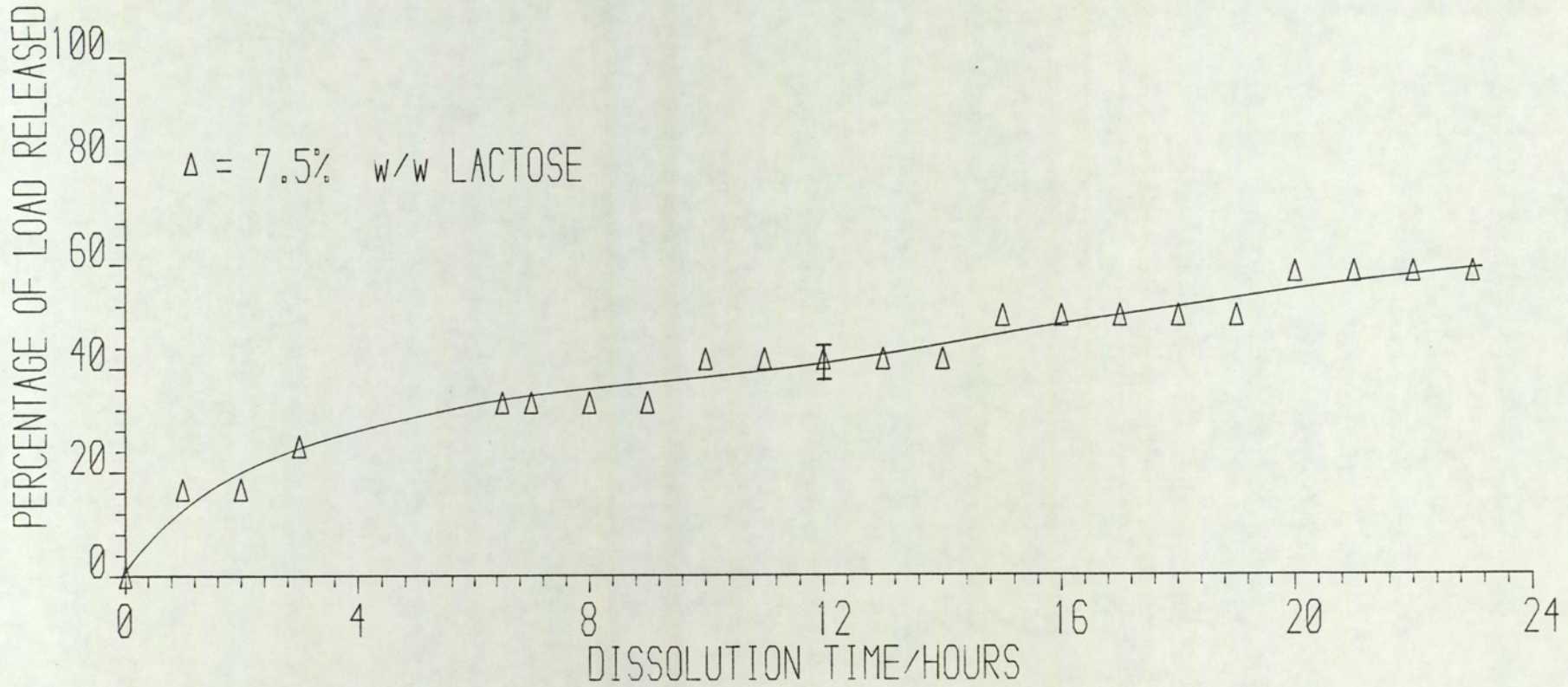


Table 6.8 Amount of dye released from tablets as a function of dissolution pH

TABLET BLEND	DISSOLUTION pH	%AGE RELEASED @ 6 Hours	%AGE RELEASED @ End of Diss^{n}
350/ Base /FITC40	1.3	30	60 @ 23 Hours
""" """	7.4	35	61 @ 23 Hours
300/ Base / F	1.3	0.6	1.7 @ 23 Hours
""" """	7.4	5.6	7.5 @ 23 Hours
350/ Base / F	1.3	0.7	1.8 @ 23 Hours
""" """	7.4	6.2	8.4 @ 23 Hours
350/7.5% L/FITC4	1.3	31	56 @ 23 Hours
""" """	7.4	33	60 @ 23 Hours
350/7.5% L/FITC40	1.3	32	66 @ 23 Hours
""" """	7.4	33	60 @ 23 Hours
350/12.5% A/FITC40	1.3	30	64 @ 23 Hours
""" """	7.4	37.5	71 @ 23 Hours
350/5% Amb /FITC40	1.3	38	63 @ 23 Hours
""" """	7.4	41	100 @ 19 Hours
350/5% C934/FITC40	1.3	36	70 @ 23 Hours

300 and 350 refer to polymer grades 300PHB (20%PHV) and 350PHB (12%PHV) respectively; F = Fluorescein; L = Lactose; A = Avicel (MCC); C = Carbopol and Amb = Amberlite. All dye loadings 7.5% w/w.

Table 6.8 (Cont)

TABLET BLEND	DISSOLUTION pH	%AGE RELEASED @ 6 Hours	%AGE RELEASED @ End of Diss^{n}
350/5% C934/FITC40	7.4	47	100 @ 23 Hours
350/10% H4M/FITC40	1.3	52	100 @ 23 Hours
""" """	7.4	53	100 @ 19 Hours

300 and 350 refer to polymer grades 300PHB (20%PHV) and 350PHB (12%PHV) respectively; C = Carbopol; and H4M = HPMC Grade E4M. All dye loadings 7.5% w/w.

show very similar release profiles to the base tablets indicating that these additives have little effect on the rate of dye release at low pH's. The difference between the polymer grades used is once again evident in the fluorescein release profiles with the 350PHB (12% PHV) copolymer having a higher release rate.

6.3.3 STATIC DYE DISSOLUTION

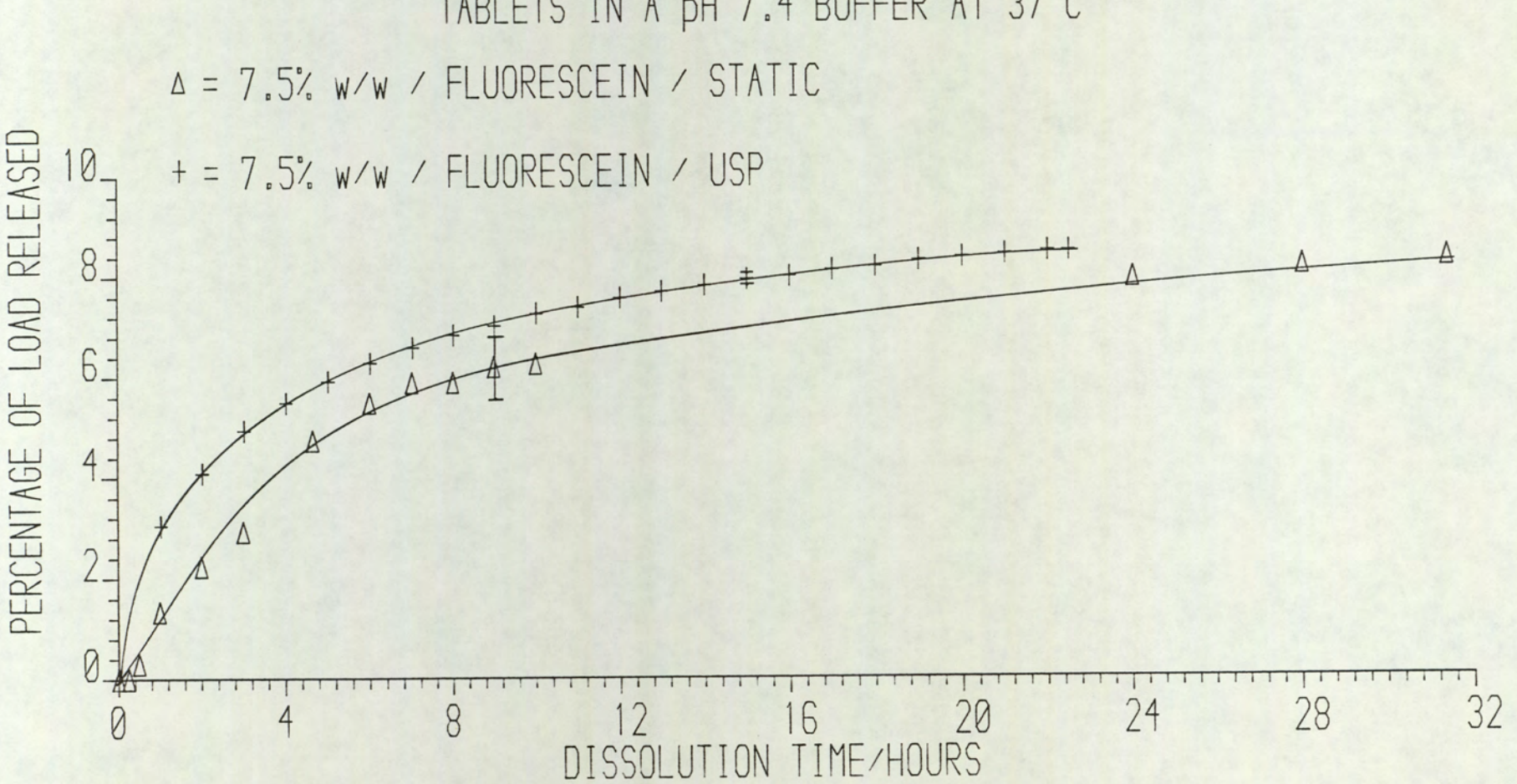
This section describes the tablet dissolution in the non-sink 'static' conditions. Normally samples were manually assayed at various dissolution times up to 32 hours.

6.3.3.1 Results and Discussion

Graphs 6.19 and 6.20 compare the rates of dye release under USP ('sink') and static conditions for polymer / dye tablet blends (no excipients). Graph 6.21 illustrates the release of FITC40 dye from the two grades of polymer tablet with added MCC, under both dissolution conditions. Table 6.9 lists the experimental release parameters derived from these graphs. A consistently lower rate of dye release under static conditions is seen for most of the samples studied. This is expected, since a decrease was seen in the USP experiments when the stirring rate was decreased four fold to 25 r.p.m. The hydrodynamic boundary layer would be expected to increase under static conditions as dissolution fluid flow over the tablet is less than for the USP system. As expected the magnitude of the release retardation in static conditions (for the MCC samples) is greater than that observed when the USP stirring rate is decreased.

One final dissolution graph - Graph 6.22 shows the static release of the smaller FITC dextran dye - FITC4. USP release appears to be less than that of the non-agitated dissolution method, however, the dye assay contained large errors. Even so, the release appears to be similar or slightly higher due to some undetermined factor.

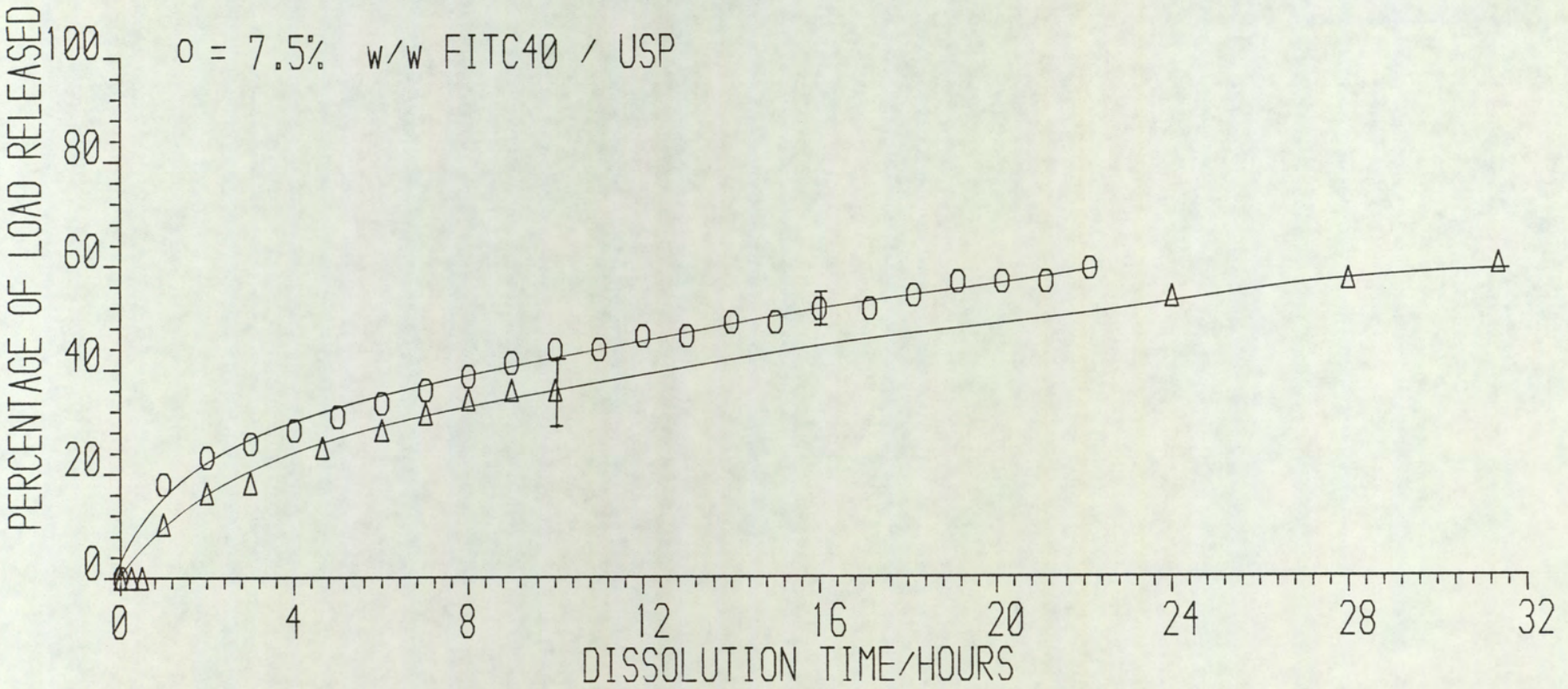
GRAPH 6.19 COMPARISON OF STATIC AND USP DISSOLUTION OF 350PHB (12%PHV) / FLUORESCEIN TABLETS IN A pH 7.4 BUFFER AT 37°C



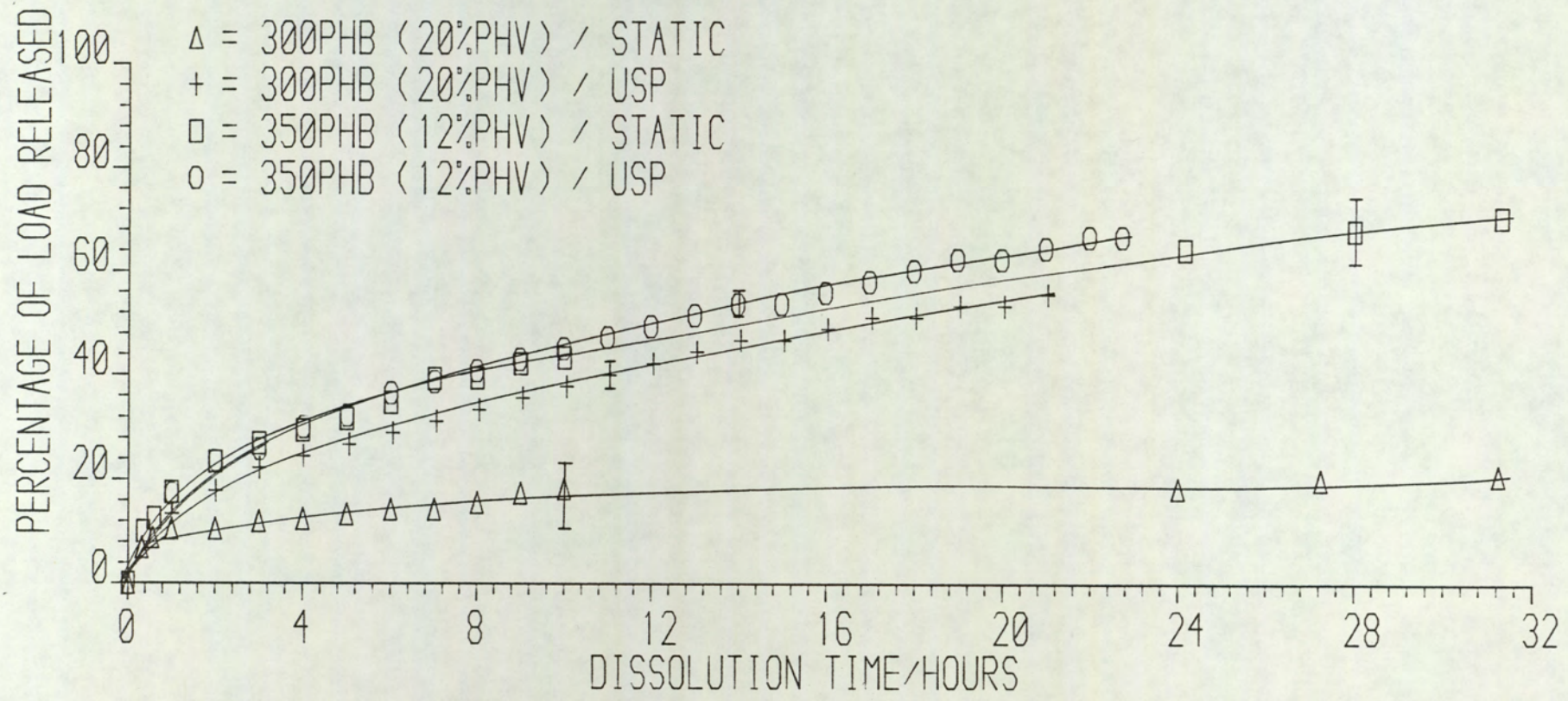
GRAPH 6.20 COMPARISON OF STATIC AND USP
DISSOLUTION OF 300PHB (20%PHV) / FITC40 TABLETS
IN A pH 7.4 BUFFER AT 37°C

Δ = 7.5% w/w FITC40 / STATIC

o = 7.5% w/w FITC40 / USP



GRAPH 6.21 COMPARISON OF STATIC AND USP DISSOLUTION OF PHB/PHV - FITC40 TABLETS WITH AVICEL (7.5% w/w) AS FILLER IN A pH 7.4 BUFFER AT 37°C



GRAPH 6.22 COMPARISON OF STATIC AND USP
DISSOLUTION OF 300PHB (20%PHV) / FITC4 TABLETS
IN A pH 7.4 BUFFER AT 37°C

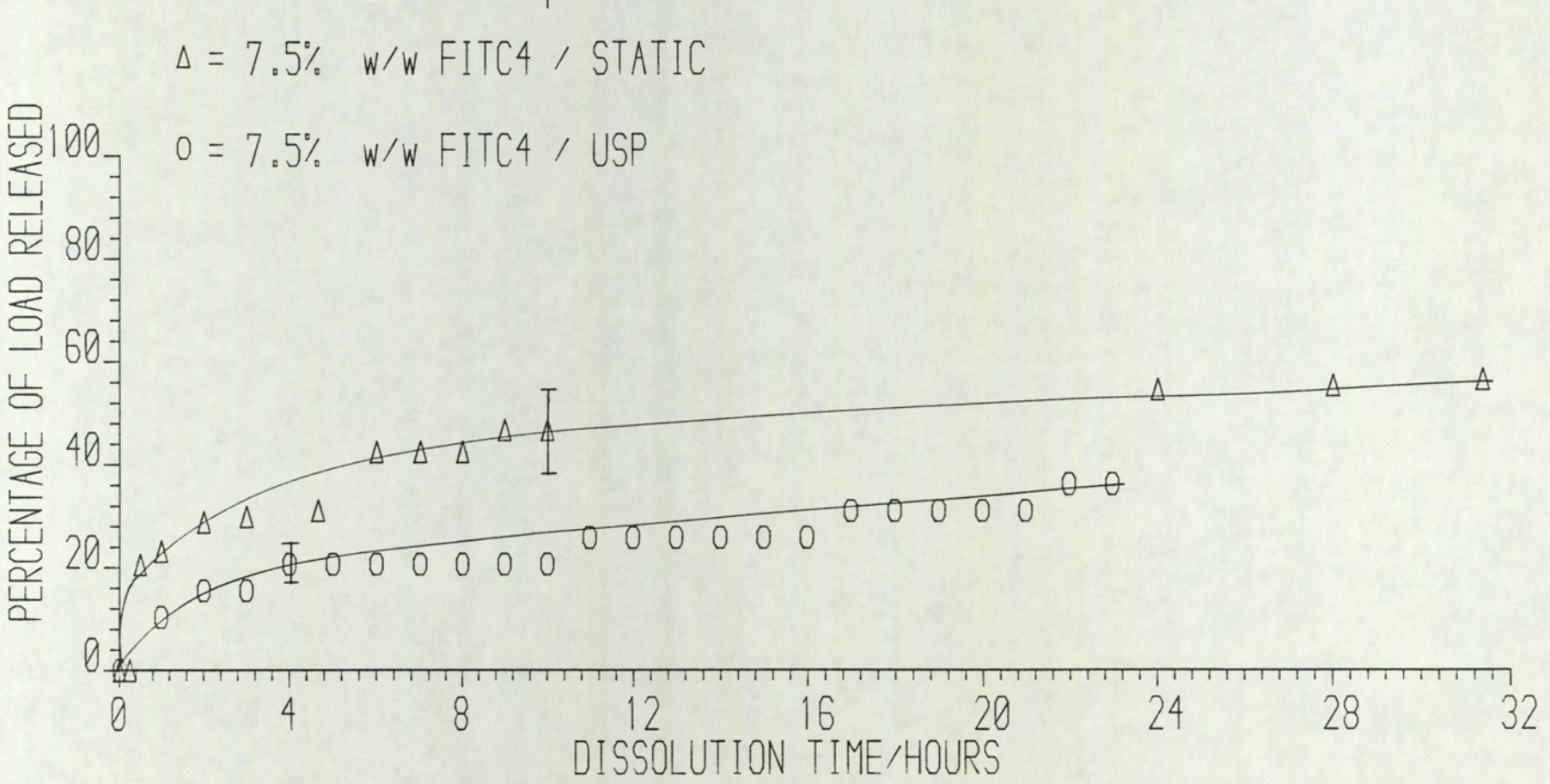


Table 6.9 Amount of dye released from tablets as a function of dissolution technique

TABLET BLEND	DISSOLUTION TECHNIQUE	%AGE RELEASED @ 6 Hours	%AGE RELEASED @ End of Diss^{n}
350/ Base / F	Static	5.3	8.2 @ 31 Hours
" " " " "	USP	6.2	8.4 @ 23 Hours
300/ Base /FITC40	Static	28	58 @ 31 Hours
" " " " "	USP	33	57 @ 22 Hours
300/7.5% A /FITC40	Static	14	20 @ 31 Hours
" " " " "	USP	32	55 @ 21 Hours
350/7.5% A /FITC40	Static	36	70 @ 31 Hours
" " " " "	USP	35	65 @ 23 Hours

300 and 350 refer to polymer grades 300PHB (20%PHV) and 350PHB (12%PHV); F = Fluorescein and A = Avicel (MCC); All dye loadings 7.5% w/w.

6.3.4 TABLET HARDNESS

Tablet crushing strength was determined using a Schleuniger tablet hardness machine. Table 6.10 summarises the range of values determined for the various blends. All tablets had hardnesses of at least 13.4 Kp with some values up to the somewhat high value of 20 Kp, with many of the tablets plastically deformed rather than simply fragmenting. Tablets containing MCC or lactose generally

formed the hardest tablets, with a substantial decrease occurring as excipient content exceeded 25% of the tablet weight. Another interesting trend is that on the whole tablets made from the 300PHB (20% PHV) copolymer were harder than the corresponding lower valerate tablets. This may partially explain the consistantly higher release rates for the 12% copolymer.

Other minor differences occur between the various dyes, with a decrease in hardness as the dye molecular weight decreases i.e the FITC40 tablets were generally more compact than the FITC4 matrices, with these in turn harder than the fluorescein tablet blends.

The unusually low release rate observed for the base blend of FITC4 and 350PHB (12% PHV) is probably explained by its low hardness value of 15.6 Kp compared to a hardness value of greater than 17 Kp for the corresponding 300PHB (20% PHV) / dye tablets. Increasing the dye load produces a similar effect as increasing the amount of excipient i.e. a decrease in tablet hardness.

6.3.5 SEM TABLET STUDIES

A detailed examination of the tablet surfaces and internal structure was carried out using scanning electron microscopy. For the internal studies tablets were cleaved in the middle to expose a cross sectional surface.

Plates 6.1 and 6.2 compare the top surfaces of USP dissoluted and non-dissoluted 300PHB (20% PHV) / FITC40 tablets respectively

Table 6.10 Tablet hardness of the various polymer blends

EXCIPIENT	RANGE OF HARDNESS VALUES		AVERAGE HARDNESS VALUE
	From / Kp	To / Kp	/ Kp
Avicel (MCC)	17.0	19.2	17.7
'Base'	15.6	19	17.7
Lactose	13.5	~20	16.4
Carbopol	16.4	17	16.4
Buffer salts	---	--	15.2
Fluorescein	14.0	15.8	15
Amberlite	---	--	14.8
HPMC	13.4	14	13.6

containing 7.5% MCC. Similar micrographs of the equivalent lower valerate copolymer (at higher magnification) are shown in Plates 6.3 and 6.4. The tablet surfaces before dissolution are very porous and release of dye would be expected to be fast. A comparison of plates 6.1 and 6.2 shows that dye release increases the size of the surface imperfections and a similar change is observed in a comparison of plates 6.3 and 6.4. There is however, only a small difference between the two grades of polymer (c.f. plates 6.2 and 6.3).

The cross-sectional micrographs show differences between the original and USP dissoluted tablets. Plates 6.5 and 6.6 (original

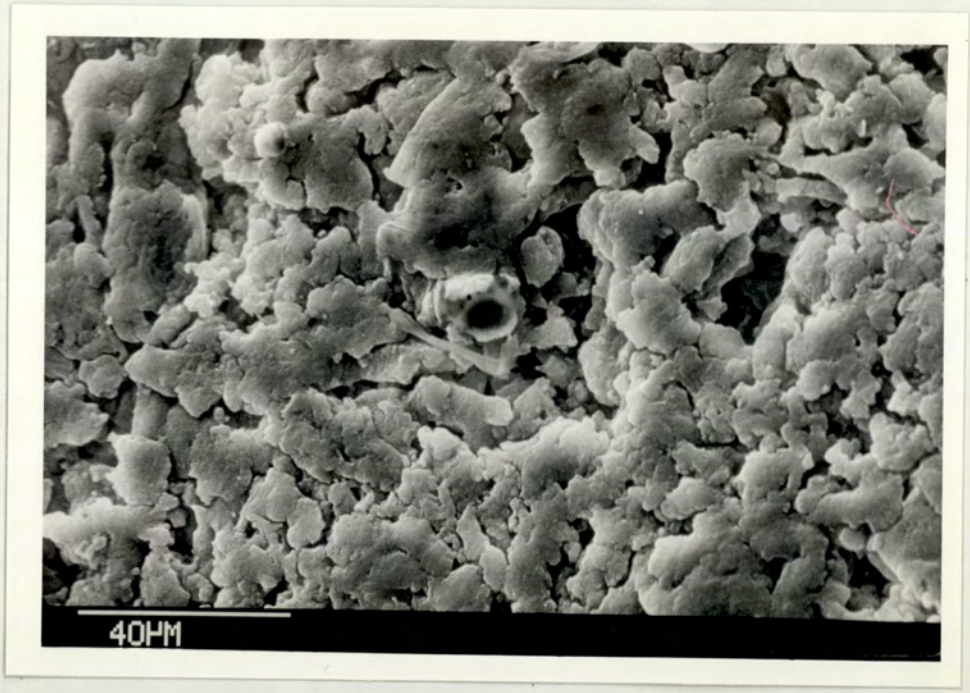


Plate 6.1 SEM of the surface of a USP dissoluted 300PHB (20%PHV) / FITC40 Tablet containing 7.5% MCC (X 500)

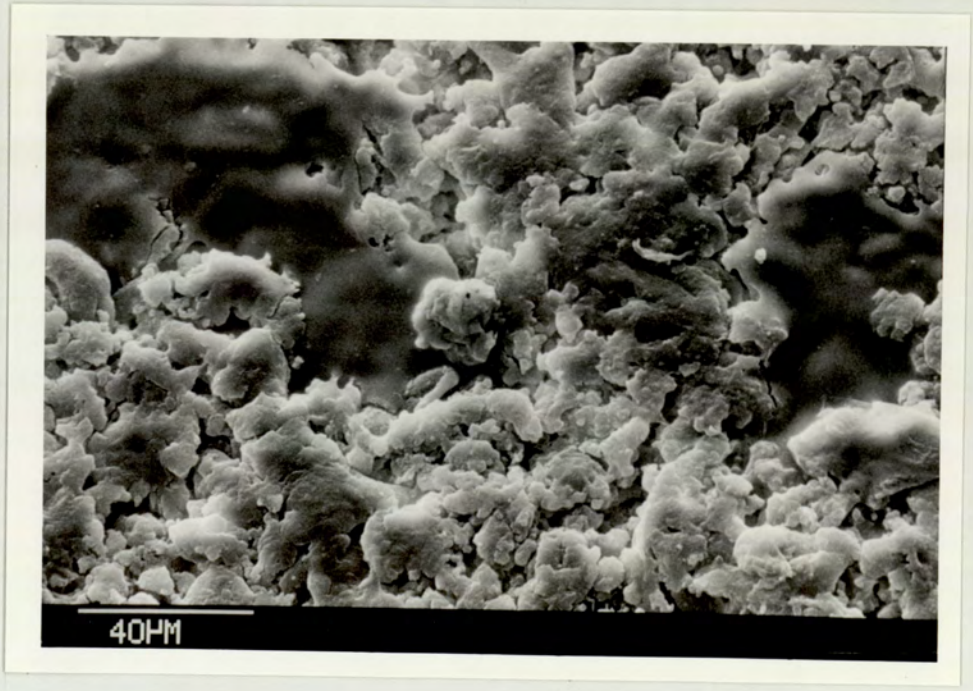


Plate 6.2 SEM of the surface of a non-dissoluted 300PHB (20%PHV) / FITC40 Tablet containing 7.5% MCC (X 500)

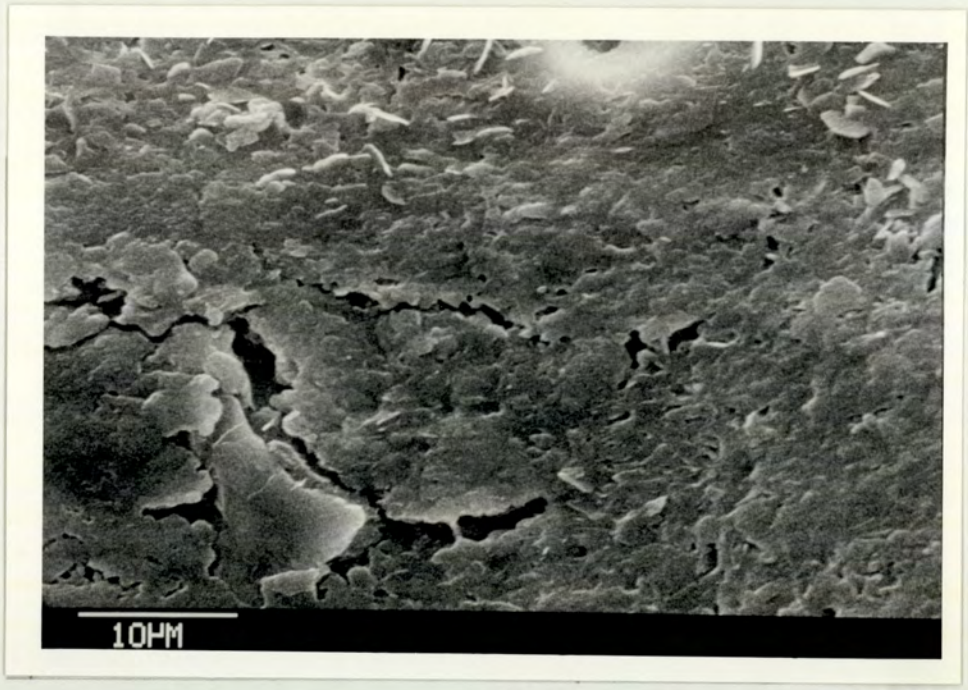


Plate 6.3 SEM of the surface of a USP dissoluted 350PHB (12%PHV) / FITC40 Tablet containing 7.5% MCC (X 2000)

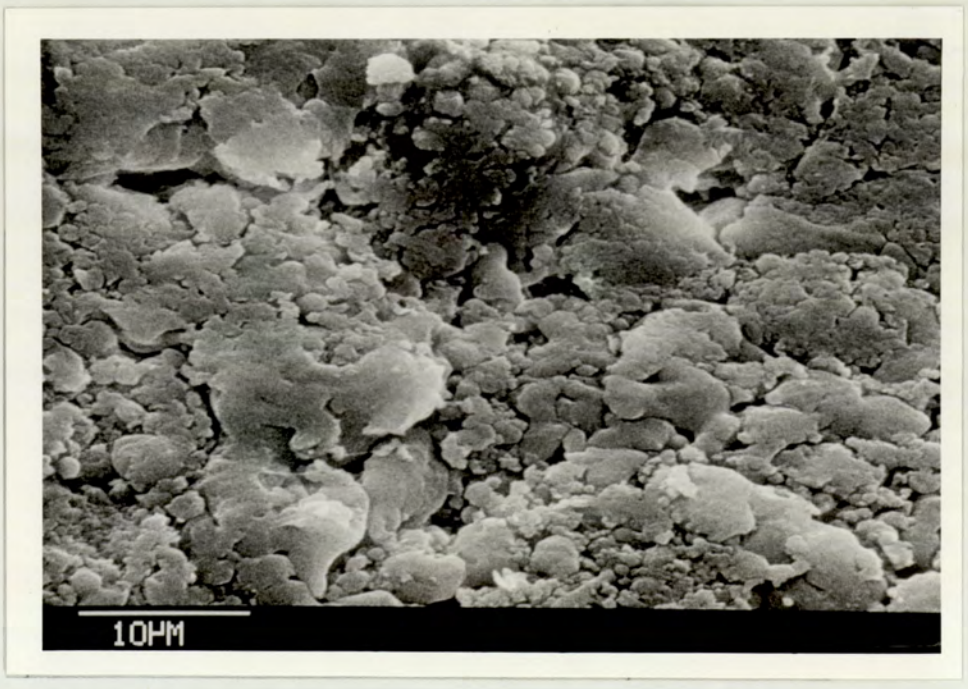


Plate 6.4 SEM of the surface of a non-dissoluted 350PHB (12%PHV) / FITC40 Tablet containing 7.5% MCC (X 2000)

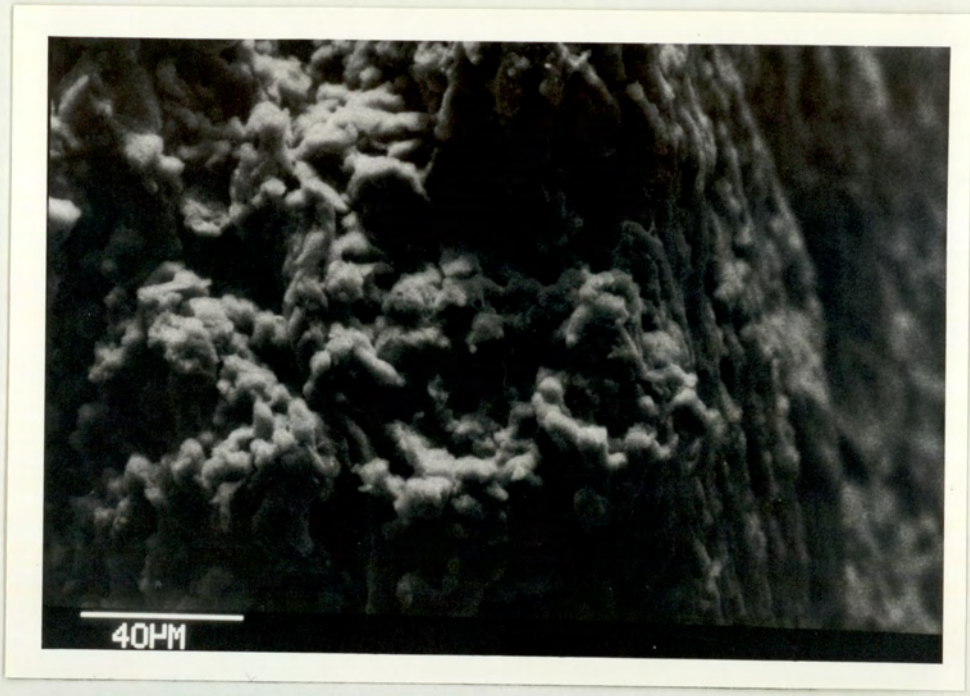


Plate 6.5 SEM of the cross section of a non-dissoluted 300PHB (20%PHV) / FITC40 Tablet containing 7.5% MCC (X 500)

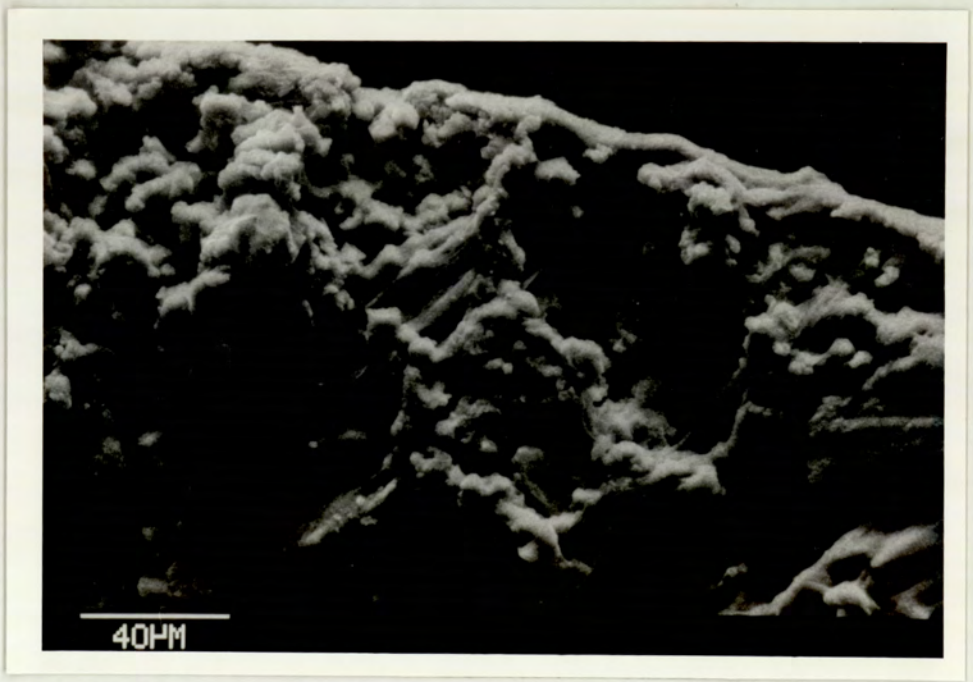


Plate 6.6 SEM of the cross section of a USP dissoluted 300PHB (20%PHV) / FITC40 Tablet containing 7.5% MCC (X 500)

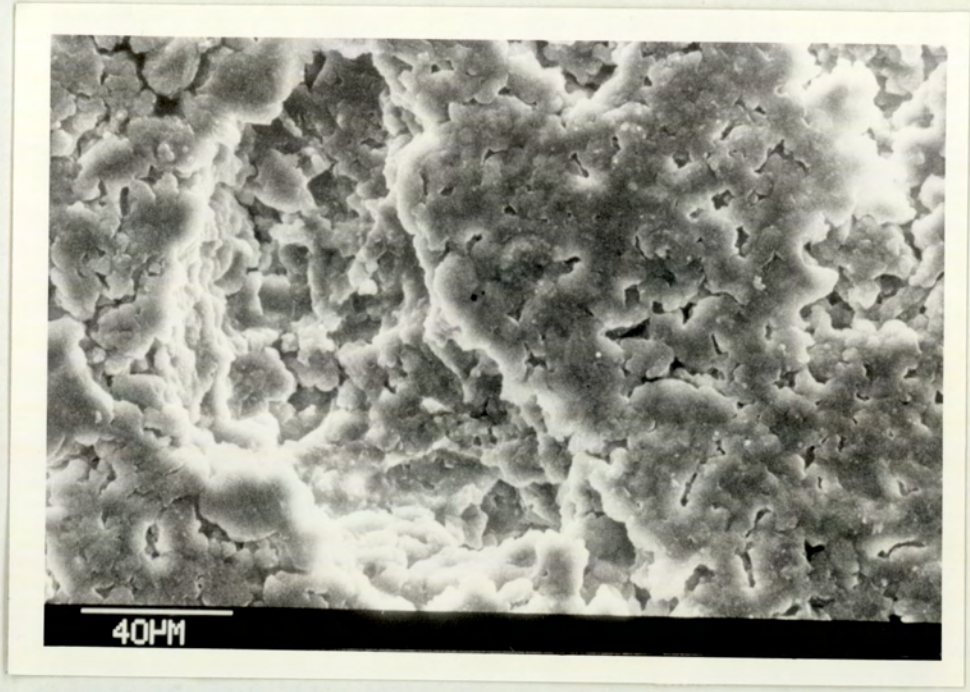


Plate 6.7 SEM of the surface of a USP dissoluted 300PHB (20%PHV) / FITC4 Tablet containing 25% Lactose (X 500)

and USP dissoluted respectively) illustrate the effect of dissolution on 300PHB (20% PHV) FITC40 tablets containing 7.5% w/w of MCC. Plate 6.6 shows the opening up of the matrix due to dye dissolution and the swelling of the MCC as it is hydrated.

One final micrograph - Plate 6.7 shows the effect of high excipient loading. This blend (300PHB / FITC4) with a 25% lactose loading releases dye at a rapid rate and this is shown on the SEM micrograph of the dissoluted tablet by the very porous matrix which is formed as the soluble excipient dissolves out of the tablet matrix.

6.4 TABLET RELEASE DISCUSSION

Drug release from the cold compressed tablets is characterised by an initial burst followed by a linear release rate with a final 'tail-off' as the dye in the matrix becomes depleted. Increasing the dye load in the tablet blends linearly increases the rate of release with a doubling of the dye load producing twice the release rate w.r.t percentage of dye load released (i.e four times the amount of actual release). This is broadly similar to work by Korstako[144] on the release of HET (a low molecular weight drug) from PHB homopolymer matrices. Addition of excipients to the polymer / dye tablet blends shows a linear dependence of the release rate on excipient loading, with little

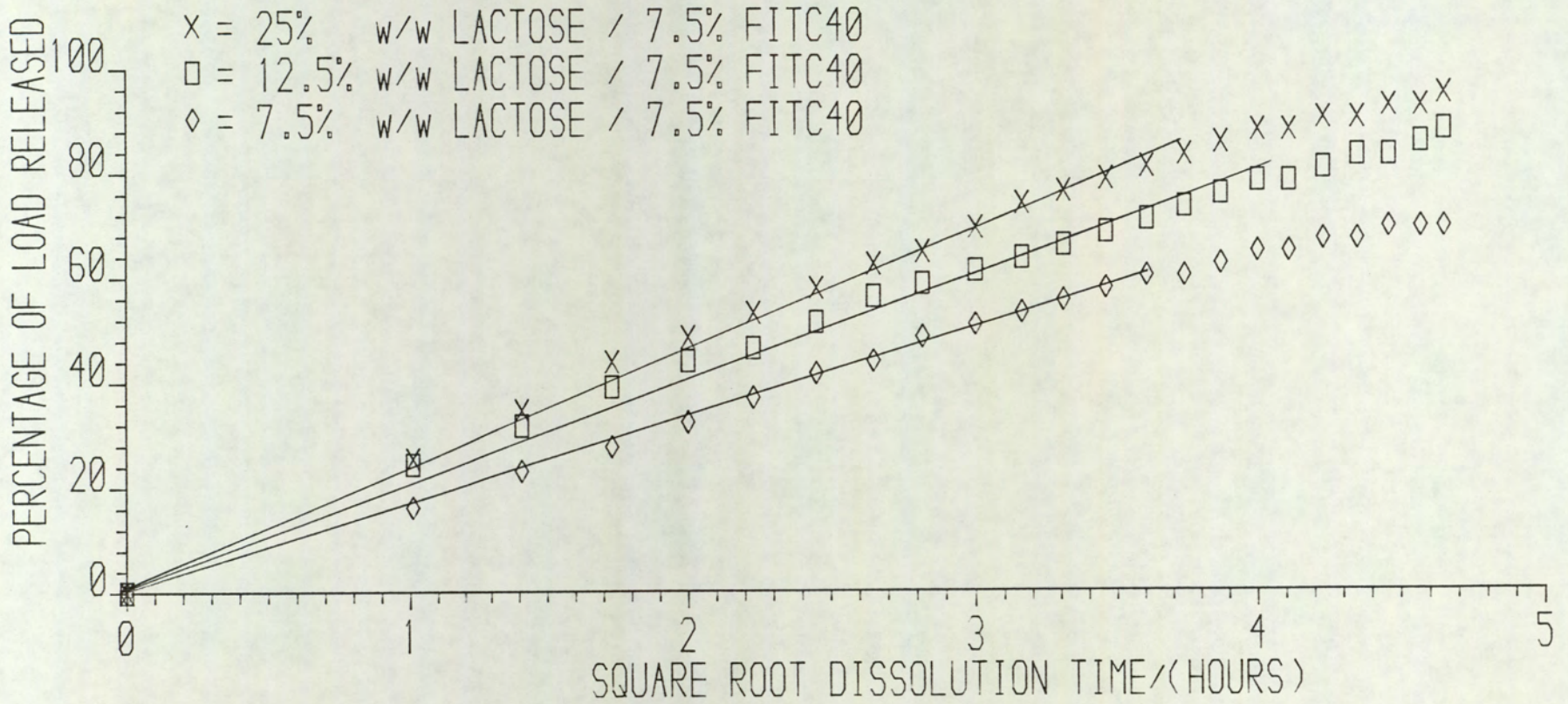
difference between the base and excipient blends at low loadings (less than 7.5% w/w).

The major trend observed is the dependence of the release rate on the molecular weight of the dye. Contrary to the expected trend predicted by Fick's law of diffusion which indicates a decrease in the rate of diffusion as solute molecular weight increases, the observed release rates increase - with the FITC 40,000 dye showing the fastest release, followed by the FITC 4,000 dye which in turn is much faster than the low molecular weight (332 Daltons) fluorecein dye. This suggests that release is controlled by the porosity and homogeneity of the matrix with the low molecular weight fluorescein blends exhibiting a low rate of release due to them being the most homogeneous polymer / dye blend.

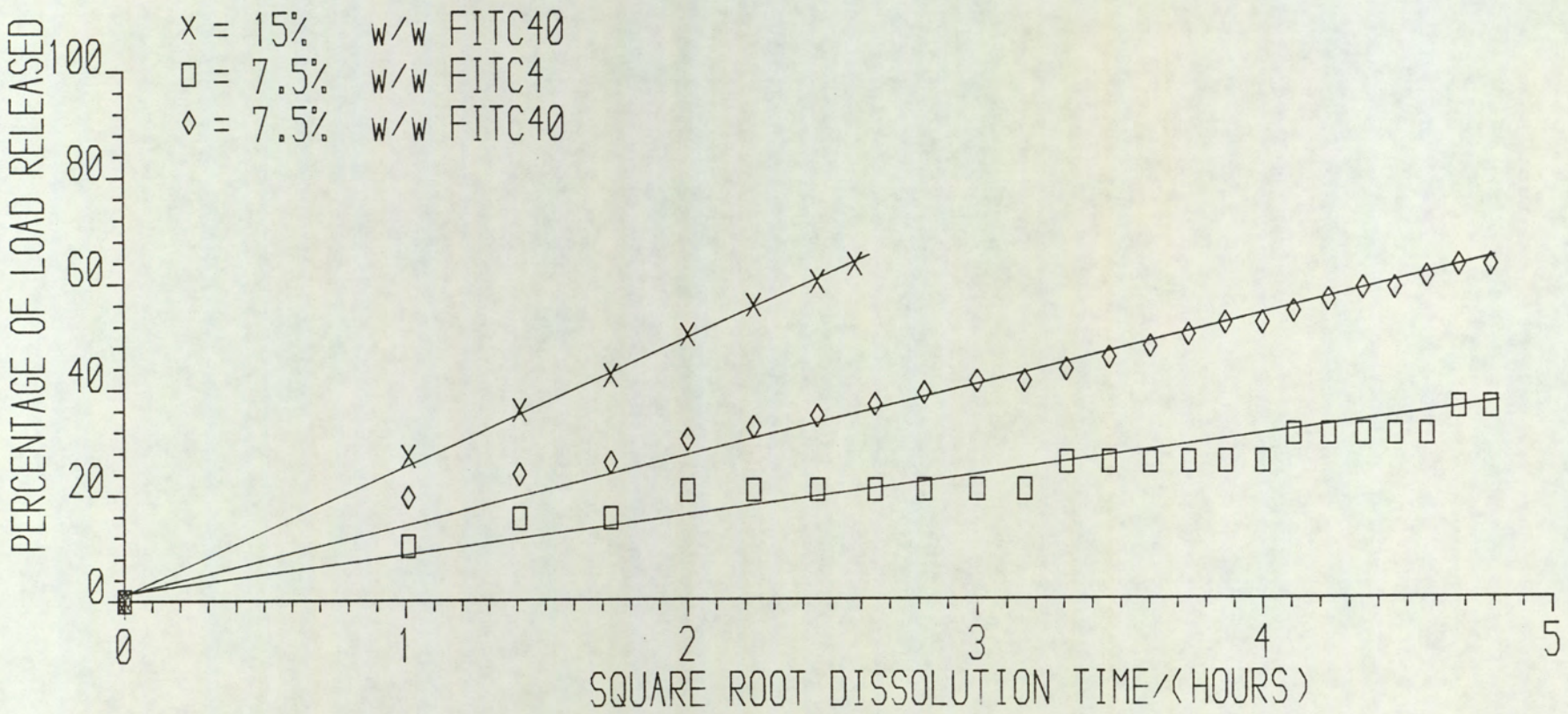
Dye release is predominantly by diffusion with plots of the square root of the amount of dye released against time (Graphs 6.23 to 6.25) showing a straight line dependence in line with the predicted release rate by a diffusional process predicted by Higuchi et al.[216-218] There is however a deviation from the $t^{1/2}$ dependence for the fluorescein system (c.f. Graph 6.25) which as already mentioned produces a linear release after the initial burst effect release.

There is a consistent difference between the two grades of polymer used, with the more crystalline 350PHB (12% PHV) grade exhibiting a faster release rate than the higher valerate

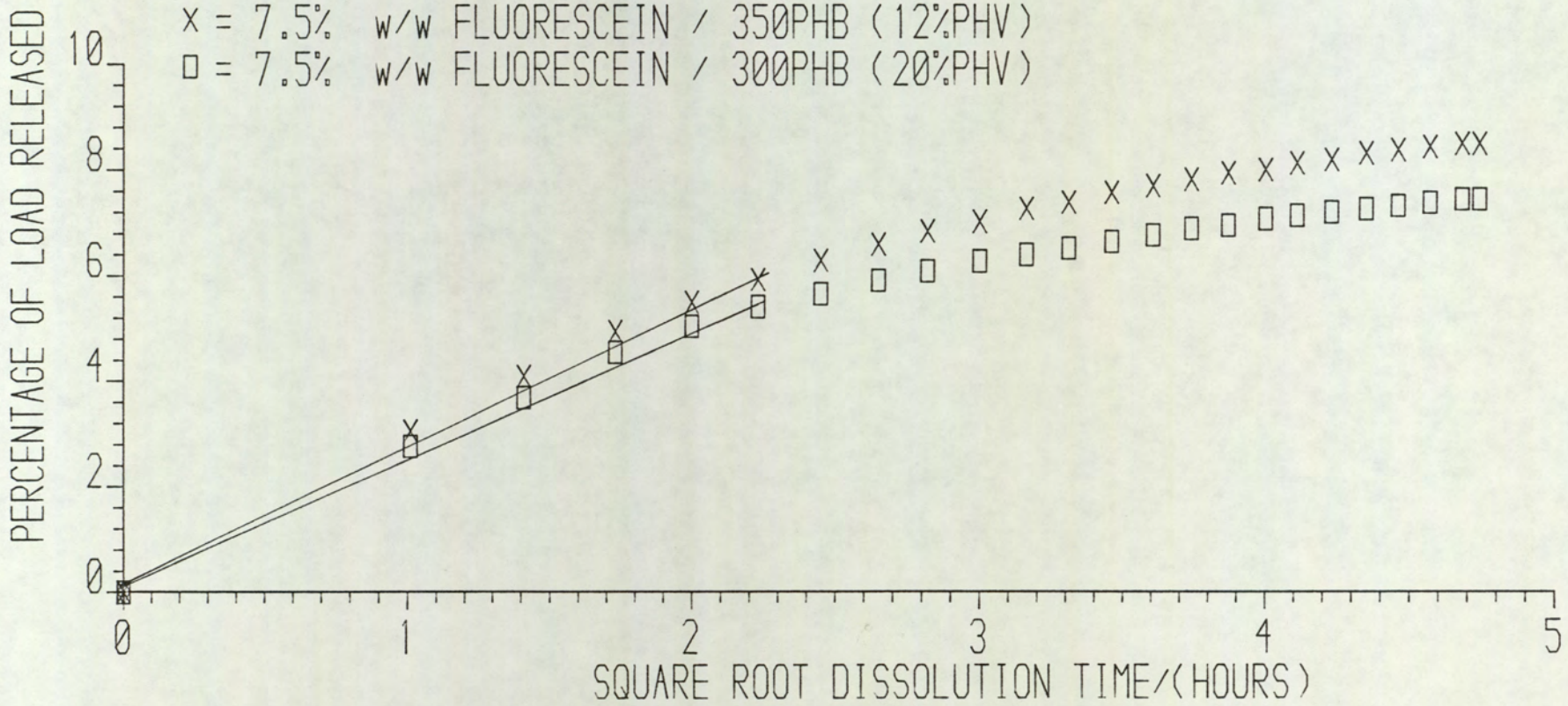
GRAPH 6.23 SQUARE ROOT PLOT OF GRAPH 6.2 DATA FOR 300PHB (20%PHV) / FITC DYE TABLETS WITH LACTOSE FILLER IN A pH 7.4 BUFFER AT 37°C



GRAPH 6.24 SQUARE ROOT PLOT OF GRAPH 6.5 DATA
FOR 350PHB (12%PHV) / FITC DYE TABLETS
(NO EXCIPIENTS) IN A pH 7.4 BUFFER AT 37°C



GRAPH 6.25 SQUARE ROOT PLOT OF GRAPH 6.12 DATA
FOR PHB/PHV / FLUORESCEIN DYE TABLETS
IN A pH 7.4 BUFFER AT 37°C



copolymer (300PHB 20%PHV). This is seen throughout the experimental systems studied, with a difference observed even at low dissolution rates and in acidic media. Apart from the crystallinity diferences between the two blends there is a difference in polymer matrix homogeneity, the 350PHB polymer grade forms softer matrices (as measured by tablet hardness) and the tablets on dissolution form more porous matrices as can be seen in the SEM micrographs. This could possibly explain the faster rate of release.

SEM studies also show that high excipient loadings create a more porous matrix and this leads to substantially higher release rates indicating that matrix porosity is the rate determining factor.

Tablets containg lactose or MCC exhibit good reproducibility with only small deviations between the experimental runs for each sample. Increasing the tablet size almost linearly increases the rate of release and studies in an acidic dissolution medium show release rates only marginally slower than in neutral pH,with the lowering of the observed rate probably due to an associated decrease in dye solubility as the pH decreases.

Solution hydrodyanamics have a marked effect on the dissolution rate of the various dyes at extended release times. An expected decrease of some 5 to 30% in the dye release rate occurs as the stirring rate of the USP experimental set-up was lowered from 100 to 25 r.p.m. This lower release rate is slightly higher

than the equivalent static release. During the initial release stages however, the amount of dye released is approximately the same for the USP low stirring rate studies and the equivalent static experiments. This suggests that initial release is from the tablet surface and does not rely on the rate of fluid influx into the matrices, which becomes important after this initial surface release.

The expected gelation effect[214,215] of tablets containing HPMC which would slow down the release rate by forming a layer through which the dye would have to diffuse was not observed, with a high release rate observed indicating that the incorporation of HPMC weakens the polymer / dye tablet matrix as it hydrates.

In this work no attempt has been made to quantitatively discuss pharmacokinetic parameters due in part to an insufficient amount of tablet data. The release rates of the dyes from the tablet matrices during regions of almost constant release (expressed in terms of the amount of dye released per unit area per unit time) are discussed comparatively with the polymer / film studies in the final Chapter.

6.5 CONCLUSIONS

Previous attempts to produce sustained release tablet matrices were based on coating the matrix with a material which behaves as a retardant on the release of the active from the enclosed tablet matrix. These systems have been extensively discussed by Conrad,[219] and examples of materials used include poly vinyl chloride and many cellulose derivatives. The tablet systems in this study are ultimately biodegradable and require no coatings. They have the potential use as non-disintegrating matrices for extended (sustained) release oral dosage forms, with the rate of the diffusional release of the 'active' varied by alteration of the excipient and active loadings. A wide range of (dye) molecular weights can be released at an approximately constant rate for several hours after the initial burst deriving from surface release subsides. Hence pre-leeching or attempts to model suitable matrix geometries[34-36] may produce a system which more closely resembles the ideal zero order release rate.

Similar to the findings of the polymer/dye film release experiments, solution pH appears to have only a small effect on the release rate. However potential candidate drugs with low solubilities in acidic conditions are not very suitable for these systems. The release rate is controlled by polymer matrix compressibility (which depends on the grade of PHB/PHV used), drug molecular weight, choice of excipient and excipient loading

and the homogeneity/uniformity of the tablet blend. Careful control of these parameters is needed to produce the desired release profiles.

In vitro degradation of the matrix is slow and there would be long term retention in the stomach for oral applications, however these matrices ultimately bioabsorb and delivery sites other than in the gut could be chosen to exploit this.

CHAPTER SEVEN

DISCUSSION AND CONCLUSIONS

7.1 DISCUSSION

The aim of this work was to consider possible erodible polymers for use as matrices for extended drug release, with particular interest centering on macromolecular drugs. Apart from the regimen of controlled release, the initial concept of such a system is one of a large polymeric matrix releasing a macromolecular entity. This is an inherently complex system due to the possible incompatabilities that can occur between two macromolecules with an additional contribution from the incompatibility to the labyrinth of the model of drug release from any degradation of the polymeric matrix.

Initial work considered the various biodegradable polymers available in order to choose candidate materials to form drug release matrices. Of the many polymer types, the polyester family were considered to be suitable for biomedical applications owing to the non toxic degradation products of these polymers since the polymers are hydrolytically degraded to simple organic acids that are present in the body, for example poly lactic acid, which degrades to lactic acid. One particular polyester was chosen for extensive studies, the poly hydroxybutyrate-co-hydroxyvalerate copolymer system. One of the reasons for its choice was that in

line with the simple poly alpha esters poly glycolic and poly lactic acid, the expected hydrolytic products are present in the body, hydroxybutyric acid being a normal constituent of human blood. Hence there should be no adverse reaction to any *in vivo* matrix system utilising this type of polymer. Other reasons for the choice of this copolymer system are based on the physical properties of the copolymers. A wide range of polymer molecular weights and copolymer compositions are commercially available and the polymers are semicrystalline with melting points in the range of 130°C for the higher valerate copolymers to approximately 180°C for the high molecular weight homopolymer. This facilitates the use of melt processing of the polymers to fabricate various types of matrix physical form.

Although there is adequate knowledge of the synthesis and processing of these polymers, at the start of this work there was little reported in the literature on the degradation mechanism. Indeed, at the time of writing there is no specific reported work on the nature of the degradation and process of the degradation mechanism, other than a few general papers on the relative rates of degradation for unspecified polymer samples in various environments,[152,220,221] with some degradation work on tabletted PHB by Korsatko.[57,144,151] At the outset of the work it was presumed that the mechanism of degradation would be ester hydrolysis by random chain scission of the acyl- (as opposed to the alkyl) oxygen bond in the polymer backbone to yield the free acid and

water. This could occur by either acidic or alkaline hydrolysis, both being bimolecular reactions.[222] It is interesting to note that of the many papers on the hydrolytic degradation of 'biodegradable' polyesters that are reviewed in the introduction only one paper[81] actually describes the probable degradation mechanism of the polyester studied, the polymer being poly glycolic acid.

Some knowledge of the mechanism of polymer hydrolysis is essential for a deeper understanding of the contribution that matrix breakdown would make to the process of drug release from a polymer/drug matrix. Initial reading of the literature leads to the oversimplified picture of release of a drug from a biodegradable polymer. The degradation appears to be by a 'peeling' off of the outer layer of polymer by hydrolysis to expose a fresh surface with concurrent release of the drug - like the opening up of an onion i.e. a mainly heterogeneous mechanism. A further simplification is that the drug is assumed to be released without modification which is an unlikely possibility for hydrophilically labile macromolecules. A homogeneous degradation mechanism however, would imply that the major part of the degradation occurred in the bulk of the polymer with only a small contribution from surface erosion. This homogenous mechanism has been shown to be the predominant degradative pathway for many polyesters and it was thought that the PHB/PHV copolymers would behave in a similar fashion.

Primary work was on the hydrolytic breakdown of the PHB/PHV copolymer series and the aqueous buffer hydrolysis studies attempted to establish a more detailed model of the degradation process, together with a useful comparison of the rate of PHB/PHV matrix hydrolysis compared with the hydrolysis rates of established biodegradable polymers. No previous attempts have been made to link matrix surface properties with those of the matrix as a whole i.e. the bulk. A correlation between the two was sought by comparing gravimetric measurements with the corresponding matrix surface energy values and goniophotometric spectra parameters as polymer hydrolysis proceeds. Apart from giving useful information to the degradation model this would enable a prediction of the extent of bulk degradation by simple non-destructive surface property measurements.

Information on the <u>in vitro</u> buffer hydrolysis of the PHB/PHV series of copolymers determined in chapters three and four shows that the rate of degradation of the various physical forms of the PHB-PHV series increases with temperature in accord with the proposed ester hydrolysis mechanism. The enhancement of degradation rate as the hydrolytic medium becomes more alkaline is also evident which is possibly due in part to the increasing solubility of the polymer degradation products at higher pHs as well as to the expected rise in hydrolysis rate.

Alteration of the physical form of the polymer matrix has a pronounced effect on the rate of degradation with the solvent cast

films being more susceptible to hydrolysis than the melt pressed discs form which in turn is reported[209] to be less stable than injection moulded samples. These differences presumably result from the lower crystallinity of these samples. Cold compressed tablets are simple compacted physical blends of polymer powder with various excipients and the large increase in matrix surface area and the total surface area (available for degradative attack) makes this form of the PHB-PHV copolymer series the least stable of all the prepared matrices. In fact the tablet matrices can be said to be a model of the melt pressed disc structure at enhanced stages of degradation (i.e. very porous); although the polymer molecular weight in this latter case would be expected to be substantially reduced by this time.

Two important factors are sample molecular weight and copolymer composition. The consistent increase in the degradation rate of the higher valerate copolymers over the low valerate polymer samples reflects the importance of sample crystallinity which decreases with increasing valerate content.[223-224] However polymer molecular weight is the dominant parameter (as outlined in section 3.3), with an order of magnitude difference in the degradation rate occurring as polymer molecular weight increases from 3.6×10^4 to 3×10^5 ($\overline{Mw}$) for solvent cast films degraded in a pH 7.4 buffer at $70^{o}C$. However PHB/PHV copolymers with molecular weights below 2×10^5 have inadequate mechanical properties for the fabrication of films and discs, hence the difficulty in making

equivalent comparisons with suture materials since the random chain scission mechanism of hydrolysis requires a substantial amount of degradation to occur to reduce the relatively high PHB/PHV molecular weights to similar values of the absorbable sutures tested (molecular weights approximately 60,000). In addition polymer crystallinity levels significantly alter the rate of hydrolysis, therefore for a true comparison between different polymers idealistic samples with the same molecular weights / molecular weight distributions and the same levels of crystallinity would have to be prepared. These equivalence problems are borne out in the confusion that exists in the literature as to the rate of poly lactic acid hydrolysis.[90] This presumably stems from the non-disclosure of the molecular weights of the polymers used in the various studies and without taking into account any equivalence difficulties the comparative studies with the established ester-based commercial biodegradable polymers show the expected slower rate of PHB/PHV copolymer hydrolysis.

The weight loss and buffer solution uptake studies show that degradation at elevated temperature and under alkaline conditions produces similar weight loss profiles to samples degraded at physiological pH and temperature over a shorter time period. Of the many degradative options available the pH 10.6 / 37^{o}C hydrolysis conditions are more suitable than pH 7.4 / 70^{o}C for predictive accelerated studies (c.f. Plates 3.1 and 3.2). Since the degradation rate increase over that of the <u>in vitro</u>

physiological conditions is lower for the low temperature alkali than for the high temperature studies at physiological pH, and the point of catastrophic matrix breakdown is more likely to be pinpointed under the former set of conditions.

Water uptake studies outlined in chapter three show the important contribution that matrix porosity makes to the rate of matrix degradation, with substantial increases between the hydrated and dehydrated sample molecular weights occurring as melt pressed disc sample weight loss exceeded 30%.

As the polymer samples were degraded in constant pH aqueous buffers nothing can be deduced about the nature of the chemical degradation mechanism of the PHB/PHV copolymer system, although the observed results are consistent with the expected random chain scission mechanism.

Polymer matrix surface studies using goniophotometry and surface energy measurements also reflect the effects of temperature and buffer pH on the rates of matrix hydrolysis, with a far higher accuracy than the gravimetric techniques within the limits of surface measurements (which are valid for the initial stages of matrix breakdown). Indeed it is the goniophotometric and surface energy parameters that indicate the first stages of the degradation.

Goniophotometry clearly distinguishes the magnitude of surface defects at sizes less than the wavelength of light. At this stage of the hydrolytic degradation a decrease in the

specular reflectance intensity signifies increasing surface rugosity. As the degradation proceeds the size of the imperfections approximately reaches the wavelength of light (~500 nm) and the shape of the goniophotometric curves becomes asymmetrical, with an increase in peak width - denoted by an increase in $W_{1/2}$. Further hydrolysis is shown by an increase in the diffuse reflectance until a perfectly matt surface is formed. Pre-treatment of the polymer matrices with an oxygen plasma is reflected by relatively large changes in the goniophotometric parameters as opposed to weight loss studies which show no apparent loss after treatment - the effect of treatment only being evident at extended degradation times under the accelerated low temperature alkaline degradation studies. This plasma pretreatment creates a roughened surface which enables a higher rate of hydrolysis owing to the resulting increase in surface area. Similarly PHB/PHV melt pressed disc treatment with blood plasma induces a larger response in the goniophotometric parameters than the associated weight loss value. However a reproducible sample mounting must be used to maintain experimental accuracy since small changes in the angle between the sample surface and the light source used produces significant differences in the deduced goniophotometric parameters.

Surface energy components determined from contact angle measurements of liquids on the degraded matrix surfaces show the expected increase in surface carboxyl and hydroxyl groups by an

increase in the total surface energy. Again the relative changes are greater for this technique than for the equivalent weight loss. However, measurement of the contact angles to within a degree proved to be difficult and this small change in the angle value corresponds to a significant difference in the calculated polar and dispersive components of the surface energy which reduces the effective accuracy of this technique. At advanced stages of degradation, SEM indicates that the crystalline regions of the matrix are the last to be eroded (c.f. Plates 4.1 to 4.3) implying preferential hydrolytic attack on the amorphous regions of the polyester matrices. This is in line with previously reported work on PGA[82] and PGA co LA.[108] Hence a combination of the techniques used in this work to follow matrix degradation provides a detailed characterisation of polymer matrix breakdown by hydrolysis.

The more appropriate <u>in vitro</u> degradation technique using blood plasma shows that the 300PHB (20% PHV) grade of polymer degrades marginally faster in blood plasma than in equivalent studies in a pH 7.4 buffer at 37^{o}C, perhaps indicating a limited esterase attack. Similar results suggesting esterase activity in the <u>in vivo</u> breakdown of PHB have been reported by Holmes.[152] However the studies in this work were on a small scale with plasma degradation studies on the melt pressed discs for a duration of only 610 hours. Further experimentation is needed to clarify the

the observed differences. It would appear though, that hydrolysis is the predominant degradative mechanism.

From the hydrolysis studies a more detailed model of the degradation process of the PHB/PHV copolymers emerges than the simple 'peeling of an onion' type described earlier. The hydrolytic (or enzymatic) degradation of a polymer matrix can be considered as a dynamic process with the following 'events' occurring: Firstly diffusion of water *into* the matrix (the rate of which varies considerably depending on polymer type[225] and the physical properties of individual matrices), followed by hydrolytic (and *in vivo* - enzymatic) erosion of the bulk, and to a smaller extent the surface of the matrix, to form pores. A 'gel' of low molecular weight polymer degradation products is produced and these diffuse *out* of the matrix. For *in vivo* systems enzymes would be expected to diffuse *in* through the formed pores at this stage of matrix breakdown. Further degradation increases the pore size and at this stage, diffusion of the higher molecular weight polymer degradation products out of the matrix is feasible. Eventually the polymeric matrix breaks down into smaller fragments and for a biodegradable polymer such as PHB further enzymatic degradation and tissue assimilation would be expected to occur *in-vivo*.

Release studies in this work were carried out using a range of dyes as the model 'drugs'. Two types of matrix were used: Solvent cast polymer / dye films and powdered polymer / dye / excipient cold compressed tablets. In all cases the release profiles are characterised by a relatively fast release followed by a tailing-off of the release rate. This is typical of a diffusional release process. However, once this 'burst' has occurred some polymer dye matrices show a resonably linear rate of release for an extended period. A comparison of the average release rates during the essentially linear 'plateau' portions of the release profiles is contained in Table 7.1, together with the results of work by Korsatko et al[57,144] on the release of low molecular weight surrogates from PHB tablet matrices.

A wide range of release rates is observed with a marked dependence on the type of polymer matrix for the duration of the approximatley linear section of the release profile. Release rates vary by some four orders of magnitude, with the USP dissoluted cold compressed PHB/PHV tablets showing the fastest release rates over a short time period and the polymer films releasing dye very slowly over a relatively long time scale.

The tablet work reported by Korsatko shows a lower rate <u>in-vivo</u> compared to the static <u>in vitro</u> release rates. He suggests that tissue encapsulation may hinder release, and one must bear this potential decrease in mind when assessing the <u>in vitro</u> dye dissolution rates from this work presented in Table 7.1.

Table 7.1 Comparison of release rates of various dyes and drugs from film and tablet polymeric matrices

POLYMER	'DRUG'	'DRUG' LOAD	RELEASE CONDITIONS	PLATEAU RELEASE RATE / $\mu g/hr/cm^2$	DURATION
260PHB*/0%PHV T	HET	10%#	Static	2.65	84 Days
260PHB*/0%PHV T	HET	10%#	Static&	1.25	126 Days
260PHB*/0%PHV T	HET	5%$	Static	2.76	44 Days
260PHB*/0%PHV T	HET	20%$	Static	9.5	38 Days
350PHB/12%PHVT	F40	7.5%	USP	120	12 Hours
350PHB/12%PHVT	F40^	7.5%	USP	150	16 Hours
350PHB/12%PHVT	F40@	7.5%	USP	431	6 Hours
350PHB/12%PHVF	FI	7.5%	USP	14.75	10 Hours
350PHB/12%PHVF	FI	7.5%	Static	11.55	23 Hours
350PHB/12%PHVF	FI	2.7%	USP	0.275	26 Hours
350PHB/12%PHBF	FI	5.3%	USP	0.37	16 Hours
350PHB/12%PHVF	FI	2.7%	USP	0.14	56 Hours
... ... F	FI	..	USP	0.013	600 Hours
PCL F	FI	0.17%	Static	0.20	92 Hours
PCL F	FI	0.18%	Static	0.083	41 Hours
750PHB/10%PHVF	F20	6.2%	Static	0.003	190 Hours
750PHB/10%PHVF	F150	3.4%	Static	0.016	254 Hours

F = Film; T = tablet; F20 =FITC 20,000; F4 =FITC 4,000 etc; FI = Fluorescein; & = in vivo all other data in vitro; * = Ref 57; # = 60 mg tablet; $ = 70 mg tablet; ^ = 12.5% MCC and @ = 10% E4M HPMC.

The best example of a consistent dye release was the dissolution of FITC40 from a 350PHB (12%PHV) tablet containing 12.5% MCC. A sustained rate of 150 µg/hour/cm^2 for 16 hours was observed after the initial burst release of dye from the matrix surface. Good controlled rates were also found from the 'base' (no excipient) tablet blends with release rates of approximately 120 µg/hour/cm^2 being maintained for over ten hours. As discussed in chapter six, varying the excipient type alters the release rate.

The PCL and PHB film dissolution studies showed less consistent release. Although the 'plateau' regions of constant release were relatively long term, the rates quoted in Table 7.1 were not as linear as the tablet release work. The PHB/PHV - fluorescein films produced the highest release rates, the PHB/PHV - FITC dextran films the slowest, with the PCL/fluorescein systems falling inbetween these two. However at extended release times (greater than 120 hours), release from the PHB/PHV - fluorescein films approximated to that from the corresponding FITC dextran dye systems. It appears that low dye loadings of fluorescein in the polymer matrices produce more consistent release with the best example observed being the 350PHB (12% PHV) / 2.7% fluorescein matrix which in static conditions released the dye at a rate of 0.275 µg/hour/cm^2 for 26 hours.

The higher dye dissolution rates of the PCL / fluorescein films compared to the equivalent PHB/PHV film matrices are probably due to differences in the physical properties between the

two polymers and the method of matrix preparation. Release of the higher members of the FITC dextran series from PHB/PHV matrices was found to be faster than dissolution of the low molecular weight dextran dyes. This is contrary to the predicted dependence on molecular weight from the general laws of diffusion. However this trend can be attributed to polymer-dye incompatabilities. These arise from the incorporation of a macromolecular entity into a macromolecular matrix. The mutual solvent incompatability of the polymer and the dye led to an elaborate co-solvent system which was unstable for dye loadings greater than 5%. This resulted in the formation of dye heterogeneities in the films as the dye loading approached this incorporation limit (c.f. Plate 5.2). The release studies did show however, that macromolecules can be incorporated into a polymer matrix within the limits described.

The cold compressed tablet studies not surprisingly showed a faster rate of dye release due to the higher porosity of the physically blended matrices. Dye release was controlled by matrix porosity and homogeneity. SEM studies illustrate the high initial porosity of the tablet matrices which increases with excipient loading and during the dye dissolution process. A wide range of dissolution rates was found, and an increase in dye load produced a considerable increase in dye dissolution rate. At low excipient loadings there was little difference between the release rate between these and the base (no excipient) tablets and certain excipients, for example HPMC, expanded the tablet matrix as they

were hydrated causing a fast dissolution rate of the incorporated dye. Similarly to the film studies dye release rate from the tablet matrices increased with increasing dye molecular weight. This is due to changes in matrix homogeneity with the larger dyes producing poorly mixed blends. The 350PHB (12% PHV) tablet blends consistently showed a higher release than the corresponding higher valerate 300PHB (20% PHV) matrices, even at low dissolution rates and in acidic dissolution conditions. This is attributed to inherent differences in crystallinity between the two polymer grades with the higher valerate copolymers forming more homogeneous and slightly less porous (c.f. Plates 6.2 and 6.4) matrix blends. The difference is also illustrated by changes in tablet hardnesses, the 12% copolymer matrices being softer. Recent work by Brophy and Deasy[226] finds differences in the release of Sulphamethizole from different PHB/PHV copolymer microparticles. The higher molecular weight PHB homopolymer matrices exhibited higher release rates than the hydroxyvalerate copolymers and the authors suggest that the relative rate differences are due to an internal plasticisation by the higher valerate copolymers which reduces matrix diffusivity. Other recent work by Juni et al[227] on drug release from PHB homopolymer microcapsules also showed this need for plasticisation of the polymer, in this case fatty acids were added to alter the release rate. This polymer matrix homogeneity effect is similar to the findings of this work.[228]

Degradation of the polymer matrix during dye dissolution from the films or tablets was not significant, with the polymer samples behaving as inert matrices from which the model 'drugs' were dissoluted. Release in all cases appeared to be a diffusional process, with little contribution from any degradation of the polymer matrix bulk. Although at the start of the work is was envisaged that a zero order release system could be made using a biodegradable polymer system such as the hydroxybutyrate / hydroxyvalerate copolymer series, it appears that the diffusional release nature of the incorporated dyes together with the very slow hydrolysis rate of the PHB/PHV copolymers make these systems unsuitable for macromolecular release by erosion of the matrix. However there have been several methods reported which attempt to obtain time independent zero-order release from essentially diffusional systems. These are based on altering matrix geometries[34-40] or by incorporating the active drug at differing concentrations throughout the matrix.[229] The application of these to the predominantly diffusional release systems observed for the PHB/PHV copolyers could obtain a less time dependent, near zero order release profile. Another method would involve pre-leeching of the excess surface 'drug' from the matrix. However, the effective drug loadings would be very low and the former methods may be more appropriate methods of achieving a more controlled release system.

The dye release mechanism from biodegradable polymeric matrices can be generally described as proceeding via a diffusional / erosional mechanism. However for these studies the emphasis is on the former. The various processes occurring during dye dissolution can be considered as: 1) An initial burst of surface dye; 2) leaching out of the dye from the polymer matrix; with 3) permeation of water into the polymer via pores and channels formed within the matrix - either in its fabrication or by hydration, swelling or hydrolysis which results in: 4) diffusion out of the 'liberated' dye. The dissolution of dye and any polymer degradation will increase matrix porosity and the process would continue via stage two at a faster rate.

Many mathematical models of diffusional release from various types of matrices have been reported. Original work was by Higuchi[216-218] with other papers by Langer and Peppas[8,230-231] and many others.[232-234] However in each case many parameters have to be derived and no attempt to apply these models other than the simple square root release plots contained in chapter six has been made in this work.

7.2 CONCLUSIONS

The hydrolytic degradation of a series of PHB/PHV copolymers has been characterised. The degradative mechanism is by a random chain hydrolysis process and is relatively slow compared to the established biodegradable polymeric materials. In line with other polyester hydrolysis studies the amorphous regions of the PHB/PHV copolymers preferentially erode. However large differences in molecular weight and in some cases crystallinity make equivalent comparisons difficult.

Hydrolytic degradation can be followed using a range of surface and bulk measuring techniques, with the former set of experimental methods which include goniophotometry and surface energy determinations, giving a precise characterisation of surface changes. These accurately magnify the changes occurring within the bulk of the polymer matrix, and are more precise than simple gravimetric measurements over the initial stages of polymer matrix breakdown in aqueous conditions.

Drug release from PHB/PHV matrices in the form dyes is predominantly by diffusion. Macromolecular dye species have been incorporated into polymer matrices, however loading is limited by polymer / dye compatibility. Release rates vary, with the more homogeneous low molecular weight dyes showing a more consistent release profile.

Tabletting studies show some potential for the PHB/PHV copolymers as non-disintegrating matrices for oral dosage. The 'drug' release rates can be varied by altering the porosity of the matrix either by including a soluble additive such as lactose or an inert swellable excipient, for example microcrystalline cellulose. In both cases approximately linear changes in release rate occur as the additive loading is increased. Release from the matrices is uneffected by solution pH, except in cases where 'drug' solubility is low. Polymer / dye homogeneity is a controlling factor in governing the release rate with the higher molecular weight dyes forming less compatible, hence faster releasing, blends. The tablet form of the PHB/PHV copolymer series therefore, could be used as an inert bioabsorbable matrix for short term drug delivery in oral dosage form, or as a longer term subcutaneous implant.

7.3 SUGGESTIONS FOR FURTHER WORK

It is hoped that the information contained in this thesis forms a basis from which other studies can be made. At the present time there is little information reported in the literature about the *in vivo* degradation of the PHB/PHV series. Work on this is needed for clarification of the degradation mechanism, although it is expected that hydrolysis will be the dominant mechanism with some associated esterase attack.

A more detailed understanding of the degradation may result from analysing sectioned layers of the polymer matrix as it degrades by GPC or by other methods, in order to gain a full understanding of polymer molecular weight and molecular weight distribution changes during hydrolysis. More information about the hydrolytic breakdown could be gained by precisely pinpointing the onset of accelerated degradation by careful goniophotometry and surface energy measurements throughout the initial stages of matrix breakdown.

A detailed investigation into the thickness of 'drug' (dye) hydrodynamic layers under various dissolution conditions could be undertaken by measurement of the appropriate parameters presented in the numerous mathematical models that have been reported. This would clarify the effect that solution flow rate over the matrix has on the rate of dye/'drug' dissolution in order for possible PHB/PHV matrix use in the relatively low dissolution rate

conditions that exist in subcutaneous environments, or in the relatively high flow rate conditions that exist in the stomach.

Alteration of the film and tablet release profiles from a diffusional to a more consistent release rate has been shown by Lee[34-40,229] and others to be possible by alteration of the drug matrix shape and / or drug loading profiles. A similar approach on the systems studied in this work may assist in the idealised search for zero-order drug delivery. An alternative to this would be to coat the the polymer tablet matrices with a hydrophilic polymer which would retard the release of an incorporated drug, although a similar delayed diffusional release rate would probably result.

Polymer matrices based on blends of the PHB/PHV copolymer series with other macromolecular materials which are less resistant to hydrolysis - for example the lower members of the polylactone or poly alpha ester series, may produce matrices with a more desirable degradation lifetime. An alternative suggestion for a bipolymeric matrix system would be to use a surface eroding polymer (for example a polyanhydride or a poly ortho ester) together with a homogeneously eroding polymer such as the PHB/PHV copolymer series to form a polymer / 'drug' matrix that erodes at two different degradation rates as each polymer component is hydrolysed.

As the PHB/PHV copolymers have been reported to have excellent <u>in vivo</u> compatibility, one use exploiting their

relatively long term biodegradation characteristics would be as surgical fixation devices e.g. bone pins and ligating clips. Another possible application could be in the form of antibiotic loaded polymer films which could be used as possible wound dressings. However for both cases the tough gauntlet of the regulatory authorities[3] has to be run in order to achieve any commercial success!

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