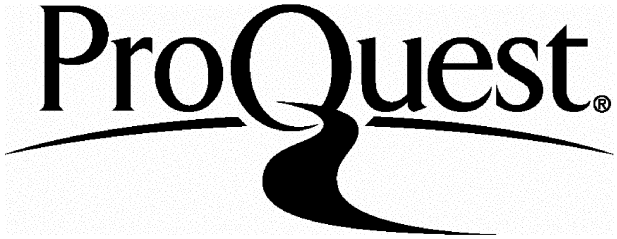
FACTORS INFLUENCING FORMATION AND THE *IN VITRO* DRUG RELEASE FROM PELLETS CONTAINING CHITOSAN

A Thesis submitted for the degree of
Doctor of Philosophy
University of London
Faculty of Medicine

by

Karen Josephine Quigley

The School of Pharmacy, University of London
SEPTEMBER 1994



ABSTRACT

Chitosan is a cationic polysaccharide material produced by deacetylation of chitin, extracted from the shells of crustaceans. Chitosan is a biodegradable, non-toxic material with a variety of pharmaceutical uses. A series of solid dosage form pellets have been produced by an extrusion - spheronization process and the ability to control the release has been investigated without a coating process.

A number of pellet formulations containing the model drug diclofenac sodium have been produced, based on varying concentrations of Avicel PH101, lactose and barium sulphate in which chitosan is incorporated as a dry powder, or dissolved in acetic acid as a gel. Incorporation of chitosan as a dry powder showed a concentration effect but did not show controlled drug release properties. The release as assessed by in - vitro dissolution studies was shown to be pH dependent. The effect of pellet size on the release profile was also investigated; a large pellet showing slower release than a small pellet. The effect of including four different electrolytes; potassium chloride, sodium chloride, magnesium chloride and potassium hydrogen carbonate at two concentrations in a standard formulation, on the formation and release properties of the pellets containing chitosan at two levels has been determined. The inclusion of the electrolyte alters the viscosity profile of the chitosan gel when assessed by a cone and plate viscometer and results in differing release profiles.

Chitosan is soluble in a number of acids and a series of seven different acids were investigated as possible solvent systems for inclusion in a pellet dosage form. The formation and release profiles of the pellets were examined. The rheological profiles of the seven gels were examined by means of flow curves and creep testing to enable fundamental parameters of viscosity and elasticity to be estimated. Oscillation rheometry provided further information on the viscoelastic behaviour of plugs of extruded material containing chitosan.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Professor J.M. Newton for his encouragement and guidance throughout these studies and the preparation of this thesis.

I would also like to thank;

Dave McCarthy for assistance with the scanning electron micrograph work,
Ms D. Sawh for her assistance with the differential scanning calorimeter,
My research colleagues both past and present,
The Dean of the School, Professor A.T. Florence for his assistance and encouragement.

Finally, I would like to thank my parents and family for their love and support during my years of study.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 7

LIST OF TABLES

CHAPTER 6

CHAPTER 1

INTRODUCTION

1 INTRODUCTION

1.1 CHITOSAN

1.1.1 GENERAL INTRODUCTION

Chitin is a polysaccharide material and one of the most widely distributed materials in nature. It is a principle component of the shells of crustaceans and insects and of the cells walls of bacteria and mushrooms. Chitosan is a poly(aminopolysaccharide) produced by the deacetylation of chitin, and the name is used for all low acetyl forms of chitin. Chitosan was first described in 1811 by Braconnot and named by Odier in 1823. The extent of deacetylation varies with the conditions used and hence the term chitosan covers a wide range of related polymers that vary in their free amine content, (Moore and Roberts, 1980).

Chitin is reported to be the second most abundant organic compound on earth after cellulose, (Ruiz-Herrara, 1978), and yet despite this only limited attention has been given to its potential uses in a large number of industries. Chitin appears to be one of the last unexploited biomasses on our planet.

Chitosan is reported to have a number of useful applications in a wide range of industries. As chitosan is a biocompatible, biodegradable, almost non-toxic material, inactive as an antigen, active as an immunological adjuvant, it has a range of potential uses. Its use as an accelerator for wound healing and as an activation for plant and animal cells has been documented. It can be processed into fibres, films, sheets, gels, tablets and beads and is usable as a digestible supporting material for slow release by oral, IV, or implant administration. Attempts have been made to apply these properties to the medical field in the form of surgical sutures and artificial organ membranes. Recently the pharmaceutical application of chitosan and its derivitives has been attempted. The release of indomethacin from chitosan gels (Miyazaki et al. 1981), the use of chitosan films for controlled release preparations of drugs (Kanke et al. 1989), the preparation of microspheres or microcapsules for the delivery of anticancer and other drugs (Nishioka et al. 1989), the permeation of drugs through chitosan membranes (Sawayanagi et al.

1982a), the development of a sustained release preparation for water soluble drugs (Sawayanagi et al. 1982b), the formulation of directly compressed tablets containing chitosan (Sawayanagi et al. 1982c), the preparation of a prolonged release tablet of aspirin with chitosan (Kawashima et al. 1985), are just some of the potential pharmaceutical uses. Chitosan also has a number of uses in the food industry and as a hypolipidemic agent. The hypocholesterolemic action of chitosans has been widely reported, (Sugano et al. 1988). Although the mechanism of the cholesterol-lowering action of chitosan is not well understood, the aminosugar polymer appears to interact with bile acid and/or cholesterol in the intestinal lumen and to stimulate fecal excretion of neutral steroids by interfering with the absorption process. Chitosan was also tested as an ingredient for domestic animal feeds, (Hirano et al. 1990). The hen's appetite and egg laying rate decreased by feeding an excessive amount of chitosan for a long term, due to the incomplete digestion of chitosan. However, it was shown to be safe, digestible and hypolipidemic at an appropriate dosage by oral administration in rabbits, hen's and broilers. The uses of chitin and chitosan will be fully discussed later.

1.1.2 ORIGIN AND EXTRACTION

Chitin is widely distributed in nature especially in marine invertebrates, insects, fungi and yeasts and chitosan is recognised in various fungi (Austin et al. 1981). Chitin is synthesized by some unicellular organisms such as diatoms, chrysoflagellates and protozoa, especially ciliates. Chitin is present in the exoskeleton of most invertebrates with the exception of all sponges, most Anthozoans and Echinoderms. The amount of chitin with respect to total dry weight is highest in Crustaceans. Ashford et al. (1977) have shown that chitin represents 14-27% and 13-15% respectively of the dry weight of shrimp and crab processing waste. Both chitin and chitosan are processed industrially from crustacean shell waste. Chitin occurs in the form of two crystalline polymorphs; α and β, α being the most stable and also the most abundant. The less common β is metastable. The extraction process consists of two main steps; (1) protein separation (2) calcium carbonate separation (Muzzarelli, 1977).
The diagram overleaf shows the process;

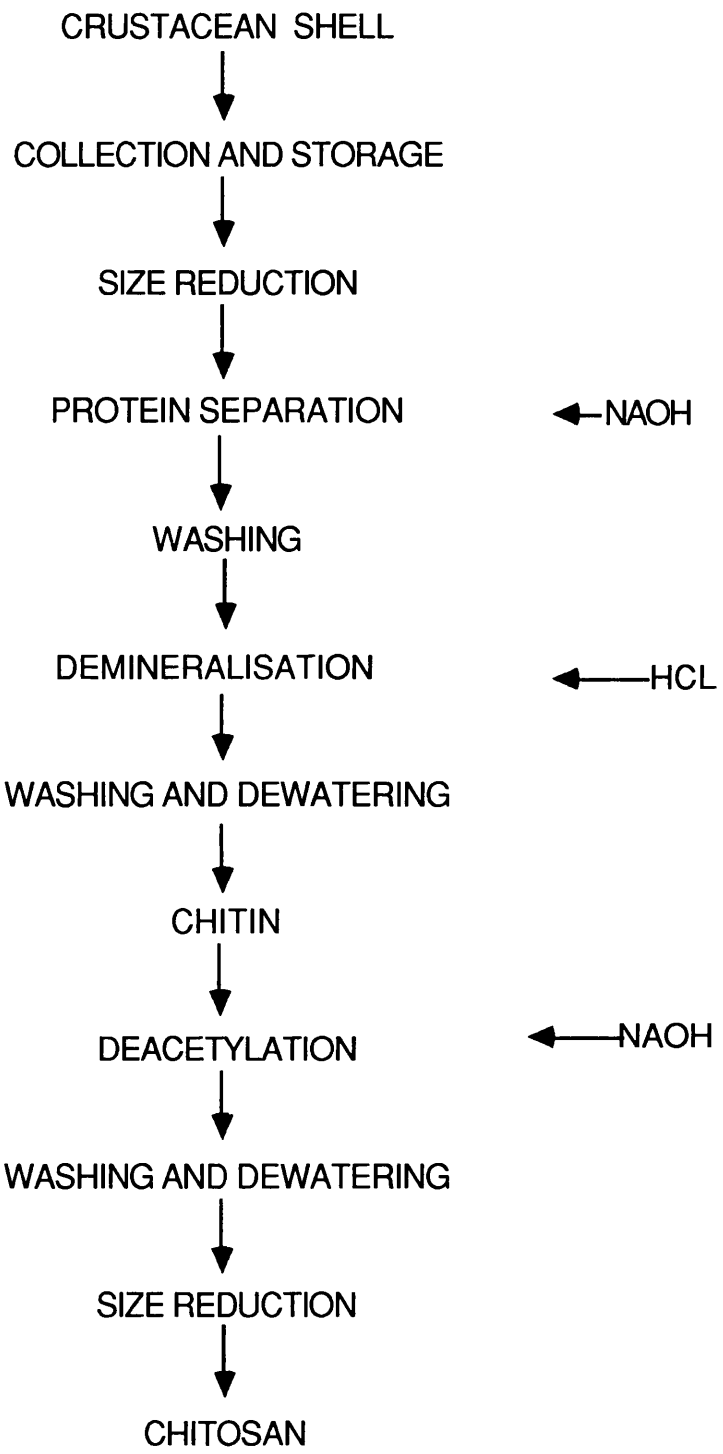


Figure 1.1 Simple flow diagram of Chitin and Chitosan processing (Knorr, 1984).

Chitin shell waste is usually ground and mixed with a dilute aqueous sodium hydroxide solution to dissolve the protein. If protein recovery is required, the pH of the protein solution is decreased to about pH 4 and the precipitate is dried. The residual material is then treated with dilute aqueous hydrochloric acid solution (~10%) to dissolve the calcium carbonate as calcium chloride. Deacetylation of chitin is achieved by treatment with hot concentrated sodium hydroxide solution (40-50%). Muzzarelli (1977) has shown that chitosan is quite stable to acid hydrolysis, and due to the treatment with dilute acid at low temperatures, only slight hydrolysis will occur during processing of chitin.

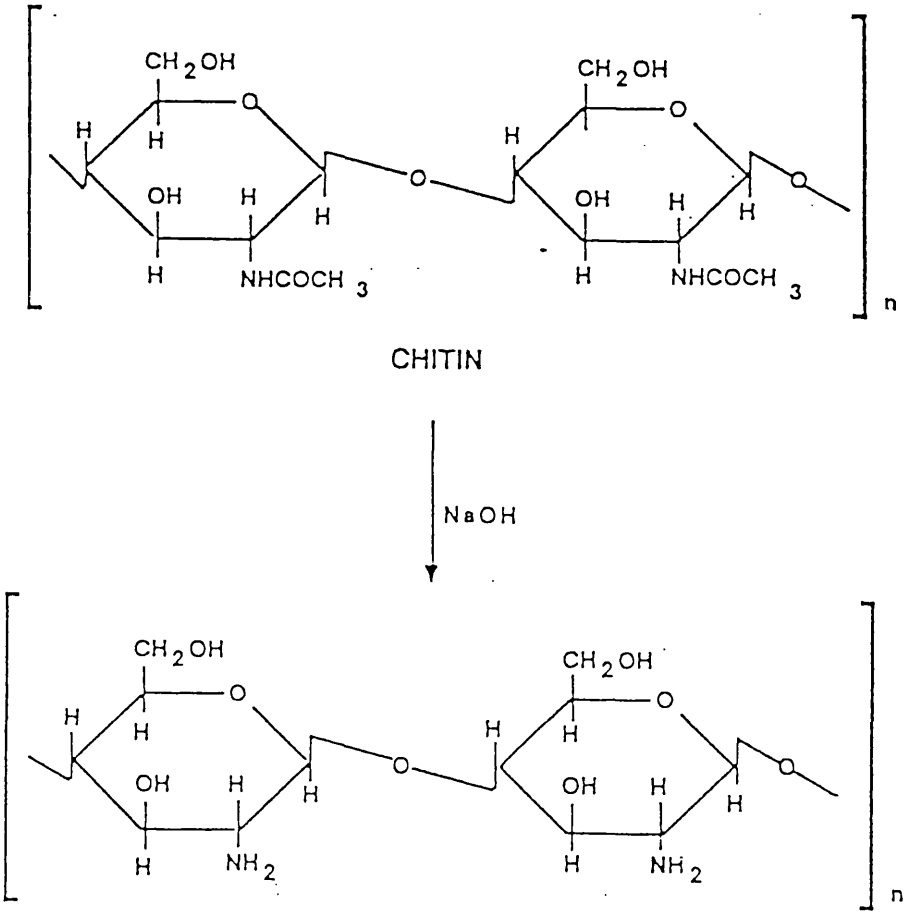
Chitosan appears as a major constituent of the hypal walls of zygomycete fungi such as the Mucor species. With its free amino groups it is a polycation but occurs in the walls together with the polyanions, polyphosphate and a glucomannan mucoran.

Several different methods have been proposed for the N-deacetylation of chitin and chitosan but they generally produce extensive depolymerization. Domard and Rinaudo (1983) proposed a new method for N-deacetylation of chitin in which a polymer free of N-acetyl groups is obtained with very little decrease in molecular weight. Different methods of determining the degree of acetylation were used including I.R. spectroscopy and conductimetry.

At present chitin is usually isolated from crab and lobster shells, owing to their high chitin content and to their relatively high availability. Other sources have been proposed and investigated e.g. marine plankton or the biological cover of marine rocky shores.

1.1.3 STRUCTURE

Chitosan is a 1-4 linked β - D - glucosamine polymer with a chemical structure; [(1 - 4) - 2 - amino - 2 - deoxy - β - D - glucan] prepared by N-deacetylation of chitin; [(1 - 4) - 2 - acetamido - 2 - deoxy -



CHITIN

CHITOSAN

Figure 1.2 Structure of Chitin and Chitosan.

β - D - glucose]. The structure of both chitin and chitosan is illustrated in Figure 1.2.

Each individual glycan chain is an unbranched linear molecule, with each sugar residue inverted with respect to its neighbour, i.e. it is a linear helix with two residues per turn.

1.1.4 PHYSICAL AND CHEMICAL PROPERTIES

The degree of deacetylation of chitosan usually ranges from 70 - 95% depending on the method used. Only a few studies have reported on the molecular conformation of chitosan and there is still no single model to describe it. The findings of a wide number of studies on different aspects of the physical and chemical properties are detailed below.

1.1.4.1 SOLUBLITY

Chitosan is insoluble in water, organic solvents and it is also insoluble in alkali and mineral acids except under certain conditions. In the presence of a limited amount of acid it is soluble in water - methanol, water - ethanol, water - acetone, and other mixtures. It is soluble in formic and acetic acids and in 10% citric acid and these are the most widely used acids for dissolving chitosan. Inorganic acids can dissolve chitosan at certain pH values after prolonged stirring and warming, but precipitated chitosan is usually easier to dissolve. Chitosan is soluble in dilute nitric acid, marginally soluble in 0.5% H_3PO_4 and insoluble in sulphuric acid at any concentation at room temperature. Chitosan is a hydrophilic material, not soluble in any common organic solvent (e.g. dimethyl formamide or dimethyl sulfoxide) but it dissolves well in acidified polyols. The best solvent for chitosan was found to be formic acid where solutions are obtained in aqueous systems containing 0.2 to 100% of formic acid, (Kienzle - Sterzer et al. 1982).

Methods for improving the water solubility of chitosan have been investigated. Kushino and Asano (1988) reported a procedure where chitosan is dissolved in an aqueous solution, concentrated by evaporation and finally spray dried at 175°C to provide a water soluble salt. Chemical modification of chitosan provides an alternative to improve its water solubility, and Muzzarelli (1988) prepared three water soluble derivitives; O-CM-chitosan, N-CM-chitosan and N,O-CM-chitosan. The O-CM-chitosan showed the best solubility over the pH range 3 - 11.

Water uptake of chitosan was found to be significantly higher than that of microcrystalline cellulose (Knorr, 1983). Possible explanations for the differences in water uptake between the chitinous polymers which range from 325 to 440% (w/w), include differences in the amount of salt forming groups, and differences in the protein contents of the materials. Austin et al. (1981) reported that protein residues remain with the chitin even after the most drastic alkali treatment. Air dried chitin readily becomes wet with water and reacts very little with solutions of sodium hydroxide in concentrations up to 17.5%. Wetting increases sharply in a 34.5% solution of sodium hydroxide which is probably due to the splitting out of the acetyl groups. Chitosan reacts readily with sodium hydroxide solutions. Filar and Wirick (1978) observed that the equilibrium moisture content of chitosan is a function of relative humidity but is not affected appreciably by sample molecular weight.

Sulphonation and nitration of chitosan yields products that are both salts and esters. Neither reaction temperature (0 - 30°C) nor time (1 - 10 hr) affect the degree of sulphonation, but it is markedly affected by the temperature of the ether used to separate the sulphonated products. The extreme stability to depolymerisation is attributed to the stabilising effect of the free amino groups in the chitosan molecule.

In general, as the concentration of ions in a polymer solution increases, the polymer solubility decreases. The amount of water available to hydrate the polymer is reduced as more water is required to keep the ions in solution. The types of ions in solution

affect polymer hydration to varying degrees, which is manifest by changes in solution viscosity. The ionic strength has an important effect on the viscosity of electrolytes, usually the intrinsic viscosity varies linearly with the inverse of the square root of the ionic strength.

Chitosan is a linear polyelectrolyte at acidic pH values. It has a high charge density, one charge per glucosamine unit. When the pH is decreased, the charge density of chitosan increases due to protonization of the amino groups resulting in expansion of the polymer chain. In solution the positive hydrogen ion from the acid associates with the amino group in chitosan producing a net positive charge on the polymer. Chitosan in its free amine form is not usually soluble in water at pH values above 6.5 and requires acid to prepare aqueous solutions. Domszy and Roberts (1985) reacted chitosan with 2,4 dinitroflurobenzene in an attempt to determine the number of free amine groups in chitosan. The extent of the reaction was determined by UV spectroscopy after hydrolysis of the reaction product and the temperature of hydrolysis should not exceed 50°C. Complete reaction of the amine groups is not achieved due to the bulky 2,4 dinitrophenyl groups shielding adjacent unreacted amine groups. This intramolecular steric hindrance would be expected to increase with an increase in the free amine content of the sample and an inverse relationship between the total free amine group content and the percentage of these that react with the 2,4 dinitrofluorobenzene was found.

1.1.4.2 MOLECULAR WEIGHT

The N-deacetylation of chitin generally reduces the molecular weight and gives a polymer that is strongly charged (Terbojevich et al. 1991). The molecular weight of chitosan can be determined by methods such as chromatography, light scattering and viscometry (Li et al. 1992). Viscometry is the most simple and rapid method. The molecular weight of chitosan is not always directly related to its viscosity due to the presence of colloidal particles. The deacetylation procedures of chitin do not lead to extended degradation of the

polymer chain as indicated by the average molecular weight obtained by light scattering, (Muzzarelli, 1987). The light scattering was carried out at 25°C using cylindrical cells in pure benzene. The refractive index increments were measured at four distinct concentrations of chitosan. At relatively high concentrations, the polyelectrolyte molecules overlap each other and the counterions are not allowed to leave the molecular domain. As dilution increases, the counterions diffuse to regions where the polymer molecules are absent and therefore the total electrical charge on each polymer chain increases and forces the molecule to extend itself to a larger configuration. An average molecular weight of 1.2 x 10*5 daltons has been reported for chitosan. The same value has been reported by Nagasawa et al. (1971) who carried out their work independently using the same method.

Nigalaye et al. (1990) reported chitosan had an average molecular weight of between 1 x 10*5 and 3 x 10*5 daltons. Chitosan displayed a wide range of viscosities in diluted acid media which depend on the molecular weight. Chitosan dissolves in dilute acid solutions but only chemically treated or acid hydrolysed chitin forms viscous solutions. Since chitosan is a cationic polyelectrolyte which forms a gel structure in acidic pH, it is different from commercial high molecular weight hydrocolloids. Chitosan has a pKa of 6.0 (Aiguelles-Monal and Peniche-Covas, 1988).

Roberts and Domszy, (1982) determined the viscometric constants, a and Km for chitosan using the Mark - Houwink equation in 0.1M acetic acid and 0.2M sodium chloride. The technique utilises the properties of the molecular weight distribution resulting from random degradation of the polymer chains. The values obtained were different to those previously reported, but similar to those reported for related ionic polysaccharides. Wang et al. (1991) also determined the values of a and Km for chitosans with different degrees of acetylation, by the light scattering method. They found that when the degree of deacetylation in the polymer increases gradually, the rigidity of the molecular chain is reduced and there is an increase in the electrostatic repulsion force of the ionic groups in the chain.

1.1.4.3 CHELATING PROPERTIES

Dye binding properties of chitosan were investigated by Knorr, (1983). Significant correlations were found between dye concentration and dye binding capacity of chitosan. Between pH 7.0 and pH 5.5 dye uptake was constant but at a lower pH chitosan was found to form gels.

Park et al. (1984) investigated the mechanism of metal ion binding to chitosan in solution. The interactions were studied by spectroscopic and viscometric measurements. The copper ion complex exhibited an absorption band at 265nm and was shown to be a 2:1 ratio complex. The binding was cooperative at pH 5 with both inter- and intra-molecular chelations depending on the concentration. The intermolecular chelation is stabilised by the addition of salts. The difference in chelating properties among metal ions suggests that the separation of a mixture of metal ions into its components is possible by ion chromatography, or dialysis using chitosan gel matrix and varying the pH of the medium. McKay et al. (1989) investigated the sorption of four metal ions; mercury, copper, nickel, and zinc, onto chitosan. The equilibrium isotherms were analysed using the Langmuir isotherm model. The effect of temperature on the saturation capacity was not very significant. Chitosan was shown to be a good sorbent for all the metals and the results ranked in the order; mercury > copper > nickel > zinc. Domard and Rinaudo, (1983) investigated the chelating properties of the polymer and, in order to do this, they prepared a fully deacetylated polymer to avoid difficulties in interpreting the mechanisms of counterion interactions.

The pK values of various samples of chitosan studied were in the range 6.0 - 6.9 (Muzzarelli et al. 1981). The free amine yields were in the range 58 - 78%. There does not seem to be any correlation between the free amine percentage, the degree of acetylation and the pK value. The reason for the range of pK values is unclear, but may depend on the structure. The pK value could therefore be used to select the chitosan most suitable for a given purpose, depending on the degree of protonation required at a given pH.

1.1.4.4 POLYELECTROLYTE BEHAVIOUR

Kienzle - Sterzer et al. (1982) used chitosan as a model polyelectrolyte to quantify the effect of pH, ionic strength and intermolecular interactions on the transport properties of polysaccharide solutions. Self-diffusion experiments were carried out at constant temperature. As the chitosan concentration increased, the intermolecular interactions became more important leading to the formation of intermolecular entanglements. The contribution of the microviscosity to the overall viscosity is small and hence the rheological response is primarily controlled by the intermolecular interactions. Semiconcentrated chitosan solutions were seen to consist of two distinct regions; a polymer rich region and a free solvent region. Thus pH, ionic strength and intermolecular interactions were shown to have a major effect on the self-diffusion coefficient through chitosan solutions. In a later study, Kienzle - Sterzer et al. (1984) reported on the hydrodynamic properties of chitosan in dilute solution which were found to be affected by the degree of ionisation. The stiffness of the molecule can be predicted as it increases with the degree of ionisation or a decrease in the ionic strength of the medium. The viscoelastic properties were affected by the polyion concentration and the charge density and the entanglement density of the molecules. In dilute solution chitosan behaves as a worm - like non - draining polymer with a flexibility that can be controlled by both changing the degree of ionisation and the counterion concentration. The conformational degree of freedom of chitosan in solution is similar to that of other semiflexible β, 1 - 4 glucan derivitives.

The flow behaviour of polyelectrolyte polysaccharides is dictated by their overall molecular conformation and the degree of H - bonding or electrostatic repulsion between neighbouring chain segments. Chitosan in solution exhibits the polyelectrolyte effect, and in the absence of salts there is an abnormal increase in viscosity due to charge repulsion and stretching out of the molecules (Nishi et al. 1986). When salt is added to neutralise this effect, there is a considerable reduction in viscosity.

1.1.4.5 GELATION

Reacting chitosan with a controlled amount of a multivalent anion will result in a crosslinking between the chitosan molecules. The network formed has the ability to retain large amounts of water, with some systems holding up to 95%. This crosslinking can be done in neutral, acidic or basic environments depending on the method applied. The ionotropic gelation of chitosan with different anionic counterions as carried out by Vorlop and Klein (1981), resulted in gels with a mechanical stability similar to those of calcium - alginate gels. A mechanical characterization of chitosan globules was conducted by Rodriguez - Sanchez and Rha (1981), who examined the relationship between preparation procedures, the strength, elastic moduli and relaxation time.

Thermally reversible gels have been produced by the addition of co-solutes (naphthalene sulphonic acid derivitives) to solutions of chitosans in dilute acetic acid (Roberts, 1989). The principle factor controlling gel melting temperature is the co-solute concentration, while chitosan or acetic acid concentration had little or no effect.

The gelation of chitosans occurs on addition of acyl anhydrides (Moore and Roberts, 1980) and hence N - acylation occurs and the acylation reaction continues after the gel has formed. This causes a continuous decrease in solubility of the polymer, causing contraction of the gel and syneresis. On addition of a co-solvent e.g. ethylene glycol, the gels eventually reach constant volumes giving rubbery flexible products. On increasing the temperature and the polymer concentration, a reduction in the time to gelation is noted. The dependence of the time to onset of gelation on the molecular weight of the acyl anhydride can be attributed to increasing steric hindrance at the reaction site with increasing size of the anhydride molecule. Gelation takes place through the formation of junction points and although the nature of the junction points has not been definitely established, it is unlikely that double helices are formed. It is more likely that hydrophobic bonding between sequences of N -acylated residues occurs, leading to the formation of micelle junction points such as have been proposed for O -methyl cellulose gels. Non-

reversible gelation occurs when between two thirds and three quarters of the amine groups have been acylated. At lower degrees of N-acylation the protonated amine groups occur sufficiently frequently along the polymer chain to prevent the aggregation of chain segments. Up to the onset of gelation, the polymer chains being polyelectrolytes are quite highly extended due to electrostatic repulsions between charged segments and to osmotic effects. As the degree of N-acylation increases the polyelectrolyte nature of the polymer becomes less pronounced and its solubility decreases. This tends to reduce the viscosity of the solution, opposing the increase in viscosity caused by the buildup of the three-dimensional network through the continued formation of junction points.

1.1.4.6 VISCOSITY

Chitosan is prepared by the deacetylation of chitin and this process results in a dramatic change in the viscosity profile. When chitosan is dissolved in dilute acetic acid, the viscosity tends to increase with increasing acid concentration (decreasing pH), while in hydrochloric acid the viscosity tends to decrease with increasing acid concentration. Solutions of chitosan tend to show a decrease in viscosity as the temperature is increased (Muzzarelli, 1977). The solutions regain their viscosities when cooled to the initial temperature. This nearly linear dependence of intrinsic viscosity on temperature has been shown with other polymers and is attributed to enhanced chain flexability and reduced root mean square unperturbed end to end distance of the chains with increase in temperature. Terbojevich et al. (1991) examined the viscosity of samples of chitosan at different ionic strengths with various degrees of acetylation. The rigidity parameter was found to decrease with an increase in the degree of acetylation. Lack of flexibility accounts for the high intrinsic viscosity, and the rigidity of the polysaccharide was ascribed to steric effects and local interactions. Ogura et al. (1982) found that 30 - 90% of chitosan solutions (degree acetylation ~ 10%) in aqueous acetic acid showed swirl like patterns under a polarising microscope when sheared between two glass plates. This suggests

that chitosan represents a family of rigid polymers regardless of the degree of acetylation.

Curves for solutions of chitosan in dilute acetic acid obtained by viscometry, are non-linear (non Newtonian and show pseudoplastic characteristics). The viscosity of chitosan was shown to depend on the varying shear rate and certain samples showed a degree of thixotrophy, (Muzzarelli et al. 1981). The viscosity decreases with a temperature increase in the range 2 - 25°C and at 15°C the sample showed negligible hysteresis. The viscosity of the solution was shown to have remained unchanged after six days storage.

Lee (1974) investigated the rheological properties of chitosan suspensions when placed in two thin cylinder Couette devices which rotated in opposite directions. These experiments indicated that particles in high velocity gradients underwent transformation and rotation, with a tensile stress developed at small angles with respect to streamlines of flow. Lee shows that shear induced deformation can occur. In shearing chitosan, two degradation processes occur; initially the molecular weight fall off is steepest, and the molecular weight rapidly approaches a limiting size with repeated passes. Repeated degradation reduces the size of the molecules to the limit produced by the critical forces applied. So, unless a greater force is applied, no further degradation will occur. When chitosan was sheared in a solution containing acetic acid and sulphuric acid, the initial molecular weight fall off was more rapid. This implies that solyvolysis may contribute to lowering the activation energy of bond scission.

Yalpani et al. (1983) reported on the rheology of a chitosan derivitive (1 - Deoxylactit -1- yl chitosan) which had been subjected to reductive alkylation. This derivitive displayed non-Newtonian features. At low shear, Newtonian properties were evident, at medium shear, dilatancy, and at a high shear, the viscosity drops (pseudoplasticity). A 2% solution of the derivitive exhibited unique oscillatory behaviour under steady shear at the transition point between the dilatant and the pseudoplastic regimes. Native chitosan (in dilute aqueous acetic acid) exhibits monotonic pseudoplasticity

with increasing shear. The addition of salt results in an increase in the apparent viscosity. The complex viscosity profiles and oscillation patterns found may be indicative of the formation and disintegration of different types of solution structures.

1.1.4.7 IR SPECTRA

The IR spectra (Ritthidej et al. 1994) of chitin is distinctive with amide bands at 1665, 1555 and 1313 cm^{-1}, all of which show perpendicular dichroism and are assigned to the C = O stretching, N - H deformation in the CONH plane and to the CN band. The chitosan spectrum differs in that the band at 1590 cm^{-1} predominates over the one at 1665 cm^{-1} and the band at 1555 cm^{-1} is absent. When chelated to metal ions, modifications occur depending on the nature of the metal ion, its concentration and the counterion.

1.1.4.8 SPECTRA EVALUATION

Saito et al. (1987) investigated the high resolution solid state ^{13}C NMR spectra of chitosan and its salts. The ^{13}C spectra differed appreciably between preparations reflecting the presence of different polymorphs. The spectra varied between extracts from shells of crab and shrimp and the different salts. Previous X -ray diffraction studies (Ogawa et al. 1987) have revealed two types of structures in chitosan salts; a two fold helical conformation similar to chitosan itself and a left or right handed helix having eight residues in three turns or a four fold helix of the dimer residues. The ^{13}C chemical shifts of the carbon atoms next to the glycosidic linkages in polysaccharides have been shown to vary considerably (up to 8 ppm). The peaks resolved into singlets and doublets and the differences between the samples were apparent. The X - ray diffraction studies show a peak at 19°58' in all samples of chitosan thus confirming its identity.

1.1.4.9 SUMMARY

From the many different papers on chitosan, the physicochemical characteristics seem to vary depending on the different origins and the different production techniques and isolation procedures. Natural species variation in chitin has been reported and currently chitosans are obtained from chitins possessing significantly different X - ray diffraction patterns, degrees of acetylation and optical properties. Therefore the product used needs to be defined, to ensure the quality of the polysaccharide and to obtain reproducible results.

1.1.5 TOXICOLOGY

Chitin is a highly chemically stable biopolymer with a wide distribution throughout nature. Chitosan has been utilized as a biodegradable and biocompatible carrier for implantable drug delivery systems using a water soluble chitosan derivitive (Machida et al. 1986). Chitosan is non-toxic (Arai et al. 1968) and Hirano et al. (1990) has reported that it is a safe, digestible and hypolipidemic material at an appropriate dosage by oral administration in rabbits, hens and broilers but excessive feeding of chitosan brings about physiological disorders due to its incomplete digestion. The growth, appetite and appearance of these domestic animals, the colour, size, weight and fatty deposit of animal livers; and the ratio of animal liver/body weight was tested. An excessive amount of chitosan fed for a long time resulted in thinner hens and the upper part of the intestinal canal was distended up to the duodenum. No visible injury was found on the surface mucous membranes in the constricted and distended parts. The liver weight and colour were normal. The physiological symptoms disappeared on cessation of feeding chitosan.

Arai et al. (1968) showed only quantities above 18g/kg of body weight/day were harmful to mice. Hirano (1989) reported the material to be almost non-toxic with an LD50 of 16g/kg body weight in mice. Its degradation products are known natural metabolites. Kay, (1982) reported that only 0.05 - 0.1% of chitosan will be present in animal diets when chitosan is used as a protein coagulating aid to

recover proteins from food processing wastes. Landes and Bough, (1976) reported that in rats receiving up to 5% of free dietary chitosan, no differences were found in growth rates, inner organs and blood serum composition when compared to control animals.

Austin et al. (1981) have shown that the growth of bifidobacteria in the gut of chickens was increased by the addition of chitin to their diet. These bacteria are also found in the intestinal flora of breast-fed infants and they block the growth of other types of microorganism and generate the lactase required for digestion of milk lactose. This may be of major importance since certain groups of humans and many animals have lactose intolerance.

Gordon and Williford, (1983) showed that at a 5% level chitosan did not affect growth or food consumption of growing rats over a three week period. Increasing the level to 10% or 20% depressed iron absorption.

Although chitin containing foods (crabs and mushrooms) are part of our food supply, more evidence from controlled chronic experiments are needed on its safety aspects.

1.1.6 APPLICATION AND USES

Chitosan and its derivitives have been used in enzyme and cell immobilisation, as flocculants, in the preparation of reverse osmosis membranes, in waste water treatment, and in personal care products (Muzzarelli, 1973). In addition, chitosan has a variety of promising medical, pharmaceutical and food applications.

Biomedical applications of chitosan include wound and burn healing, soft and hard contact lenses, and artifical kidney membranes. It has been used in ophthalamic wound healing, artifical skin, haemodialysis membranes and for drug targetting, (Chandy and Sharma, 1990). Its use as an adsorbant for removal of immunoglobulins from blood in auto-immune disease therapy is the subject of a patent. Other applications include; the reconstruction of

periodontal tissue (Muzzarelli et al. 1988), as a matrix for the immobilisation of dextranase, bioabsorptive sutures, moisturising and softening properties for skin and hair (Li et al. 1992), its use in vascular surgery , tissue culture and tissue regeneration (Malette et al. 1985), promotes regular fibrin formation and favours epithelialisation (Sapelli et al. 1986), and as a haemostatic agent, its film forming characteristics seal the grafts (Malette et al. 1983).

Chitosan has numerous applications in the food, paper (to improve the wet strength) and cosmetic industries (Li et al. 1992). It has been used as a film in the preservation of fruit and vegetables. Its use in animal feeds and fertilisers have been documented, as has its application in plugging gas well drilling sites, and as a gelled electrolyte for batteries with high ionic conductivity. Chitosan can be added to cropland to stimulate natural microbes that provide protection to certain crops, and as a seed coating it results in increased crop yields. Chitosan is also used as a non-toxic polymer in hair treatment and skin care, that can form clear films that adhere to skin or hair due to its cationic nature.

Chitosan's main use continues to be as a non-toxic flocculant in treatment of wastes (sewage, sludge, breweries, canning, etc) and as a chelator of toxic metals including radioactive metals. As a natural polymer it is preferred over synthetic polymers which may contain hazardous monomers. The largest single use of chitosan is the clarification of wastewater in Japan, and its ability to chelate iron as well as its flocculating activity, makes it a very useful pool and spa clarifier (Sandford, 1989).

Anti-ulcer, anti-tumor and antacid properties have also been claimed (Hillyard et al. 1964). Anti-tumor activity was shown against sarcoma in mice using liposomes containing oligomers of chitin or chitosan. Chitosan has been shown to decrease cholesterol absorption (Ikeda et al. 1989) more so than guar gum or cellulose and the effect was more significant when administered with safflower. Dietary fibre also significantly affected triglyceride absorption and absorption tended to be low in the chitosan group. This hypocholesterolemic activity of

chitosan has been shown in rabbits and rats, and is the subject of a patent.

1.1.6.1 PHARMACEUTICAL APPLICATIONS

A wide number of pharmaceutical uses have been attributed to chitosan. Chitosan beads have been formulated as a biocompatible matrix to deliver drugs. Due to chitosan's ability to be depolymerized by lysozyme, an enzyme found in various mammalian tissues, drug impregnated chitosan beads can be used as a bioerodible system to deliver pharmaceuticals. Bodmeier et al. (1989) developed a delivery system for micro- or nano-particles in bead form. The particles were entrapped in beads formed by ionotropic gelation of the charged polysaccharides in solutions of the counterion. The chitosan beads showed pH dependent release and disintegrated in 0.1M HCl. The disintegration time was studied as a function of the solution viscosity of the polysaccharide, gelatin time, counterion concentration and the method of drying. Murata et al. (1993) prepared chitosan reinforced alginate gel beads, and release rates from the gel beads were slower after an initial lag time when incubated with chitosan compared to the gels incubated without chitosan. This was assessed by incorporating a dye (Brilliant Blue G) into the alginate gel beads and then measuring the amount retained by each alginate gel after a dissolution test. Erosion of the gel beads was supressed by chitosan treatment.

Shin et al. (1987) tried to enhance the dissolution properties of poorly soluble drugs by cogrinding chitosan with the drug. This was also shown by Shiraishi et al. (1990) who showed enhanced dissolution rates of acidic, basic and neutral drugs using low molecular weight chitosan from kneaded mixtures. These workers propose that the enhanced dissolution rate of kneaded mixtures may be due to improved wettability and to changes in the crystallinity, microcrystal size and shape. Low molecular weight chitosans and their interactions with indomethacin in solution and the solid state were studied by Imai et al. (1991). The data suggested that the acetyl

and amino group of chitosan played an important role in the complexation and the dissolution rates were enhanced.

The use of chitosan in a sustained release pharmaceutical for the treatment of gingivitis in the form of a sheet, film or stick is the subject of another patent. The preparation of chitosan particles by spray drying of acidic chitosan solution (JP 63 20,302), showed that when spray dried under pressure of $1kg/cm^2$ and a flow rate of 5-6ml /min. at 130°C the particles were spherical with an average diameter of 2μm. Kanke et al. (1989) prepared three types of chitosan films (by evaporating the solvent from the chitosan/drug mixture); a monolayer, a doublelayer and an N -acetylated layer containing prednisolone and pores were observed by scanning electron micrographs. The results showed that the monolayer type films retarded drug release as the films became thicker. Following N-acetyl treatment of chitosan films, the release occurred more slowly than from the corresponding doublelayer films, probably due to the presence of extremely small pores. Miyazaki et al. (1990) investigated the potential of chitosan films containing diazepam as a controlled release dosage form and found that it was a suitable alternative to tablets. The film is composed of a drug-chitosan mixture in a ratio of 1: 0.5. The ground mixtures were dissolved in 10% acetic acid which is then evaporated off. Chandy and Sharma (1991) prepared films of chitosan by an evaporation technique, where chitosan was mixed with steroid drugs. The release profile of the steroids from the film matrix was studied and also the weight loss and the tensile strength of the films. They conclude that the chitosan films show first order release patterns and may be useful in contraceptive applications.

The formation of gels by reaction of chitosan with glutaraldehyde is reported by Roberts and Taylor, (1989). They found that by adding neutral electrolytes they increased the rate of gelation and the cross-linking mechanism involves the formation of a Schiff base. Miyazaki et al. (1981) prepared dried gels of chitin and chitosan using indomethacin and papaverine as model drugs and the drugs dispersed in the chitosan gels were released in a zero order fashion. Knapczyk (1993) prepared a water soluble / miscible gel ointment

base containing 93% deacetylated chitosan in a solution of lactic acid. Gels containing 66% deacetylated chitosan were shown to be less stable. The storage and stability of the gels containg different drugs were investigated, and they found a number of stable formulations.

Acarturk, (1989) used chitosan to prepare a prolonged release tablet formulation of diclofenac sodium. Tablets were made by direct compression and wet granulation methods and tested by dissolution and showed controlled release properties. Sawayanagi et al. (1982b) used chitosan in the preparation of a sustained release pharmaceutical for water soluble drugs e.g. propranolol. Zero order controlled release profiles were seen from the directly compressed tablets. Kawashima et al. (1985) prepared a prolonged release tablet of aspirin with chitosan. The drug release became prolonged with increasing chitosan concentration, with the physical state of the chitosan (in liquid solution or gel), or with decreasing pH in the dissolution media. Sawayanagi et al. (1982c) has suggested that chitosan would be suitable as a diluent for direct tabletting after comparing the flow properties of powders containing chitosan, crystalline cellulose or mannitol. Knapczyk, (1993) states that when chitosan is used as a filler or binder it does not affect mass flow, however when it consists of 50% of the tablet mass, rapid tablet disintegration resulted. Upadrashta et al. (1992) evaluated chitosan as a binder for chlorpheniramine tablets prepared by wet granulation in comparison with other binders and found that tablets containing chitosan showed rapid dissolution profiles. The results showed that chitosan is an effective tablet binder ranking above methylcellulose and sodium carboxymethylcellulose.

Chitosan has been used as a matrix for sustained release pharmaceuticals containing angiotensin converting enzyme inhibitors or ascorbic acid. The tablets showed zero order release over 8hr in neutral and acidic media, and this is the subject of another patent (US 4,738,850). Chitosan has also been used to covalently crosslink semipermeable membranes for microcapsules. The preparation of ultrafine spherical chitosan powder as a pharmaceutical additive is the subject of another patent (JP). Chitosonium malate, a salt of chitosan was investigated as a sustained release matrix tablet by

Akbuga, (1993). Tablets were prepared by direct compression and wet granulation and as the chitosonium malate concentration increased, the drug release rate decreased.

Nigalaye at al. (1990) developed a hydrocolloidal matrix system containing complexes of chitosan for preparation of sustained release tablets. If the concentration of chitosan was greater than 50%, it was found that an insoluble, non-erosion type matrix was formed and when the concentration was less than 33% the tablets were fast releasing. Chitosan in a concentration of ~ 10% acted as a disintegrant. Dose dumping in alkaline media was avoided by formulating a hydrated erosion type matrix of chitosan, carbomer - 934P and citric acid. Meshali and Gabr (1993) investigated the effect of interpolymer complexes of chitosan on the release of chlorpromazine. These solid complexes of chitosan with acacia or pectin were dried to be used as tablet matrices. The effect of the different polymers on drug release was dependent on the gel property of the polymer and the drug-polymer interaction. Takahashi et al. (1990) formed interpolymer complexes of chitosan with sodium alginate and sodium polyacrylate. Differences in the systems were mainly due to the rigidity or flexability of the polymer chains. The chitosan - sodium alginate complex was stable to pH change while the other complex was quite sensitive to pH change. Shiraishi et al. (1993) also formed a chitosan - polyelectrolyte complex with indomethacin as the model drug which showed a sustained release profile using different molecular weight chitosans. The plasma concentrations of indomethacin after oral administration of the chitosan gel beads to beagle dogs exhibited the sustained release pattern. A negative correlation was observed between the molecular weight and the release rate constant. It was suggested that the absorption of other drugs could be controlled by the selection of chitosan hydrolysate of optimal molecular weight which is predicted from the in vitro dissolution tests. Adusumilli and Bolton (1991) prepared chitosan citrate complexes of several viscosity grades. These complexes formed gels on dispersion in water. Their ability to control drug release from matrix tablets was evaluated using a factorial design and they were found to be effective sustained release dosage forms and could be directly compressible.

Sawayanagi et al. (1982a) also investigated the permeation of drugs through chitosan membranes. The permeability decreased with an increase in the molecular volume of the drugs. The activation energy was found to be close to that of the diffusion of organic drugs in water. The observed effect of pH was attributed to the cationic state of the membrane. The drug permeation was controlled by diffusion through pores especially in the case of drugs with small molecular volumes, but it also depends on the charge state of the chitosan membrane.

Nishioka et al. (1989) prepared cisplatin albumin microspheres containing chitin and treated with a chitosan-acetic acid solution. They suppressed the decomposition by protease, but the microspheres treated with 70% deacetylated chitosan showed the greatest susceptability to lysozyme. Meshali et al. (1989) encapsulated acidic and basic non-steroidal anti-inflammatory drugs with cellulose derivitives and chitosan and studied the interaction of the drugs with chitosan. Thanoo et al. (1992) evaluated cross-linked chitosan microspheres as a controlled release matrix. The microspheres were prepared by the glutaraldehyde cross-linking of an aqueous acetic acid dispersion of chitosan in paraffin oil using dioctyl sulphosuccinate as the stabilizing agent. Drug release rates were influenced by the cross - linking, density, particle size and the initial drug loading in the microspheres.

Watanabe et al. (1990) indicated that 6 - O carboxymethyl chitosan may prove useful for the slow release of drugs and cytokines including vaccines from carrier gels.

Takayama et al. (1990) investigated bioadhesion properties and drug release phenomena of tablets consisting of a mixture of chitosan and sodium hyaluronate. An interaction between the two polymers appears to affect the release rate following water penetration into the tablet. Lehr et al. (1992) investigated the mucoadhesive properties of chitosan and found it fairly mucoadhesive in comparison to the reference sample, Polycarbophil (a high molecular weight copolymer), especially in a neutral or slightly alkaline environment.

Miyazaki et al. (1988) report the slow release of indomethacin from chitosan granules tested in rabbits. The chitosan granules were superior to the conventional capsules in terms of reducing the peak in plasma concentration and maintenance of the drug concentration in the plasma, and when placed in an acid medium they gradually swelled and floated. The release rate could be controlled by changing the mixing ratio of chitosan and the drug. Hou et al. (1985) also used chitosan granules to achieve a sustained release effect with indomethacin and these swelled and floated on an acid medium at pH 1.2. The effect of cross-linking was also examined and found to prolong the release rate. They conclude that chitosan is useful as a vehicle for sustained release preparations of indomethacin in granular form. Inouye et al. (1989) formulated sustained release intragastric buoyant granules based on chitosan using the drug prednisolone and tested these in beagle dogs. Tablets containing an effervescing agent were prepared and drug release from tablets containing 50-60% deacetylated chitosan was more sustained than from tablets containing 93% deacetylated chitosan. Henriksen et al. (1993) studied the drug release from granules (prepared by wet granulation) containing chitosan or chitosan malate, using three water-soluble drugs. The granules showed good flow properties and granules containing 25 - 75% chitosan malate showed an increased sustained release effect as the chitosan concentration is increased. The granules containing chitosan showed more rapid dissolution, due to fast disintegration and a wetting effect. The chitosan malate interacted with the phosphate buffer and this influenced the release rate. Machida et al. (1989) prepared buoyant chitosan granules that had internal cavities and showed sustained release of prednisolone. The release properties were controlled by changing the composition of the granules and/or the thickness of the chitosan membrane.

Recently, a number of workers have prepared pellets containing chitosan using extrusion-spheronization technology. Goskonda and Uparashta, (1993) prepared beads using Avicel RC-591, with different viscosity grades of chitosan, and found that the higher viscosity grades of chitosan yielded beads with rough surfaces and slower release characteristics. Beads swelled in 0.1M HCL but the

structure remained intact. Tapia et al. (1993) prepared two different sizes of sphere without coating, 1mm and 2mm using chitosan (Seacure 242) and Avicel PH101. The release of the model drug from the spheres was found to be slower than from formulations without added chitosan. The release profiles followed first order kinetics. No common mechanism for drug release was found and the stirring speed on dissolution did not affect the release rate.

Although chitosan may be less advanced than other polymers with regard to research and its utilization, it has potential in a number of areas including pharmaceuticals. Some further basic studies are required and a standardised raw material is needed before its full potential in the pharmaceutical field can be realised.

1.2 CONTROLLED RELEASE TECHNOLOGY

1.2.1 INTRODUCTION

Controlled drug delivery is the phasing of drug administration for a condition so that the optimal amount of drug is given in the time required. The use of spherical particles for pharmaceutical dosage forms is dated by historical interest in the hand - rolled pill, O'Connor and Schwartz, (1989). Spherical granules flow freely and pack uniformly and are therefore suitable for producing controlled release pellets of the multiple unit dosage form. Multiple unit dosage forms spread out uniformly in the gastrointestinal tract. This results in a more reproducible drug absorption and reduces local irritation compared to single unit dosage forms. A number of oral solid dosage forms are produced mainly in the form of coated pellets (Mehta, 1986), with a low surface to volume ratio and good flow characteristics that can be filled into gelatin capsules or compressed into tablets. The advantages of these dosage forms include; highly reproducible transit through the gastrointestinal tract and as they are widely distributed over a large surface area the risk of damage to the intestinal mucosa is minimised. The optimum drug therapeutic concentration is maintained with minimum blood fluctuation and reproducible release rates over extended time periods may be

obtained. These drug delivery systems are particularly suitable for drugs with short half lives. A wide range of drugs with short half-lives, including propranolol, indomethacin, theophylline and nitroglycerin have been formulated by this method. Pellets are usually coated for aesthetic reasons, enteric release, taste masking, stability or to control release of the active ingredient (Deasy, 1984). The matrix and reservoir systems are the most commonly used methods in controlling drug release.

Most controlled release systems are classified (Lee and Robinson, 1978) into a number of different types, either chemically controlled systems or diffusion controlled. Diffusion controlled systems may be further classified into membrane reservoir and matrix systems, including porous inert matrix tablets, eroding matrix tablets, tablets with a diffusion controlling membrane and swellable hydrophilic matrices. A spherical pellet which is smooth and dense has a minimum surface area per unit volume and can be characterized by its diameter. Most of these sustained release pellets employ dissolution as the rate limiting step as a drug with slow dissolution is inherently sustained. To achieve optimum therapeutic effects it is often desirable to have zero order drug release. Membrane reservoir systems, where the drug core is surrounded by a rate - controlling membrane, are often employed for this purpose. A number of methods involve coating individual particles or granules with a slowly dissolving outer layer. By varying the thickness of the coat it is possible to control the release, as the time required for the coat to dissolve is a function of its thickness and aqueous solubility.

1.2.2 MATRIX TYPE SYSTEMS

Matrix type systems utilize conventional pharmaceutical techniques and are relatively simple to manufacture. Release of a drug from the dosage form may be controlled by the rate of penetration of the dissolution fluid into the insoluble matrix. The drug is usually compressed with some sort of slowly dissolving carrier and the porosity and tortuosity of the matrix will affect the rate of drug release as will the drug solubility. The drug release rate decreases with time in a matrix formulation due to the increased diffusional

distance and decreased surface area available. Matrix systems usually show first order release behaviour with a continually diminishing release rate. This is a result of the increasing diffusional resistance and decreasing area at the penetrating diffusion front as matrix diffusion proceeds. Higuchi (1961), derived an equation to describe drug release from a porous (granular) matrix system which stays intact during dissolution (no swelling or erosion);

$$Q = [\, D\varepsilon/T\,(2A - \varepsilon C_S)\, C_S\, t]^{1/2}$$

where Q = weight in grams of drug released per unit surface area, D = diffusion coefficient of drug in the release medium, ε = porosity of the matrix, T = tortuosity of the matrix, C_S = solubility of the drug in the release medium and A = concentration of drug in the tablet. Therefore, to achieve zero order release from a matrix, one needs to select a geometry that compensates for the increase in diffusional distance with a corresponding increase in surface area available. This square root of time relationship has become the accepted standard in evaluating drug release from inert matrices. A plot of the amount of drug released versus the square root of time should be linear if the the drug release from the matrix is diffusion controlled. This assumes that a pseudo steady state is maintained, that perfect sink conditions apply and excess solute is present, drug particles are smaller than those in the matrix, the diffusion coefficient remains constant and that there is no interaction between the drug and the matrix. In practice the diffusion of a drug often occurs in combination with a swelling or erosion process and this complicates the release model. Hopfenberg (1976), describes the release from erodible slabs, cylinders and spheres, where surface erosion is the only factor allowing drug release to occur. Lee (1984) describes the release from an erodible or swellable polymer matrix in the presence of moving boundaries. The swellable system has also been investigated by a wide number of other workers including, Peppas and Korsmeyer (1987) and Ranga and Devi (1988).

A number of retardant materials are used in matrix formulations, either insoluble or hydrophilic types. The drug is embedded in a matrix core of the retardant. Many different polymers, waxes, gums and clays have been reported in the literature as retardant materials. The choice of drug and retardant material will affect the rate and mechanism of drug release from the device. Drug diffusion through polymers is affected by morphological and structural characteristics of the polymer.

1.2.3 EXTRUSION/SPHERONIZATION

The preparation of spherical pellets with a high drug content may be achieved by extrusion- spheronization, as described by Conine and Hadley (1970) and Reynolds (1970). Extrusion is defined (Fielden and Newton, 1992) as the process of forming a raw material into a product of uniform shape and density, by forcing it through an oriface or die under controlled conditions. The processing sequence involves dry blending of the powders with the active ingredient, wet granulation of the mass, extrusion and subsequent spheronization and drying of the pellets formed (Figure 1.3). The process has diverse applications in a range of industries. Extrusion is a continuous process and the force required to extrude is dependent on the rheological properties of the extrudate and the die itself. Advantages of the method for preparing pellets include; regularity of pellet shape, uniformity of size, a smooth surface and good flow properties. A good extrusion mixture consists of a cohesive and suitably plastic mass, which is non-adhesive to both the equipment used and itself.

Extrusion is mainly classified into systems under temperature control or semisolid systems whereby solvents are added to the materials to alter the consistency of the mix. The various types of extruders have the common feature of forcing the extrudate through a narrow die. The different types are discussed below. Commercial extruders include the screw feed extruders, the gravity extruder and the prefilled type i.e. the ram extruder.

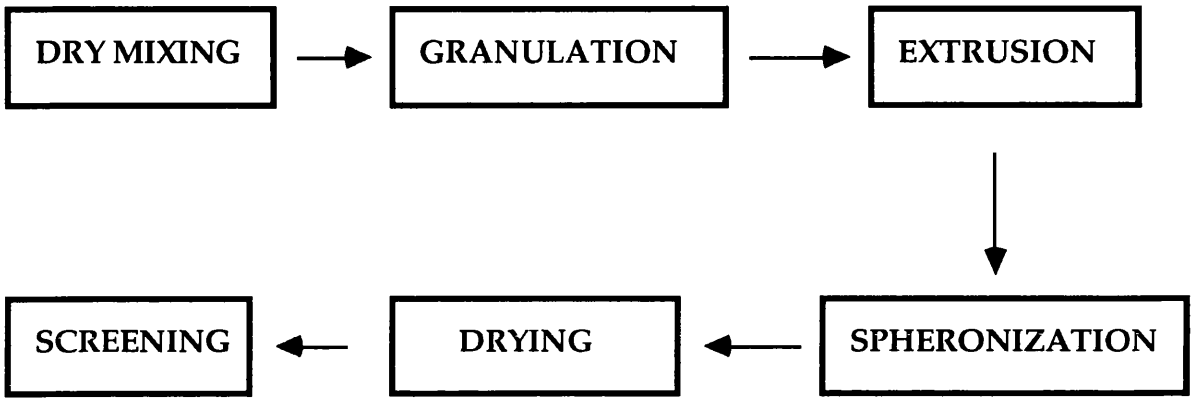


Figure 1.3 Flow Chart for the Formulation of Pellets.

1.2.3.1 SCREEN EXTRUDER

The process consists of feeding the material via a gravity feed hopper into a channel between the flights of a helical screw, where the material is forced in contact with the wall of the extruder, as well as being sheared. The system utilizes a screw feed mechanism consisting of single or twin helical screws rotating in a barrel to transfer the damp mass from the feed hopper to the die. The die consists of a thin steel plate perforated with numerous holes, positioned radially or axially to the screw feed. This process has been widely used with polymers and in the food industry (Jasberg et al. 1979), as it has a high output rate and is continuous. The screens are easily changed and cleaned. A disadvantage of the system is that the screw mechanism can exert a high shear on the material, generating

excessive friction and heat, as the wet mass passes between the screw and the barrel. The low length to radius (L/R) ratio of the die holes can also result in low compaction in the extrudate and distortion of the surface finish, i.e. shark-skinning. The temperature rise created by the frictional effects can have a detrimental effect on some materials, and the design has been modified to use a shorter helical screw, a radial screen and wider helical channels to reduce the frictional effects, (Fenner, 1970).

1.2.3.2 ROTARY CYLINDER EXTRUDER

This type of extruder consists of a feed hopper and two parallel counterrotating cylinders. The granulating cylinder is perforated and acts as a die. Material is fed from the hopper to the die region between the cylinders and adheres to the knurled surface of the solid cylinder, building up a thin layer which is pressed through the die cylinder. Although the extrusion is a continuous process, the material flow through each cylinder is intermittent due to the rotation of the die. Pressure is built up in the perforations and is dependent on their length and diameter. Since the cylinders only apply pressure to a small quantity of material in the feed zone, there is little tendency to create moisture gradients.

1.2.3.3 ROTARY GEAR EXTRUDER

The gear extruder operates on a similar principle to the cylinder extruder, and consists of two hollow counterrotating gear cylinders with counterbored dies or nozzles. The material is fed by gravity from a feed hopper between the two adjacent cogs of each gear. Scrapers cut off the extrudate in the centre of the cylinders and the material is compacted as it passes through. Higher throughput rates can be attained with this type of extruder since output is achieved through both rotating gear wheels. The diameter of the holes can be varied to produce a range of pellet sizes. The machine may be furnished with cooling equipment, circulating water through the compaction gears for processing materials that require

temperature control. A disadvantage of this system is the improper feeding of material often occurs due to the bridging of material.

1.2.3.4 RAM EXTRUDER

Systems that possess no continuous feed mechanism are batch systems and are referred to as prefilled systems. Industrial ram extruders are commonly used in the plastic and rubber industries and for materials that require critical in-process control and in the extrusion of moist powders and clay like materials. The simplest type of ram extruder is where the material is packed into a barrel. The material is then extruded through a die by the application of a load on the piston which is inserted in the barrel above the material. Semi-continuous extruders have been designed using a twin barrel and ram arrangement in which material is fed to each barrel in turn by a screw system. A ram extruder designed by Ovenston and Benbow, (1968) consists of a stainless steel barrel ~ 20cm in length, with interchangeable dies able to be bolted onto the end. A fluon seal ring positioned at the lower end of the piston provides a low friction seal to prevent material escaping. The material is packed into the barrel and partially consolidated to a plug by inserting the piston. The barrel (with die attached) is mounted on the 'C' piece and a load applied to compress the material in the barrel and extrude it. The crosshead may be driven at various constant rates and the force acting on the material during extrusion is recorded as a function of the displacement of the piston, and a force - profile is produced. A typical profile (Figure 1.4) shows three distinct regions; compression stage, steady state flow stage and a region of forced flow.

In the compression stage, the material is compressed into a plug prior to flow and the pressure builds up while the material density is maintained. At the end of this stage, the pressure builds up until it is high enough for the material to yield and commence flow. This is the region of steady-state flow where the force remains constant and may lead to the region of forced flow. This leads to a gradual rise in extrusion force with displacement and may be caused by the proximity of the ram tip to the die face.

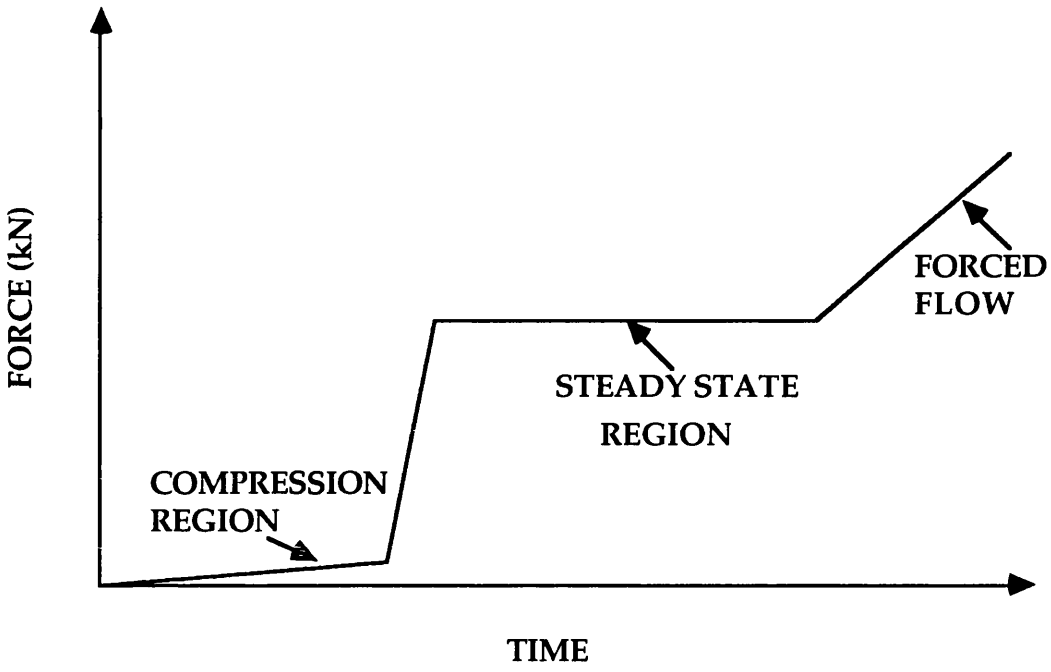


Figure 1.4 Force - Displacement Profile.

The force displacement profile can be altered by altering the die diameter, the L/R ratio or the extrusion rate. The rheological properties of a formulation can be studied by extruding formulations through dies of different die length to radius ratios at different speeds. Bagley, (1957) showed that it is possible to represent the pressure curves required to produce steady-state extrusion through a die as a function of the length to radius ratio of the die. The existence of steady state flow is dependent on the wet powder formulation. The magnitudes of two rheological parameters from the force displacement profiles, the 'upstream pressure loss' and the 'mean wall shear stress' can be related to the quality of the pellets, (Harrison, 1982). A reduction in the 'mean wall shear stress' has been found to eliminate shark-skinning; a roughness of the extrudate

surface. The 'upstream pressure loss' P_o represents the finite pressure loss associated with end effects. The die wall shear stress τ_w is expressed as;

$$\tau_w = \frac{(P_T - P_o)\, R}{2L} \qquad (1.1)$$

where P_T is the total pressure on the piston, P_o is the upstream pressure loss, R is the radius of the die hole and L is the length of the die hole. The Bagley plot, which is a graph of the ram pressure exerted on the material against the L/R ratio of various dies will yield τ_w as half the slope of the line and P_o as the y intercept. Bagley also described an end correction factor (n_b) to compensate for pressure loss upstream of the die entry. Depending on the velocity of the material entering the die the end correction factor will vary. The relationship between the upstream pressure loss and the entrance correction factor is given by;

$$n_b = P_o/2\,\tau_w \qquad (1.2)$$

Increasing the shear rate may increase or decrease the entrance correction factor and increasing the extrusion rate increases the ram pressure obtained for any given L/R ratio of die. Deviations occur when L/R ratios of dies above a value of 16 are used. Harrison et al. (1984) attributes this to a moisture gradient occurring in the die during extrusion.

There is obviously no one type of extruder that is suitable for all types of material and the type of instrument chosen should reflect this. The design modifications of the various instruments render them more suitable for certain materials or formulations. Thus the properties of the formulation and the final objective of the study indicate the most appropriate type of instrument.

Lactose and microcrystalline cellulose are the most widely used excipients employed in extrusion-spheronization technology. Microcrystalline cellulose is known as an excellent excipient for pellet production by either wet granulation or extrusion - spheronization. On addition of the granulating liquid it gives a cohesive plastic mass , i.e. it has good rheological properties and can be extruded to give a uniform product. The production of a sphere of the desirable size and shape depends on the production of a good extrudate. Fielden, (1987), demonstrated significant differences in the flow characteristics and quality of the extrudate formed from mixtures containing equal parts of microcrystalline cellulose and lactose. The ability of microcrystalline cellulose to retain within its structure a large quantity of water (15.4ml of water per 100g solid) was shown in the same study.

1.2.4 SPHERONIZATION

Traditional spheronization processes may be divided into methods which form spheres by individually shaping particles and those which rely on the build up of smaller particles into spheres, utilising surface forces, (Reynolds, 1970). The process of spheronization by pelleting can be divided into three stages; nucleation, transition and ball growth. The material is shaped by utilising the friction and surface forces to form spheres. The spheronizer described by Reynolds consists of a stationary vertical cylinder, open at the top with smooth internal walls and a diameter of 30 -100cm. At the base of the cylinder a rough surface plate rotates and the speed of rotation can vary between 400 - 1600rpm. The product from the extruder is fed onto the revolving spheronizer plate (Conine and Hadley, 1970), either cross-hatch or radial geometry, where it is initially broken down into short lengths, ideally equal to their diameter. As the rolling progresses, the short lengths are shaped into spherical particles. The pellets are impelled under centrifugal forces to the periphery of the plate where their residual momentum causes them to rise up the stationary wall and then to fall as their momentum is dissipated.

The uniformity and regularity of the spheres produced and the time taken for processing is dependent on the plasticity and moisture content of the extrudate, the temperature, the speed of rotation of the plate and the type of plate employed (Chapman, 1985). There is an optimum speed below which there is no densification and only cylindrical shapes are produced, and above this speed densification occurs so fast that large agglomerates or 'golf balls' are produced. With very high speeds and long residence times, small spheroids result. Generally spheres are larger and more spherical with an increase in residence time and spheronizer speed. Formulae with good extrusion characterisics do not necessarily produce good spheres. A product which contains pellets of varying degrees of roundness is probably due to insufficient plasticity. Baert and Remon, (1993) showed that the amount of granulating liquid influences drug release and is correlated to the pellet hardness and density. A slower release rate was observed with increasing amounts of granulating fluid. Rowe, (1985) describes the mechanisms of pellet formation; the extrudate breaks to form cylinders with rounded ends before forming dumbells, ellipses and finally spheres. The forces involved cause densification to occur, usually within the first two minutes. The residence time, speed of rotation of the plate and the geometry of the grooves on the plate were all found to affect the process.

The influence of spheronizer load on the size distribution and quality of the granules was studied by Barrau et al. (1993). It was discovered that although increasing the spheronizer load reduced the sphericity of the granules slightly, it increased the yield of granules in the majority size range and produced harder and smoother granules. Therefore the spheronizer load can influence the porosity and quality of the pellets, due to the increase in intergranular contact and thus pressure as the load increases. Malinowski and Smith, (1975) evaluated the effects of water level, extruder speed, screen size, spheronizer speed and dwell time on the pellets using a factorial design and found that water level and spheronizer speed had significant effects on the granules formed. Baert et al. (1993) studied the parameters important in the spheronisation process, including the water content, spheronizer speed, and spheronization time. The

quality of the spheres was evaluated by the roundness of the pellets and the yield. The greater the amount of water used or the lower the spheronizer speed, the higher the yield of spheres. A longer spheronization time yielded rounder spheres. The temperature and relative humidity were not considered.

1.3 RHEOLOGY

1.3.1 INTRODUCTION

Rheology is the study of the deformation and flow of matter (Ferry, 1980), and rheological measurements are commonly used to characterize liquid and semi-solid systems. The parameters used to describe Newtonian and non-Newtonian materials (viscosity, elasticity, thixotrophy, yield values, apparent viscosity) are essential for theoretical considerations of the influence of variables on flow properties. Deformation of a body can generally be divided into reversible deformation or elasticity and irreversible deformation called flow. Elastic deformation is a function of stress whereas the rate of deformation for flow is a function of shear. Many different instruments and techniques have been used to measure the rheological properties of materials.

1.3.2 CONTINUOUS SHEAR

Information about the rheological properties of a pharmaceutical semi-solid may be obtained by;

(1) Subjecting the material to continuous shear at variable shear rates or shear stress, and obtaining flow curves which indicate the relationship of shear rate to shear stress between suitable limits for that material.

(2) Examination of the material in its rheological ground state (Sherman, 1970). Creep testing is in this category. Minimal internal stresses and strains are present in the test so no organised structures

such as floccules in emulsions or networks in gels are disrupted. Structures are flexed but not broken. At the end of the experiment the material exists in its original state; the test is esentially non-destructive in nature. The results of such investigations may be interpreted on the basis of linear viscoelastic theory. Analysis of the data to obtain physical and rheological parameters will be considered later.

In continuous shear testing the material is sheared isothermally in a narrow annulus between vertical co-axial cylinders or a cone and plate in a simple shearing motion and the end-effects are relatively small. In mixing and forming operations the rheological properties are of primary importance and must be held within established limits for successful processing.

1.3.2.1 ROTATIONAL VISCOMETRY

Different types of instruments have been used to measure the viscosity of materials (Ferry, 1980, and Van Wazer et al. 1963). The first instruments devised by Couette in 1890, measuring viscosity, used stress driven (gravity) flow, many of which are still used, for example, flow cups, U - tubes and capillary tubes. In capillary viscometers the liquid is forced through a fine bore tube and the viscosity is determined by measuring the volume, flow rate, the applied pressure and knowing the dimensions of the tube. Rotational viscometers, which may be of concentric cylinder, cone and plate, rotating disc or conicylindrical geometries are widely used to obtain flow curves. The general theory is that a rotating body immersed in a liquid experiences a viscous drag or retarding force and this is a function of the speed of rotation of the body. These instruments are used as continuous measurements can be made for extended periods of time and time - dependency effects may also be measured. This is not possible with capillary type instruments.

In rotational viscometry, the material under test is enclosed between two parts of the viscometer that rotate relative to each other about a common axis of symmetry. As one member rotates, the test

sample transmits a torque to the second member, which then tends to be moved also. The relationship between this torque and the relative angular velocity is used to characterize the flow properties of the experimental material. As only one of the cylinders is rotated the thermostatic control of the apparatus is greatly simplified.

The concentric cylinder apparatus has the advantage of involving a nearly constant shearing stress. For a Newtonian material if the rate of shear across the gap is not constant, the results will not be reliable. The test sample is set in the annular space between the concentric cylinders and a known torque is applied to one of the cylinders. The cylinder containing the sample is rotated, and it is free to rotate against a restoring torque which increases in proportion to the rotation.

Rotational viscometry is a versatile technique to measure the flow properties of a material. The shear stress and shear rate should be expressed at the same point in the viscometer or corrections apply. The temperature increases during shear, and turbulence and end - effects can influence the flow behaviour. Many different types of commercial viscometers are available (Ferry 1980), some of the more common ones being the Stormer viscometer (the shear stress is held constant), the Merrill - Brookfield (useful for high shear rates), and the MacMichael (for low friction).

1.3.2.2 CONE AND PLATE VISCOMETER

Rotational viscometry is usually used to measure the properties of fluids in flows which approximate a simple shearing motion. The flow may be contained between the cone and plate. In the cone and plate viscometer, the cone rotates at a constant angular velocity while the plate remains fixed. In the parallel plate geometry, the upper plate rotates at a constant angular velocity while the bottom plate is fixed. The cone and plate viscometer is used to measure the shear viscosity and the normal stress difference. These measurements require great care in minimising or elimimating pressure errors. The assumptions of this method are that the angular motion has persisted

for long enough to eliminate transient effects associated with the start up of the flow, that the cone angle is small and that there is negligible fluid inertia or edge-effects. The fluid is held in the gap between the plates by surface tension and hence there will be little or no end - effects. The sample size required is small, and when the angle is <4° the edge effects are minimised, but at high shear rates the temperature of the sample may rise. As the gap between the cone and plate is small it may not be suitable for studying the flow properties of highly structured materials.

The Carri-Med CSL used throughout this work, differs from conventional instruments in a number of ways. Firstly, samples are examined in one continuous process without the need to interrupt the process to fit different values of torque, as the stress is applied electronically. Secondly, as the stress is applied and released at will, the actual behaviour of the sample in the region of non-destructive deformation can be measured. Essentially the main difference is that shear stress replaces shear rate as the controlled variable. Deformation is measured in the region of elastic or viscoelastic flow, often below an apparent yield point, if one is present. The controlled stress technique can give a more complete analysis of the particular material being examined.

Variable shear rate viscometers are widely used as it is easier to provide a constant shear rate and measure the shear stress than vice - versa. When shear stress is the independent variable, yield values, i.e. the minimum shear stress to initiate flow may be directly measured. Materials which have a complex structure or viscoelastic properties can be studied using very low shear stresses. The variable shear rate instruments may have either concentric cylinder or cone and plate geometries. The Stormer viscometer (Van Wazer et al. 1963) and the air turbine viscometer (Davis et al. 1968) are two examples. In the Stormer viscometer, the torque is applied by use of strings, pulleys and weights. The instrument is not designed to measure fast time-dependent phenomena accurately. The period of shearing is also limited by the travel of the weight.

A concentric cylinder viscometer in which an air turbine is used to centre, support and apply a constant stress to the inner cylinder has been constructed by Davis et al. (1968) and other workers. This instrument may be used for continuous shear or creep work.

From a discussion of the various types of instruments used and the technique of continuous shear, it can be seen that there are limitations to both the technique and the different instruments used for the rheological measurement of materials. Correction factors applied may often only be approximations and no single instrument is universal in its application to pharmaceutical materials. Comparison of results between different instruments may not be valid.

1.3.3 THE RHEOLOGICAL MODELS

A number of different models have been developed to aid with the identification and analysis of the sample undergoing continuous shear. The resultant graph of shear stress against shear rate which may or may not have a yield value, can then be fitted to the mathematical models shown below (Figure 1.5) and the regression coefficient and standard error of the fit calculated. The mathematical model may represent a theoretical concept with regard to the structure of the material and gives an expression for the non- linear relationship between shear stress and shear rate in some materials.

The Newtonian model is the simplest model, a straight line through the origin, where the shear stress is equal to the shear rate multiplied by the viscosity. In Newtonian flow the shear viscosity does not vary with shear rate, and the only stress generated is the shear stress, σ, the two normal stress differences being zero.

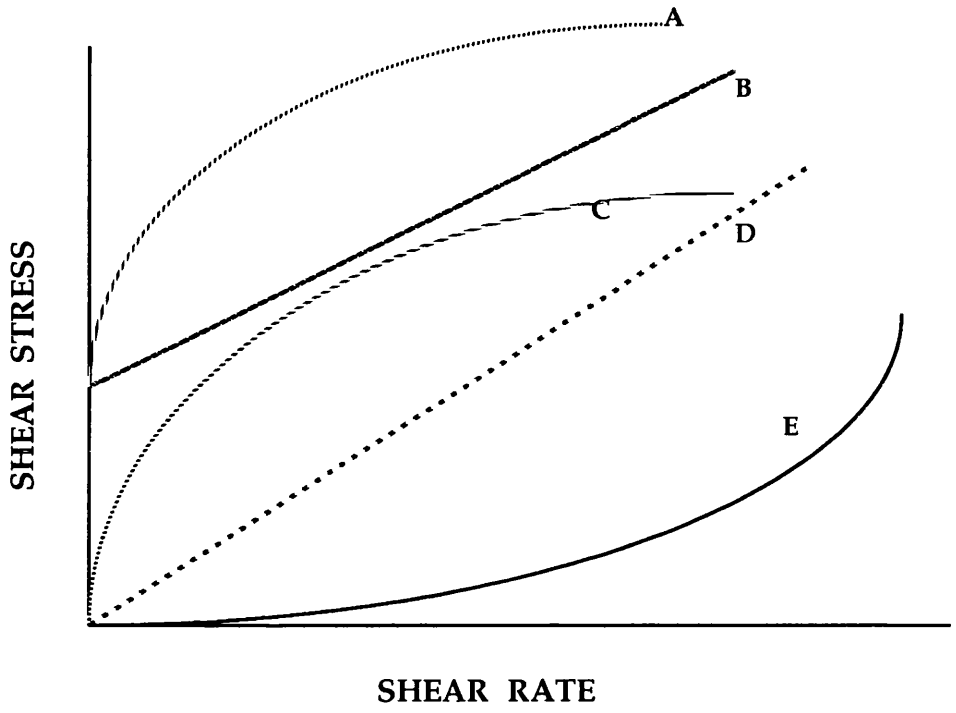


Figure 1.5 The Rheological Models.

A = Hershel - Bulkley Model
B = Bingham Model
C = Power Law Model (Pseudoplastic)
D = Newtonian Model
E = Power Law Model (Dilatant)

The Pseudoplastic model (shear thinning) is determined by taking the logarithm of shear stress and shear rate and fits the best straight line to the data. The slope of the logarithmic data gives the power of the equation and the regression coefficient gives an

indication of the degree of deviation from Newtonian flow. The regression coefficient is not an indication of the goodness of fit but simply states whether the parameters are related.

Dilatant flow (shear thickening) analysis is similar to pseudoplasic except that the power law index is greater than one.

The Bingham model has a yield stress value below which the material will not flow, and the shear stress is equal to the yield stress plus the Bingham plastic viscosity multiplied by the shear rate. The equation usually fits a straight line and the regression coefficient indicates how linear the data is, and the yield value gives the best fit for the curve as a whole. The yield value obtained from this model is an arbitrary value required to fit the data.

The Hershel - Bulkley model is the most versatile as it incorporates three variables. The direct measured yield value is given, the stress that produces the first non-zero shear rate data point. Initially the dynamic yield value is calculated from fitting the best straight line to the first 5% of the data points and extrapolating back to the stress axis. This value is then subtracted from each subsequent stress value. The logarithm of these data points are then fitted to the best straight line and the slope and intercept of this line will give the power index and the viscosity coefficient of the curve.

1.3.4 ELASTICITY

An ideal elastic body is defined (Ferry, 1980) as a material that deforms reversibly and for which the strain is proportional to the stress, with recovery to the original volume and shape occuring immediately on release of the stress (Hooke's Law). The ratio of stress to strain is called the elastic modulus. Four constants corresponding to the common types of deformation are employed;

1. Young's Modulus Y (change in normal stress divided by the relative change in length), which describes the distortion of the materil under simple tension.

2. Shear Modulus G (change in tangential stress divided by the change in the resulting angle of extension).
3. Bulk Modulus K (change in hydrostatic pressure divided by the resulting relative change in volume).
4. Poisson's Ratio μ (lateral contraction divided by extension in simple tension).

The ideal theory of elasticity gives a good approximation of the rheological behaviour under small deformations of reasonably hard, rigid materials but does not describe the behaviour of semisolids. Many of the newer polymers exhibit response characteristics outside the scope of the theories of elasticity and viscosity and hence the theory of viscoelasticity developed.

1.3.5 VISCOELASTICITY

A wide number of materials exhibit both elastic and viscous rheological properties and are termed viscoelastic. Linear viscoelastic behaviour applies in cases where viscous effects obey Newton's Law and elastic effects are Hookean, and is limited to materials undergoing small strains.

A Newtonian viscous fluid responds to a suddenly applied state of uniform shear stress by a steady flow process. In other materials however, this induces an instantaneous deformation followed by a flow process which may or may not be limited in magnitude with time. A material which responds in this manner is said to exhibit both an instantaneous elasticity effect and creep characteristics, and combines features of both theories. The prominence of viscoelastic behaviour in polymers is associated with the versatility of movement of flexible threadlike macromolecules. The frictional force involved in sliding a layer of polymeric material along a hard surface is related to the viscoelastic properties of the material. Relaxation and creep tests provide the simplest and most direct means of obtaining the mechanical properties involved in the linear theory of viscoelasticity. Stress is assumed to be distributed

uniformly throughout the material and dynamic effects may be encountered in obtaining data at short times. One of the characteristics of a linear viscoelastic material (Ferry, 1980), which is illustrated by the Voigt and Maxwell models is that it obeys the Boltzmann superposition principle.

1.3.6 THE MAXWELL MODEL

This model is used to represent a material, which when stretched can respond elastically, but which also exhibits viscous flow. The model (Figure 1.6) consists of a purely elastic spring with a shear modulus G in series with a dashpot containing a purely viscous liquid of viscosity η. Under an external force, the stress in the spring equals that in the dashpot. The total strain is equal to the sum of the strain in the spring and in the dashpot.

$$Y = Y_1 + Y_2 \qquad (1.3)$$

where Y = total strain, Y_1 = elastic strain, Y_2 = viscous strain. When a shear stress, σ is applied the spring extends instantaneously and thus according to Hooke's Law,

$$Y_1 = \sigma / G \text{ (shear modulus)} = J\sigma \qquad (1.4)$$

where $J = 1/G$ = elastic compliance.
According to Newton's Law;

$$Y_2 = \sigma/\eta \qquad (1.5)$$

where $Y_2 = d.Y_2/dt$ (i.e. shear rate)

Therefore,

$$Yt = Y_1(t) + Y_2(t)$$

$$= \sigma(t)/G + \sigma(t)/\eta \qquad (1.6)$$

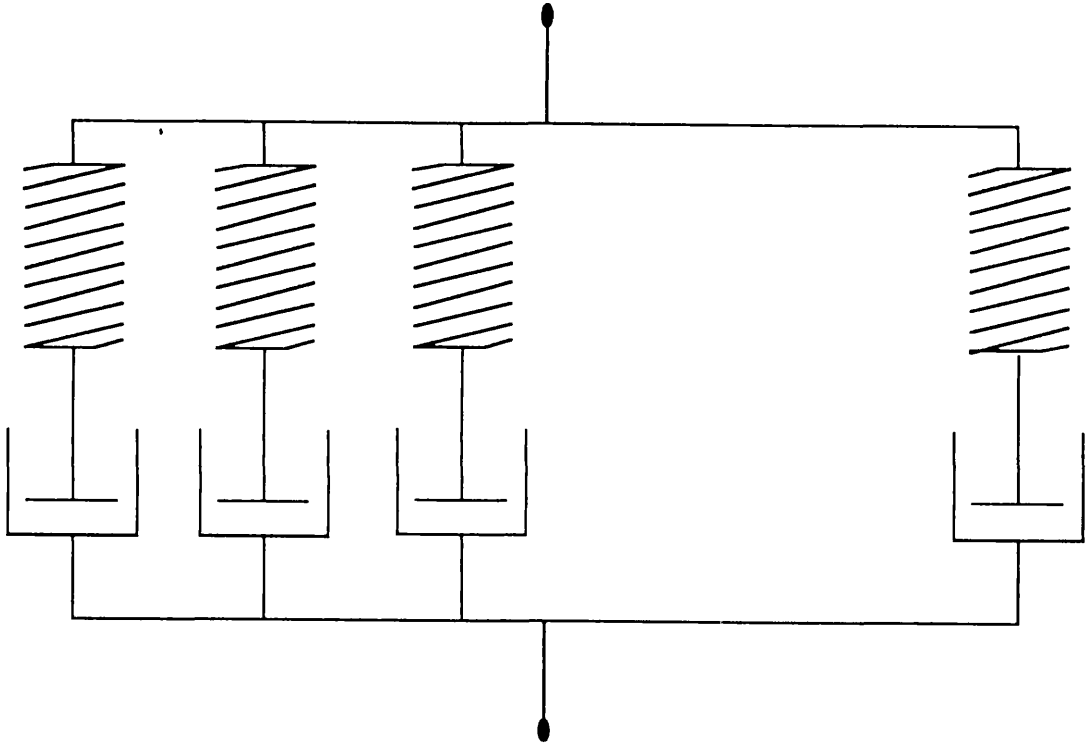


Figure 1.6 THE MAXWELL MODELS IN PARALLEL.

For a constant shear stress, $\sigma(t) = 0$, the Maxwell model reduces to a Newtonian dashpot. The ratio of shear viscosity to shear modulus has the dimensions of time and is called the relaxation time γ,

$$\gamma = \eta/G = \eta J \tag{1.7}$$

The Maxwell model is particularly useful for representing stress relaxation phenomena, in which a body is constrained at constant deformation, and the stress required to maintain this deformation diminishes or relaxes with time. This corresponds in terms of the model to the gradual release of the spring by the motion of the dashpot. As it is unlikely that a real material would be represented by a single Maxwell element, the model may be generalised to consist of a finite number of parallel Maxwell elements (discrete spectra of relaxation times) or an infinite number of elements (continuous spectra of relaxation times).

1.3.7 THE VOIGT MODEL

The Voigt model (Figure 1.7) has been used to chacterize the behaviour of many materials in creep analysis. A spring and a dashpot in parallel represent a material whose response to an external force is not instantaneous, but is retarded by a viscous resistance. Under an external force, the strain in the spring equals that in the dashpot, Y, and the total stress σ, is the sum of the stress in the spring, σ_1 and in the dashpot σ_2.

$$\sigma = \sigma_1 + \sigma_2 \tag{1.8}$$

By applying the laws of Hooke and Newton, an equation relating stress and strain as functions of time is obtained.

$$\sigma(t) = GY(t) + \eta\, Y(t) \tag{1.9}$$

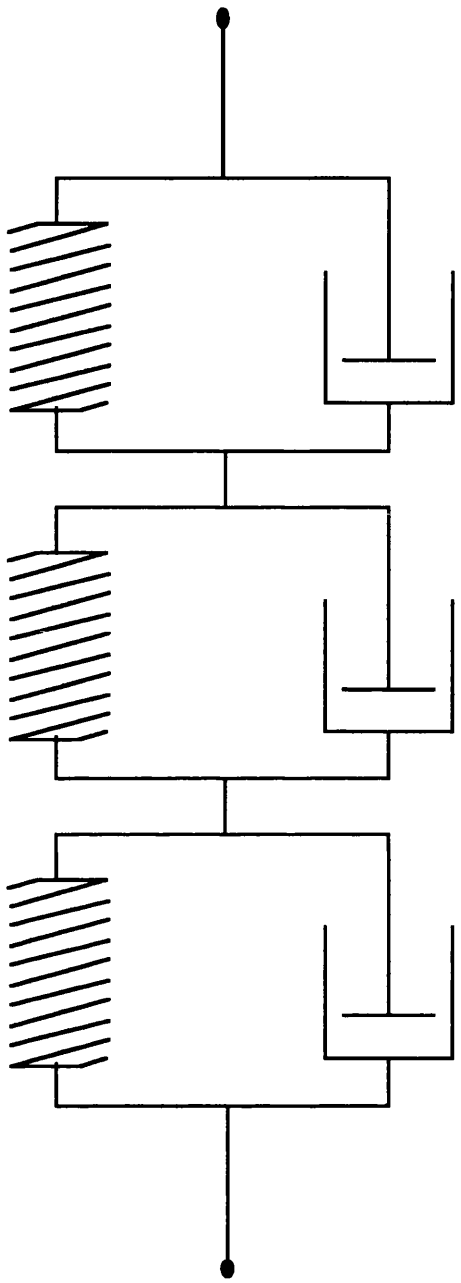


Figure 1.7 THE VOIGT MODELS IN SERIES.

For a constant strain, Y(t) = 0, the Voigt model reduces to an uncoupled spring. The quantity $\eta/G = \gamma$ has the dimension of time as for the Maxwell model and is designated the retardation time. It is the time required for the strain in the spring to relax to 1/e of its initial value, when the stress is removed.

It can be noted that the retardation time will be short, i.e. the strain recovers quickly, if η is small compared to the rigidity modulus (G) η characterizes the internal friction of a system. If the value of η is high, the internal friction will retard greatly the strain recovery and the retardation time will be increased. The viscoelastic behaviour of materials is usually represented by more than one retardation time, by the generalised Voigt model consisting of a set of Voigt models in series. If a constant shear stress is applied to this model, then the total strain, Y(t) is a function of time and is the sum of the strains in each constituent element. It is assumed that no two elements in the generalised model possess the same retardation time, since such elements can be replaced by a single equivalent Voigt element.

If one of the Voigt elements in a series is ascribed a zero modulus, the element degenerates to a simple dashpot which undergoes viscous flow. A Voigt model containing such an element corresponds to a viscoelastic liquid. Another form of degenerate Voigt element is that with a zero viscosity (an unretarded spring). The inclusion of such an element in a Voigt model corresponds to a certain amount of instantaneous elastic behaviour in the material.

1.3.8 LINEARITY AND THE BOLTZMANN SUPERPOSITION PRINCIPLE

One of the characteristics required of a linear viscoelastic material, which is illustrated by the Voigt and Maxwell models is that it obeys the Boltzmann superposition principle (Ferry, 1980).

This may be summarised by briefly stating that if a material is subjected to a series of known stresses, the net effect on the strain is

the sum of the effects of each of the individual stresses. Van Wazer et al. (1963) expressed this in a more formal way;

"The value of a characteristic function of a system is equal to the sum of all the changes induced in the system by the driving functions which have been applied to it throughout its past history".

If a material in a particular experiment fails to obey the Boltzmann principle, then the material is outside the field of linear viscoelastic theory.

1.3.9 CREEP TESTING

In creep testing the sample experiences a sudden change in the shear stress imposed with the resulting time-dependent shear rate being measured. It is valuable both theoretically and practically to consider a system in the rheological ground state, that is without the method of testing significantly altering the structure. This enables fundamental parameters like elasticity and viscosity to be estimated.

Most of the instruments used in creep work operate on a similar principle. The sample is loaded between two cylinders or plates of a thermostatically controlled viscometer. The material is then left to rest for a suitable length of time to allow all stresses in the material to relax. A torque is then applied to the inner cylinder or one plate and the resulting small movement is recorded by a device like a displacement transducer, the output from which is then fed to a recorder. Instantaneous delivery of torque to the cone or cylinder may be achieved by the use of an electromagnetic release mechanism.

Warburton and Barry, (1968) used a modification of the Weissenberg Rheogoniometer for creep work in which a chemical balance was used to apply a torque. When the balance arm was raised, a force equivalent to the mass in the pan was delivered to the inner cylinder. The resulting movement was measured using a displacement transducer which was attached to the cylinder by means

of a 10cm arm. Barry, (1974) describes a similar instrument which has an air turbine to centre, support and drive the inner cylinder. The constant stress rheometer was also used by Davis et al. (1968) for both continuous shear and creep work.

The parallel plate system employed in the more modern instruments, (such as the Carri-Med CSL used for this study), provides a convenient measuring system for stiff materials, and the gap can be adjusted to overcome any clogging problems and to increase the strain sensitivity of the instrument. The Carri-Med CSL has a wide shear rate and stress operating range which makes it very suitable for creep measurement.

1.3.9.1 ANALYSIS OF THE CREEP CURVE

In creep measurement the sample is treated as if it were an elastic solid. The stress is applied and the deformation that results is recorded. The ratio of the stress to the shear rate is the Newtonian viscosity, if the sample is a Newtonian fluid. If the sample is not Newtonian, but incorporates a viscoelastic structure then the deformation of the structure is characteristic and can be deduced from the curve of strain against time. Creep analysis must be carried out in the linear region, and the material should be in the completely relaxed state before any stress is applied. In order to apply the theory of linear viscoelasticity to the creep test, the strain response must be proportional to the applied stress, i.e., strain/stress = compliance (J), and is a constant. The creep compliance, J (t) is defined by;

$$J(t) = \frac{Y(t)}{\sigma} \qquad (1.10)$$

where Y (t) is the shear strain after time t, and σ = shear stress. A plot of J (t) versus time as shown in Figure 1.8 is known as a creep compliance curve. The same curve is produced regardless of the magnitude of the applied stress, provided the test is run in the linear viscoelastic region.

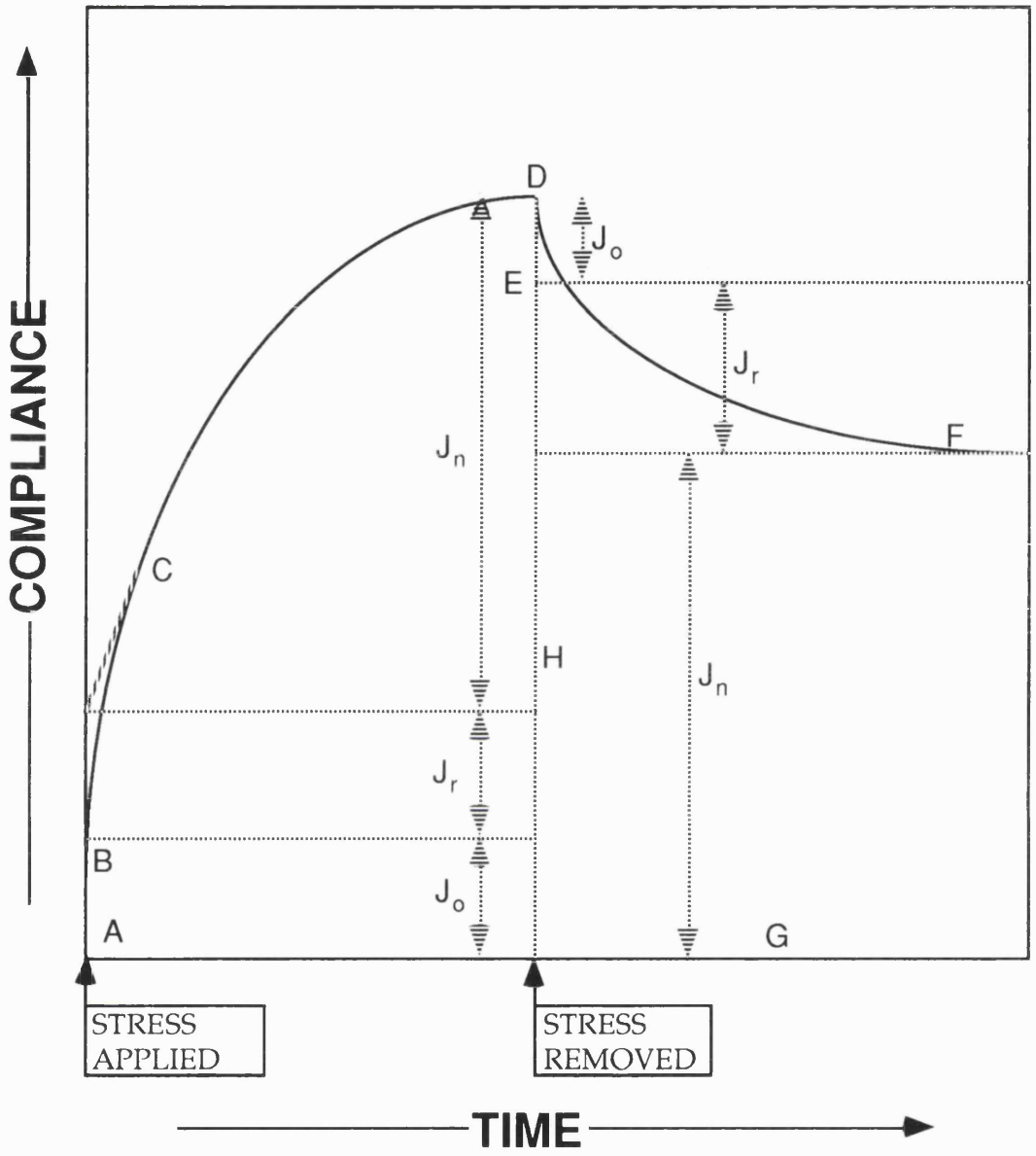


Figure 1.8 Typical Creep Compliance and Recovery Curve for a Viscoelastic Material (Sherman 1970).

(1) A - B is the region of instantaneous compliance in which bonds between the primary structure units stretch elastically.

$$J_o = \frac{1}{G_o} = \frac{Yo\,(t)}{\sigma} \qquad (1.11)$$

where G_o = instantaneous elastic modulus, Y_o (t) = instantaneous strain at time zero, σ is the applied shear stress.

(2) B - C is the time dependent elastic region with a compliance J_R . The slope of the linear part of the curve gives the inverse of the retardation time. The intercept of the extrapolated linear region on the axis gives J_1, the creep compliance.

(3) C - D is the linear region of Newtonian compliance, J_N.

(4) D - E is the region of instantaneous elastic recovery as is of the same magnitude as A - B.

(5) E - F is the region of retarded elastic recovery which is equivalent to B - C of the up curve.

As bonds were irreversibly broken in the C - D region of the curve, this part of the structure is not recovered and is represented by F - G.

The analysis of the creep curve fron the Carri-Med CSL divides the analysis into an examination of the retardation and relaxation times. The structure of a sample can be described in terms of springs to represent elastic behaviour and dashpots to denote viscous, flowing behaviour. The structural information obtained by this technique can be directly related to the bonds and mechanisms which operate in the test sample. When the stress is initially applied to the sample there is an immediate deformation due to the undamped elastic behaviour and the sample is described in terms of the number of viscoelastic Voigt units fitted. The rate at and the extent to which an individual Voigt unit moves depends on the

damping effect of the dashpot and the strength of the spring, i.e. its rigidity modulus. The ratio of these is called the retardation time. Once equilibrium is reached, initially by the spring and dashpot with the shortest retardation time, movement ceases. Voigt units with longer retardation times continue to move until finally all movement ceases. Therefore the Voigt units reach equilibrium in ascending order of retardation times and as the analysis proceeds the movement or compliance of each Voigt unit is overlaid by the compliance of the Voigt unit with the shorter retardation time. The computer data analysis characterizes the curve by measuring the initial compliance at the bottom of the curve, the viscosity at the top, and then fitting a number of exponential expressions, as the behaviour of a single Voigt unit is exponential.

1.3.10 OSCILLATION RHEOMETRY

Conventional oscillation tests involve the application of either free or forced oscillatory strains in tensile and shear geometries. A wider frequency range is available with forced oscillations. The Carri-Med CSL instrument where the test material is contained between parallel plates as used in this work, is of the forced oscillation type. The basic principle of oscillation for a viscoelastic material is based on Newton's Law for an ideal viscous fluid and Hooke's Law for a perfect elastic solid. Newton's Law states that ;

$$\text{Stress} = \text{viscosity} \times \text{shear rate}$$

and Hooke's Law states;

$$\text{Stress} = \text{elasticity} \times \text{strain}$$

The stress is applied in a sinusoidal fashion and since stress is proportional to strain from Hooke's Law this implies that a sinusoidal stress wave applied to an ideal elastic body will generate a sinusoidal strain wave completely in phase. For a Newtonian material if the sinusoidal stress wave is applied then the resulting

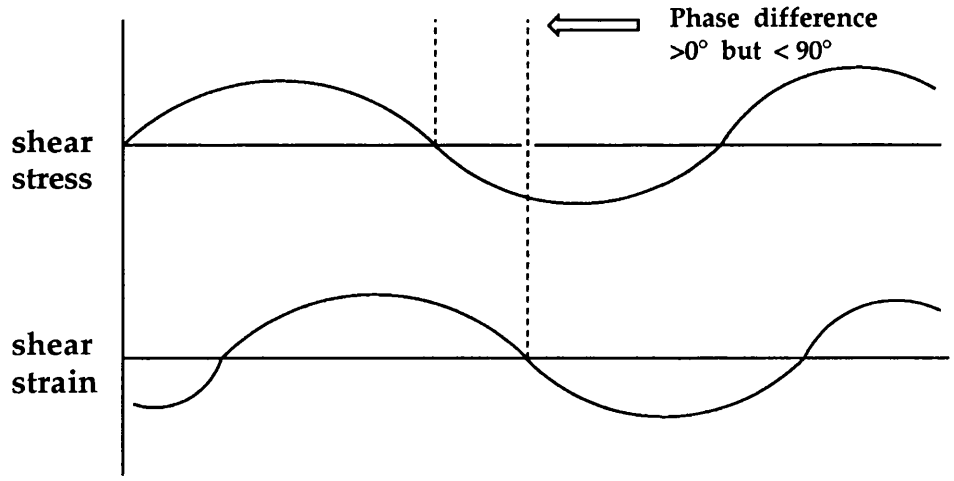


Figure 1.9 Phase Difference for a Viscoelastic Material.

strain wave will be 90° out of phase. A viscoelastic material will therefore show a phase difference (Figure 1.9) between 0 - 90°.

The amplitude of the displacement wave and the phase difference between it and the imput torque wave can be calculated from the data obtained. These are then converted to stress and strain data and from this the viscosity and elasticity of the sample at the selected frequency can be determined. Oscillatory measurements assume that the viscosity and modulus are constant with respect to stress or strain. Most materials are only constant at relatively small strains and hence low stresses. In the linear region, strain is proportional to stress and G' is constant. When a high stress is applied eventually the linear relationship breaks down and the value of G' will plummet. It is conventional to present results of oscillatory tests in terms of the dynamic viscosity, storage modulus and loss modulus and these and other common terms are defined below (Ferry, 1980);

The storage modulus - G' is defined as the stress in phase with the strain in a sinusoidal shear deformation divided by the strain, i.e. it is a measure of the energy stored and recovered per cycle, when different systems are compared at the same strain amplitude.

The loss modulus - G'' is defined as the stress 90° out of phase with the strain divided by the strain, i.e. it is a measure of the energy dissipated or lost as heat per cycle of sinusoidal deformation, when different systems are compared at the same strain amplitude. At a high frequency as G'' approaches zero, this implies perfect elastic behaviour. At low frequencies, G'' for a viscoelastic material should be directly proportional to the angular velocity.

The dynamic viscosity - η' is related to the loss modulus G'' by $\eta' = G''/\omega$, where ω is the angular velocity.

The complex modulus G* is a measure of the ratio of stress amplitude to strain.

The storage compliance J' is a measure of the energy stored and recovered per cycle when different systems are compared at the same stress amplitude.

The loss compliance J'' is a measure of the energy dissipated or lost as heat per cycle of sinusoidal deformation, when different systems are compared at the same stress amplitude.

The loss tangent tan δ is dimensionless and conveys no physical magnitude but is a measure of the ratio of energy lost to energy stored in a cyclic deformation. At low frequencies tan δ is large for uncrosslinked polymers.

$$\tan \delta = G''/G' = J''/J' \qquad (1.12)$$

Although linear viscoelastic properties can be measured in many ways, the small amplitude oscillatory shear test is one of the most widely used ones. No predominant test method has emerged

for non linear behaviour. The Storage and Loss Modulus give an indication of the elasticity and viscosity of the sample. At high frequencies of oscillation, the elastic nature of the material will predominate, while at low frequencies the viscous nature will predominate, as the sample is given more time to flow. The phase angle δ, when approaching zero implies the test material is behaving as an elastic solid. If the phase angle is large, approaching 90° then the sample is behaving as a viscous fluid. Thus the phase angle measurement shows whether a material is behaving as an elastic or a viscous body or as a combination of the two parameters.

CHAPTER 2

MATERIALS & METHODS

2.1 MATERIALS

2.1.1 CHITOSAN

Chitosan used throughout this work was supplied by Protan Biopolymers under the product name Seacure 252. The batch number is 019-7XX-32C . Product specifications of that batch as supplied by Protan, are shown in Table 2.1. The material is stored in a cool dry place.

Appearance	Off-White Powder
Viscosity	170mPas
Loss on Drying	8.1%
Particle Size	90% through 0.23mm
Deacetylation	78%
Insolubles	<1%
Ash Content	<1%
pH	7.2
Arsenic	<3ppm
Lead	<10ppm
Total Viable Count	<1000 in 1 gram
E. Coli	negative in 1 gram
Salmonella	negative in 1 gram
Yeast and Mould	<200 in 1 gram

Table 2.1 Product Specifications of Chitosan (Protan).

2.1.2 MICROCRYSTALLINE CELLULOSE

Avicel PH101 MCC, (Lot 6015, FMC Corporation USA) is a purified, partially depolymerised alpha cellulose derived from fibrous plants. Avicel PH101 was used throughout this work. It is a white, odourless, tasteless, relatively free-flowing powder which is virtually free from organic and inorganic contaminants. It is insoluble in water, in dilute acids, and in most organic solvents. It has a low

moisture content (typically < 5%), and a particle size of ~ 50 μm, according to the manufacturers (FMC) specifications.

2.1.3 LACTOSE

Lactose NF (hydrous), Sheffield Products, Norwich NY , (BN ON A22 -37), is a disaccharide material obtained from the whey of milk. It is a white/ off-white odourless crystalline powder, soluble 1:6 in water.

2.1.4 DICLOFENAC SODIUM

Diclofenac sodium (Heumann Pharma Gmbh, Nurnberg Germany, BN91-03329) is an important analgesic and non-steroidal anti-inflammatory, with anti-pyretic properties. Its mode of action is via the inhibition of prostaglandin synthesis. The drug is used primarily in the treatment of rheumatic disorders eg., rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis in a dose of 75 - 150mg daily. Diclofenac is associated with gastro-intestinal side effects, and rapidly eliminated from the plasma after dosing, which implies that the development of extended release dosage forms may be beneficial in patients. Its pKa value is 3.8 and it has a solubility of 1.5×10^{-5} M at pH 2 at 25°C.

2.1.5 BARIUM SULPHATE

Barium sulphate XR -H6 (BN 7727-43-7), Sachtleben Chemie Gmbh.

2.1.6 ACIDS USED IN THE STUDY

ACETIC ACID

BDH Analar grade (Batch 7016930N)- a clear colourless liquid with a pungent odour, miscible with water, alcohol and glycerol. The pKa = 4.75 at 25°C.

ASCORBIC ACID BP - Takeda Chemicals Japan (Batch HBP486), pKa = 4.1, 11.79 at 25°C.

CITRIC ACID - BDH Analar grade (Batch K17352736), pKa = 3.1, 4.7, 6.4 at 25°C.

TARTARIC ACID - BDH Analar grade (Batch 212A63863), pKa = 2.98, 4.24 at 25°C.

MALEIC ACID - BDH Analar grade (Batch 327ZA24563), pKa = 1.9, 6.33 at 25°C.

HYDROCHLORIC ACID - BDH Analar grade (Batch 3197050L), pKa = -6.1 at 25°C.

LACTIC ACID - BDH Analar grade (Batch K18874344), pKa = 3.85 at 25°C.

2.1.7 ELECTROLYTES

SODIUM CHLORIDE - BDH Analar (Batch K16866633)

POTASSIUM CHLORIDE - BDH Analar (Batch 6093240M)

MAGNESIUM CHLORIDE - BDH Analar (Batch 149A594833)

POTASSIUM HYDROGEN CARBONATE - BDH Analar (Batch 204A602519)

2.1.8 SODIUM HYDROXIDE (pearl) - BDH Analar (Batch 6444270N)

2.1.9 WATER - Deionised water was used throughout the study.

2.2 METHODS

2.2.1 PREPARATION OF THE MIXTURES FOR PROCESSING

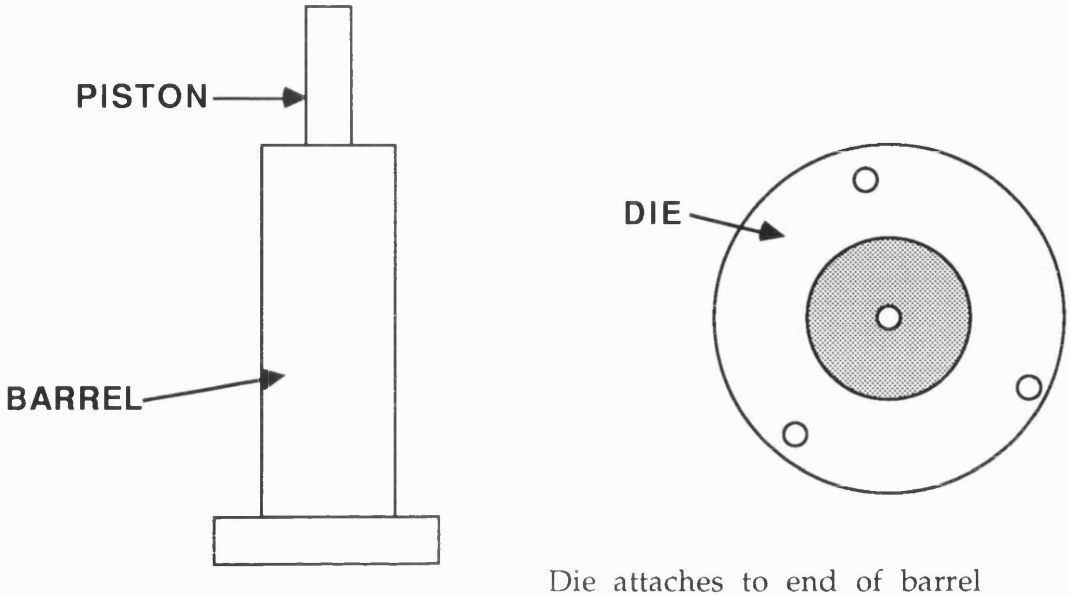
Various formulations were prepared in a planetary mixer (Kenwood Chef) in which the mixing paddle is set off centre and is carried on a rotating arm. In general, the previously weighed powders were mixed initially for a period of 10 minutes at the lowest setting, subsequently the liquid was added and mixing continued for a further 5 minutes. In other formulations, the chitosan content was incorporated as a gel into the powder mixes and excess water then added to form a sufficiently plastic mix to extrude.

A small clearance between the vessel wall and the paddle necessitates 'scraping down' several times to ensure a uniform mix. The wet mass was then left to stand overnight to allow water equilibration or extruded immediately, depending on the formulation.

2.2.2 RAM EXTRUSION

The uniformly mixed formulation was packed into the extruder barrel to a constant volume, and then fitted to the physical testing instrument (LLoyds MX50). The hardened steel barrel has an internal diameter of 25.4mm and a length of 180mm. The lower end of the barrel allows the connection of various sized dies by means of three screws to a flange. A rubber 'O' - ring seal is placed in the groove between the barrel and die to prevent water escaping and provide a low friction seal. A schematic diagram of the barrel and die is shown overleaf in Figure 2.1.

Hand pressure was applied via the piston to compress the mass in the barrel with the requisite sized die attached; 1 x 4 mm or 2 x 2 mm most commonly throughout this study. The filled barrel was then placed on the C - piece and aligned underneath the piston. The crosshead of the LLoyds MX50 with a 50kN load cell, is then lowered until the piston just comes in contact with the material in the barrel. This is taken to be zero on the displacement transducer. The



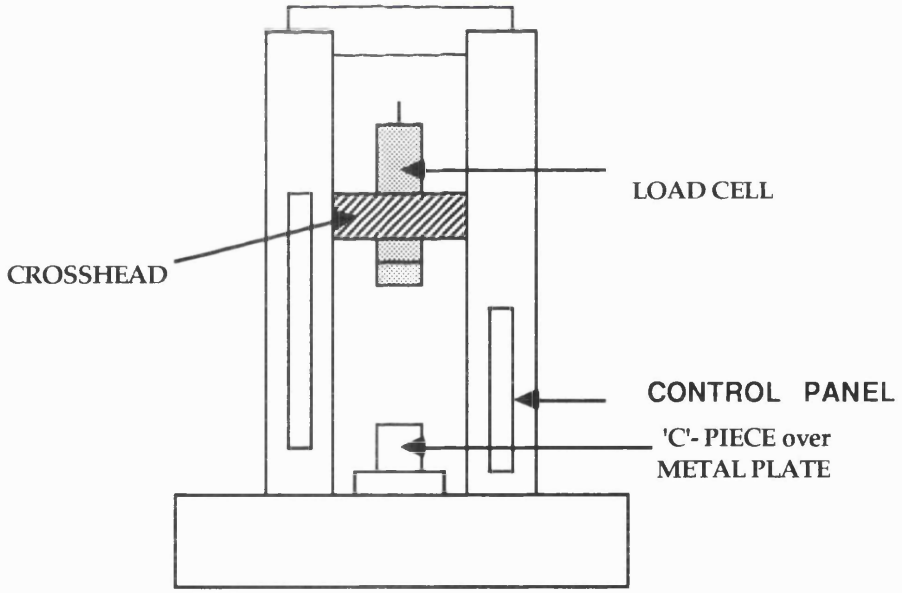


Figure 2.1 Diagram of the Barrel and Die and the LLoyds MX50.

crosshead then moves at a preselected speed and initially the material in the barrel is compressed and then extrudes. The extrudate is collected in a plastic bag in the C - piece and the force applied is recorded on the computer screen which displays the compression force vs time profile. The crosshead is stopped in the steady state region. The extrudate (~ 200g) is then transferred to the spheroniser.

2.2.3 SPHERONIZATION

The spheronizer used throughout this work is a Caleva (K.A. Greeves, Ascot Berks., UK) and the plate used has a radial cut geometry. Approximately 200g of extrudate was placed on the radially grooved plate (22.5cm diameter) and rotated at 1000rpm for a set time interval. Extrudate for each formulation was spheronised immediately the required quantity had been extruded. A residence time of 10 or 15 minutes was used depending on the quality of the extrudate and its ability to form spheroids.

2.2.4 DRYING

The resulting product was then collected, weighed and left to dry overnight at 50°C to a constant weight in an open air oven and the percentage weight loss on drying was noted. The spheres were then stored in polythene bags and labelled.

2.2.5 DISSOLUTION

Dissolution tests were undertaken using the USP paddle method (Pharma Test), using 1000ml of phosphate buffer pH 7.4 (USP). Diclofenac sodium has a low solubility at acidic pH values and therefore it is difficult to obtain sink conditions and hence limited dissolution experiments at acidic pH are reported.

Experiments were carried out at a temperature of 37°C, a stirring speed of 100rpm. The pellet test sample size of 0.5g was kept

constant for each formulation studied and none floated on the surface.

Filtered samples were taken automatically (Pharma Test PTFC sample collector) at various time intervals over a 12 - 18 hour period. Samples were then assayed using a UV spectrometer (Perkin Elmer 554) at 275nm. Each experiment was performed in triplicate and the mean values obtained. Percent drug released was calculated from the absorbance values using a standard calibration curve for various concentrations of diclofenac solutions.

2.2.6 ANALYSIS OF THE PELLETS

The resulting product is initially analysed visually, with regard to size and shape of the pellets. The product can range from cylindrical to spherical in shape and the size distribution may be similar or extremely diverse.

2.2.6.1 SIEVE ANALYSIS

The size distribution of the pellets was determined by sieving. A nest of sieves (6 - 8) with a progression based on a /2 change in screen diameter between adjacent apertures was used and, the pellets (100g) loaded onto the top sieve. A nest of 5 - 8 sieves in standard progression is then placed on the sieve shaker (Endecotts, London), and vibrated for a time period of 15 minutes. Passage through a sieve requires that the particle has a cross-sectional area which is less than that of the aperture and is measured on a weight basis. From the amount remaining on each sieve, the size distribution of the pellets was determined.

2.2.6.2 DENSITY MEASUREMENT

The density of the pellets was measured using an air comparison Beckmann pycnometer (Model 930). This measures the volume of solids rapidly and precisely. The pycnometer consists of

two chambers assumed to be equal in volume when the connecting valve is closed, and the same pressure is maintained in each. The true volume of the sample (i.e. the volume enclosed by its outer surface and excluding its outer pores) is thereby measured. The mass of pellets is weighed and the volume of the sample determined from the pycnometer and hence the density calculated.

2.2.6.3 SCANNING ELECTRON MICROSCOPY

Samples of pellets, either whole or halved, were mounted onto an aluminium stub and then coated with a fine layer of gold (4 min. at 13 mAmp) using a sputter coater (Emitech K550). Cross sections were obtained by cutting the pellets with a razor blade. The prepared samples were then viewed under a Phillips XL20 (Phillips Analytical UK) scanning electron microscope at an accelerating voltage of 10 - 15kV. The size and surface morphology of the pellets can then be characterized from the micrographs obtained.

2.2.7 DIFFERENTIAL SCANNING CALORIMETRY

The Differential Scanning Calorimeter 7 (Perkin Elmer) was used for the calorimetric measurement, characterisation, and analysis of the thermal properties of both chitosan and diclofenac sodium in this study. The instrument is programmed to scan a temperature range by changing at a linear rate over one to eight temperature ramps, for the study of the endothermic and exothermic reactions. The energy absorbed or evolved by the sample is compensated for by adding or subtracting an equivalent amount of electrical energy to a heater located in the sample holder. The continuous and automatic adjustment of heater power is necessary to keep the sample holder temperature identical to that of the reference sample and this provides a varying electrical signal equivalent to the varying thermal behaviour of the sample.

The sample holder is composed of two low mass platinum - iridium sample cells embedded in a large aluminium heat sink. A

rubber ring seal is used to seal the furnaces from the external atmosphere. Vented platinum lids are used to cover the sample and reference.

Dry powder samples (~6mg) of both diclofenac sodium and chitosan were examined by differential scanning calorimetry. The samples were run at a rate of 20°C/min. over a temperature range of 40 - 380C, both individually and together. Table 2.2 shows the three samples tested; diclofenac powder and chitosan as supplied and a sample of chitosan and diclofenac powder mixed in a turbuler mixer. The table shows the peaks associated with each, the height and area of the peak. Chitosan shows two separate peaks, a broad peak at 109.9 °C and a more defined one at 297°C. Diclofenac shows a sharp peak at 298°C and both powders mixed show peaks at 286.1°C and 98.3°C. A graph of heat flow vs temperature is shown in Figure 2.2 for the sample of mixed powders. The results show that there does not appear to be any significant interaction between the two powders as the peaks of the individual components are evident. Diclofenac is a relatively pure sample with narrow peaks and the chitosan sample has broader peaks as would be expected of a natural polymer.

SAMPLE	CHITOSAN	DICLOFENAC	CHIT/DICLOF.
WEIGHT	6.318mg	6.336MG	6.075mg
X1	232.0 C 56.33 C	217.2 C	183.6 C 63.3 C
X2	315.6 C 164.6 C	301.8 C	290.3 C 151.6 C
PEAK	297.0 C 109.9 C	298.42 C	286.1 C 98.3 C
AREA	786.4mJ 1005.4mJ	1060.268 mJ	1094mJ 237.2mJ
LH	124.5J/g 159.1J/g	167.34 J/g	180.1J/g 39.0J/g
HEIGHT	9.62mW 6.42mW	32.9 mW	23.5mW 1.9mW
ONSET	271.7 C 71.6 C	289.8 C	278.1 C 72.3 C

Table 2.2 Thermal Analysis Results.

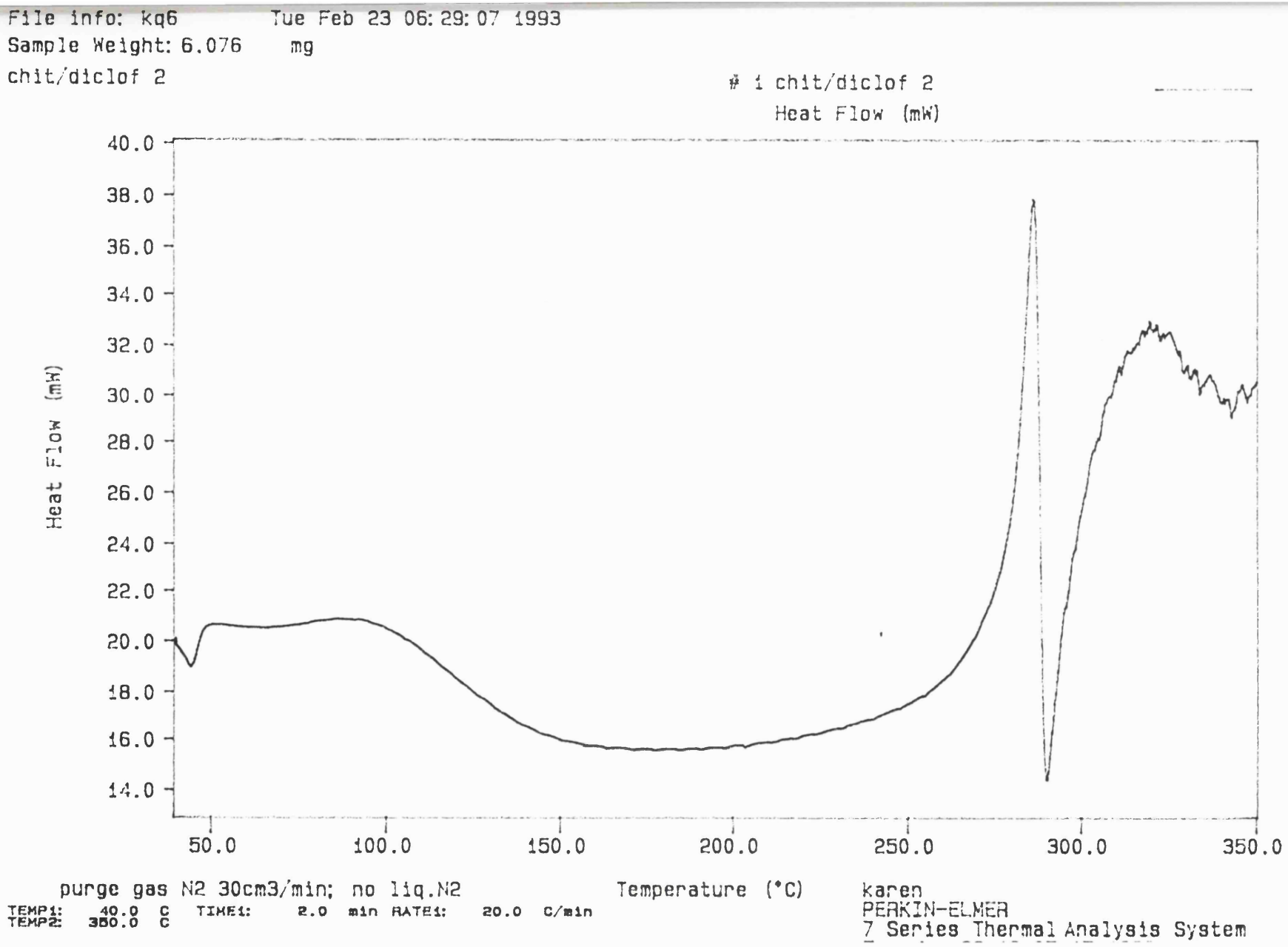


Figure 2.2 Thermograms of Mixed Diclofenac Sodium and Chitosan Powders.

2.2.8 PARTICLE SIZE DETERMINATION

Particle size control may be exerted for a number of reasons; to improve solubility, bioavailability or to ensure a more homogenous mix of powders. Various methods were employed for the chitosan powder as detailed below.

2.2.8.1 HAMMER MILL

The hammer mill induces size reduction by impact of the particles of chitosan. The four hammers are hinged on a central shaft and enclosed within an outer case. The powder then passes through a mesh of a selected size to be collected. This method did not produce any appreciable reduction in the size of the chitosan particles.

2.2.8.2 BALL MILL

A ball mill is an example of a comminution method which produces size reduction by impact and attrition of particles. The ball mill is mounted on a horizontal rotating axis at a set speed , and the cyclinder contains balls of various sizes which occupy 30-50% of the total volume. The larger balls tend to break down the coarse material and the smaller balls help to form the fine product and decrease the void spaces. A sample volume of 1kg of chitosan was subjected to this treatment for eight hours and the resulting particle size measured using the Laser particle sizer.

2.2.8.3 MALVERN PARTICLE SIZER

The Malvern Laser Particle Sizer (Model 2600C, Malvern Instruments, UK) is based on the principle of laser light scattering. The optical measurement unit comprises an optical transmitter, a receiver and an optical bench, which are optically aligned and calibrated. A low power visible laser transmitter produces a parallel monochromatic beam of light which illuminates the particles and is

diffracted by them to give a stationary diffraction pattern regardless of particle movement. Therefore by integration over a time period, and using a continuous flux of particles through the illuminated area, a representative bulk sample of the particles contributes to the final measured diffraction pattern.

Once the diffraction pattern is measured the computer uses a non-linear least squares analysis program to find the size distribution which gives the most closely fitting diffraction pattern. This method of analysis has the advantage that sufficient data is collected to perform statistically significant calculations and the bulk of the sample is adequately represented. Particles are measured suspended in a suitable medium (water for chitosan), which does not interact with the sample. The particles are easily dispersed in the medium and no aggregation was visible.

SIZE MICRONS	% UNDERSIZE	SIZE BAND	% IN BAND
564.0	100		
261.7	68.4	564.0-261.7	31.6
160.4	27.5	261.7-160.4	40.9
112.8	19.5	160.4-112.8	8.0
84.3	10.5	112.8-84.3	9.0
64.6	7.2	84.3-64.6	3.3
50.2	5.8	64.6-50.2	1.4
39.0	3.6	50.2-39.0	2.2
30.3	2.7	39.0-30.3	0.9
23.7	2.0	30.3-23.7	0.7
18.5	1.5	23.7-18.5	0.5
14.5	1.0	18.5-14.5	0.5
11.4	0.7	14.5-11.4	0.3
9.0	0.5	11.4-9.0	0.2
7.2	0.4	9.0-7.2	0.2
5.8	0.1	7.2-5.8	0.3

Table 2.3 Particle Size Distribution of Chitosan Sample using the Malvern Lasersizer.

The results for the sample of chitosan tested are shown below in Table 2.3. The experiment was carried out using a concentration of 0.04% w/v chitosan at a beam length of 14.3mm. The specific surface area was shown to be 0.0551sq.m./cc. The printout obtained from the instrument also gives the 10% and 90% particle undersize values as 81.1μm and 277.3μm respectively.

2.2.9 SWELLING INDEX

The swelling index is the volume in ml occupied by 1g of a material after it has swollen in an aqueous liquid for 4 hours. This test was adapted from the British Pharmacopeia (1993) for the swelling of the chitosan gels in various acids. A quantity of chitosan (1g) is placed in a 25ml ground - glass stoppered cylinder graduated in 0.5ml divisions. The chitosan is then dissolved in 1.6M concentration of each of the acids to 25ml in the stoppered cylinder. The mixture is shaken vigorously every 10 minutes for 1 hour and then allowed to stand for 3 hours. The final volume occupied by the chitosan gel is then noted. The swelling index is calculated from the mean of three tests.

2.2.10 RHEOLOGY MEASUREMENTS

The interaction of chitosan with different solvent systems gives rise to changes in solution viscosity. The formation of a gel results due to an interaction with an acid and a rheological measure of this bond is required. The response of the system to different stresses and strains is measured using a cone and plate rheometer. The behaviour of a plug of material from various extrusion formulations of chitosan may also be determined, via oscillation rheometry.

2.2.10.1 CONE AND PLATE VISCOMETRY

The Carri-Med CSL 500 Rheometer was used to study the deformation and flow properties of the various chitosan gel

formulations. The instrument is composed of (1) a microprocessor controlled induction motor drive coupled with (2) a minimum friction low inertia air bearing and (3) a high resolution digital optical displacement encoder. The shear stress can be applied and released at will, therefore the behaviour of the sample in the region of non-destructive deformation can be measured, which is not normally possible with constant shear rate instruments. Samples may be examined in one continuous process throughout the complete rheological spectrum as the stress is applied electronically and hence the dynamic range is not limited by mechanical factors. It is suitable for creep, oscillation and flow measurements, and has a maximum torque of 50,000 microNm. A diagram of the apparatus is shown in Figure 2.3.

2.2.10.2 FLOW VISCOMETRY

Samples were prepared by dissolving the required concentration of chitosan in the molar strength of the solvent in the test series, to form a gel. The gel under test is placed on the plate of the apparatus and allowed to equilibrate at the preset temperature (20°C) and the base plate then raised. Small samples are used to avoid edge effects. The deformation of the sample on the application of different stresses is shown as a graph of shear stress vs shear rate. The flow curve analysis program allows the fitting of various mathematical models to the data obtained and arrives at the best fit of the data to the equations. The quality of the fit is indicated by the regression coefficient and by the standard error of the fit. Automatic analysis consists of an analysis of the up and down curve and the peak hold data and also gives an indication of the degree of thixtrophy in the sample.

2.2.10.3 OSCILLATION RHEOMETRY

A parallel plate system is used to measure the dynamic viscoelastic behaviour of plugs of material. This involves the application of a harmonically varying strain at various frequency

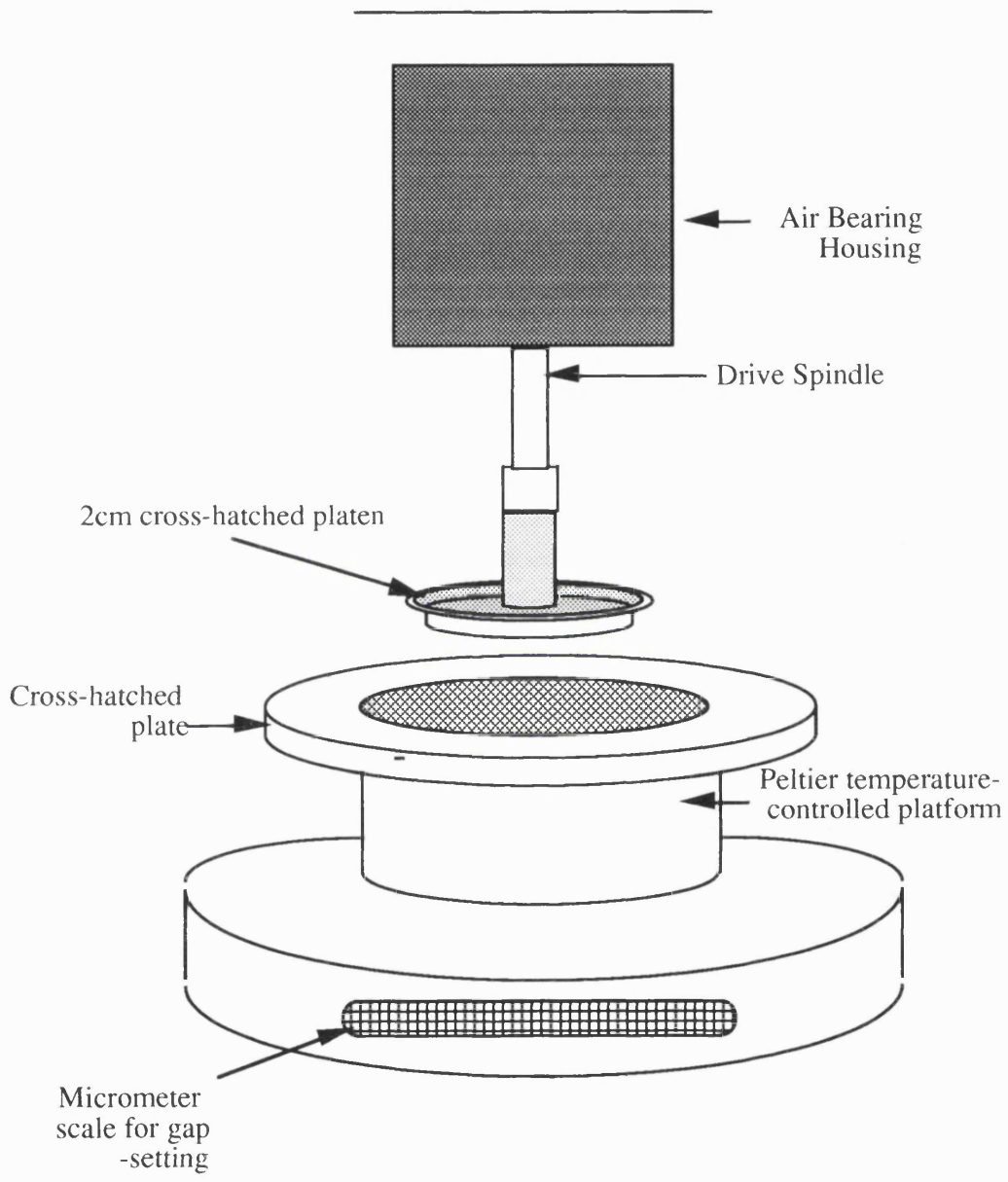


Figure 2.3 The Carri-Med CSL 500.

values. Plugs are formed by mixing the powders (with the chitosan dissolved in the different acids to form gels) in the same formulations as for the extrusion batches and this plastic mass is then extruded against a blank die to form plugs of less than 10mm in height. If the sample is too thick the stress wave will not reach the bottom of the sample.

Oscillatory measurement of viscoelastic properties is based on the assumption that the material's behaviour is linear and this is usually only correct for relatively small strains and hence low stresses. Therefore, in order to increase the strain sensitivity of the instrument when used in the oscillation mode the gap is adjusted to be as wide as possible. The gap is set to zero initially and then adjusted for the height of the plug minus 0.7mm, the depth of immersion found to be most suitable by other workers on this instrument (MacRitchie, 1993), for these types of plugs.

The cross hatch plate is employed as the most suitable for a material which is prone to slip due to the production of a surface film of water. The plug of test material is contained between the parallel plates and subjected to a frequency sweep between 0.1 - 10 Hz. A fresh plug of material is employed for each test and the temperature is kept constant at 20°C. Each formulation is also subjected to a torque sweep between 10 - 5000 microNm at a frequency of 1 Hz. A manual measurement is then conducted at a frequency of 1Hz and a value of torque for which the modulus and viscosity are constant for the test plug. Each test is then repeated on a plug of the same material. The moment of inertia for the parallel plate system was 4.34 microNm for stainless steel which has a low coefficient of thermal expansion and is compatible with most test materials.

The computer generates a digital sine wave from the torque and frequency values selected and the rheometer then applies a corresponding stress wave to the sample. Normally the computer will take the rheometer through five cycles of oscillation and provided that a stability criterion is met during the last few cycles the last cycle will be stored and used for analysis. The amplitude of

the measured displacement wave and the phase difference between it and the imput torque wave is then calculated. These are then converted to stress and strain data and from the amplitude ratio of the input stress wave and the measured strain wave and their phase difference, the viscosity and the elasticity of the sample can be deduced at the selected frequency. Two sinusoidal wave forms result from an experiment and are characterised by their amplitude and wave difference. Initially, one needs to specify the desired frequency and torque amplitude of the imput waveform, also the dimensions and moment of inertia of the measuring geometry being used.

2.2.10.4 CREEP MEASUREMENT

In creep testing the sample experiences a sudden change in the shear stress imposed with the resulting time dependent shear rate being measured. It is valuable as a static method of estimating the viscosity and elasticity of the test material. Seven different gels of chitosan were allowed to equilibrate, subjected to stresses for 2 minutes and allowed to relax for 2 minutes. The gels were prepared in the same manner as those employed in the flow tests and the temperature kept constant (20°C) throughout the experiment. Each sample was allowed to rest for ten minutes once placed on the test plate and the results are the mean of three determinations. In order to apply the theory of linear viscoelasticity to the creep test, the strain response must be proportional to the applied stress, and the creep tests are then performed at a shear stress in this linear region. The gels were subjected to an applied stress of .29840, .59680 and 1.1936 Nm^{-2} (equivalent to a torque of 5, 10 and 20 microNm). Creep tests provide values for the compliance (Jo) under different stresses. The creep curve can then be analysed mathematically by the computer software, to obtain values for viscous and elastic components. The curve is analysed in two parts; the retardation and relaxation components. The analysis technique used in the program attempts to model the data to a theoretical curve made up of three different components which contribute to the total compliance as a function of time;

- An instantaneous compliance Jo, from the initial movement the sample makes once the stress is applied.
- A Newtonian viscosity from the constant shear component of the sample at a large value of time.
- A spectrum of decayed exponential movement of the sample with each Voigt unit having different compliances and relaxation times, made up from a dashpot with a viscosity η_i and a spring with compliance J_i.

The instantaneous compliance is set from the curve intercept on the compliance axis of the graph and the Newtonian viscosity is derived from the slope. A total of at least fifteen data points is required for analysis.

CHAPTER 3

FORMULATION STUDIES

3.1 INTRODUCTION

The objective of this study is to investigate the use of chitosan as a vehicle for a controlled release, non-eroding matrix formulation via extrusion / spheronization techniques, through which the drug is distributed in a non-uniform manner. Chitosan has been shown to control the rate of drug release in a number of different formulations, by a wide number of independent researchers (see Section 1.1.6.1). This study is aimed at producing controlled release matrix pellets containing diclofenac sodium as the model drug. Spherical particles or pellets have the lowest ratio of surface area to volume and can be characterized by their diameter, Mehta, (1986). This promotes a more uniform absorption of drug with reduced mucosal irritation and this renders it especially suitable for delivery of drugs with gastrointestinal side-effects. Diclofenac sodium, used in this study is a non-steroidal anti-inflammatory drug which, when given by the oral administration route can cause gastrointestinal side-effects. In order to minimise these side-effects and to provide an effective blood level over a period of time it is formulated as a sustained release product. Microcrystalline celluloses and lactose are the most commonly used excipients in extrusion - spheronization and the initial formulations consisted of these materials, with chitosan added in varying amounts as a dry powder, in order to investigate the effect of chitosan concentration on drug release rate, in the absence of a solvent system for chitosan.

3.2 EFFECT OF CHITOSAN CONCENTRATION

3.2.1 INITIAL FORMULATIONS

The basic formulation studied initially consisted of;

Chitosan 10 parts
Diclofenac Sodium 2 parts
Avicel PH101 48 parts
Lactose 40 parts

and the amount of water is approximately 1.2 times the amount of Avicel. This is used as a reference point and the moisture content can then be varied depending on the quality of the extrudate and its ability to form a good spheronized product. The powders were dry mixed as described in Section 2.1.1, including the chitosan powder and the quantity of water required to form a suitably plastic mix to extrude was determined by trial and error. No solvent was employed for the chitosan powder. The initial formulations prepared are indicated in Table 3.1; the dry powders expressed as a percentage. The analysis and dissolution profiles of these are presented later.

FORMULATION	CH004	CH100	CH010	CH025
Chitosan	10	-	5	15
Avicel PH101	48	38	51	45
Lactose	40	60	42	38
Diclofenac Sodium	2	2	2	2
Water	248ml	236ml	248ml	254ml

Table 3.1 Composition of the initial Formulations.

The first formulation shown, CH004 contains Avicel, lactose and chitosan and is regarded as the standard. The subsequent formulation, CH100 consisted of removing the chitosan concentration and doubling the lactose concentration to see what effect this would have on the pellets and the release rate of the drug. Another formulation, CH010 halved the chitosan concentration while keeping the rest of the mix constant. The final formulation in this series, CH025 increases the chitosan concentration by half while keeping the rest of the mix constant with respect to the standard one. This should show what effect if any, the chitosan concentration has on the release rate and if chitosan slows the release rate. The resulting plastic mass is packed into the barrel of the ram extruder and

extruded through a 1 x 4 die as described in Section 2.1.2. The resulting extrudate is then spheronized and the pellets dried overnight in an open air oven.

3.2.2 ANALYSIS OF THE PELLETS

The % weight loss on drying was 18.7% for CH004, 37.1% for CH100, 37.3% for CH010, 14.5% for CH025. The % weight loss for CH010, and CH100 was almost twice that of the basic mix. Increasing the chitosan concentration showed the least weight loss on drying and lowering the chitosan concentration or the absence of chitosan from the formulation appears to dramatically increase the amount of moisture lost from the pellets on drying.

The pellets were sieved and the results are presented in Table 3.2 expressed as the cumulative percent oversize.

SIEVE SIZE	CH004	CH100	CH010	CH025
2mm	0	5.2	0	0
1.7mm	0.1	33.6	0	0.1
1.4mm	37.4	45.4	44.7	46.2
1.0mm	60.8	15.7	54.1	51.3
710um	1.6	0	1.2	2.4
Base	0	0	0	0

Table 3.2 Sieve Analysis of the initial Formulations.

The pellets formed were of a smiliar size distribution in general, except for CH100, the formulation without any chitosan, which had a wider size distribution and a larger diameter pellet. The pellet size showed 95 % within two sieve sizes for each formulation except CH100, which is indicative of a fairly uniform size distribution. The pellets formed had a spherical shape.

3.2.3 DISSOLUTION PROFILES

The pellets were then tested in the dissolution apparatus and the percentage of the drug released against time noted. All dissolution experiments were repeated with different batches of the same formulation and the weight of the sample was kept constant. A sample of diclofenac sodium equivalent to the expected amount contained in the sample of pellets tested was placed in the dissolution medium, and this is used as a reference, as 100% drug dissolution is reached within ten minutes. The initial batches formulated were left to stand overnight as the wet mass, to allow water equilibration and extruded on the following day. However, on extruding a batch the same day as it was formulated, no difference in the dissolution profile resulted and so subsequent batches were formulated and extruded on the same day. The dissolution experiments were carried out in phosphate buffer at pH 7.4 using the USP paddle method (Section 2.1.5).

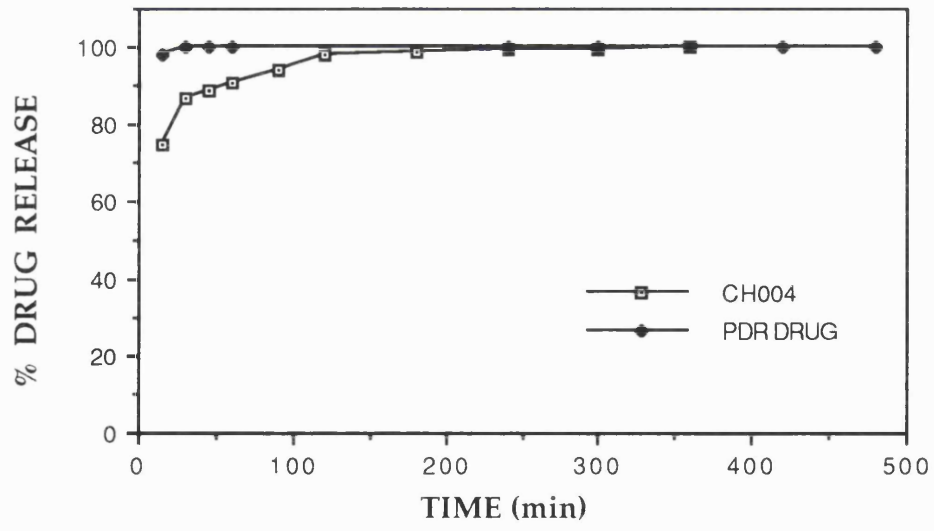


Figure 3.1 Dissolution profile of the standard formulation (CH004) and Powdered Drug; Diclofenac Sodium at pH7.4.

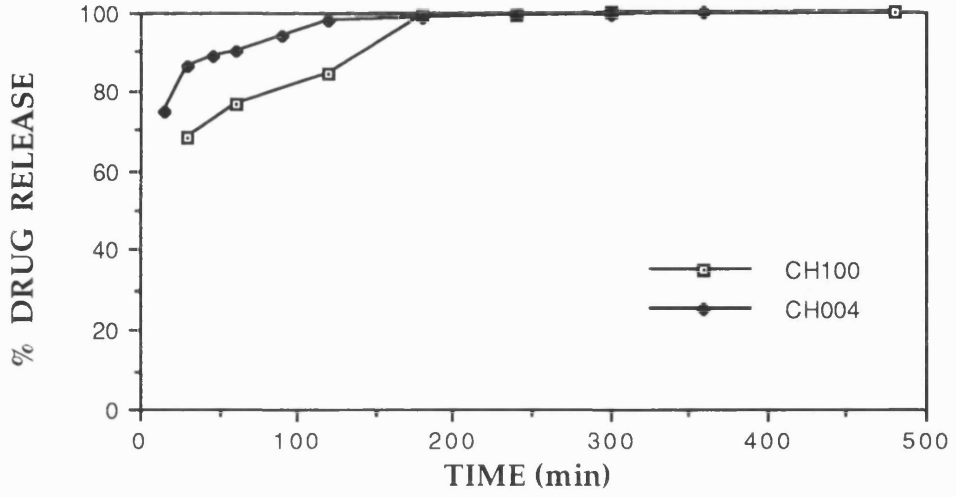


Figure 3.2 Dissolution profiles of CH100 (chitosan replaced by lactose) and the standard formulation (CH004) in phosphate buffer pH7.4.

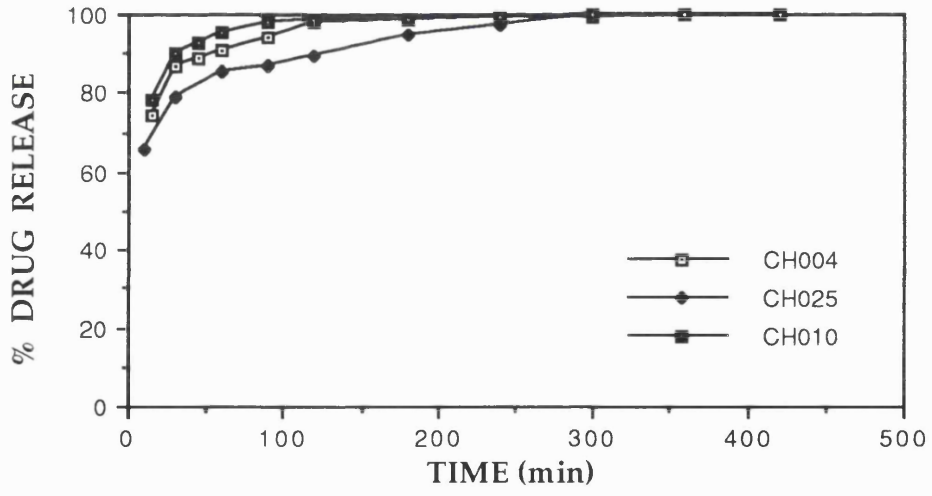


Figure 3.3 Dissolution profiles of formulations containing increasing amounts of Chitosan (CH010, CH004, and CH025 respectively) in phosphate buffer pH7.4.

Figure 3.1 shows the dissolution profile of the standard formulation (CH004), compared to an equivalent amount of powdered drug, diclofenac sodium in the dissolution medium. The powdered drug reaches 100% dissolution within the first ten minutes while the pellet containing chitosan shows a slower release profile, with 90% dissolved within the first hour, it is still a fast releasing preparation.

The formulation CH100 contains no chitosan and the lactose content is doubled while the Avicel content is reduced. This formulation is compared to the standard one (CH004) in Figure 3.2. The dissolution profile shows that the formulation containing chitosan is faster releasing than that which does not contain chitosan. The formulation without any chitosan has a larger pellet size and this may influence the release rate as the drug has a larger surface area to diffuse into the dissolution medium.

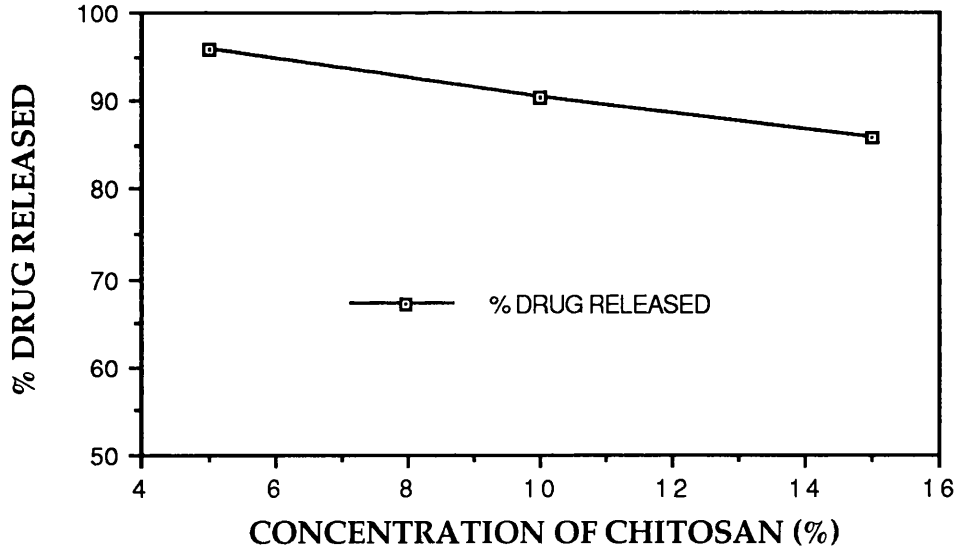


Figure 3.4 The effect of Chitosan concentration in the formulations on the drug release at time, 1hr.

A graph of the dissolution profile of batch CH010 compared with the standard formulation (CH004), and CH025 shows (Figure 3.3) that halving the amount of chitosan (CH010) in the pellets allows the drug to dissolve faster. After 30 min. ~85% of the drug from the standard mix, CH004 has been dissolved, while ~ 90% of drug from batch CH010, and only ~80% from CH025. The effect of chitosan concentration on the release at a set time point (1 hr) is shown in Figure 3.4. Chitosan concentration is shown to effect the release rate; as the concentration of chitosan increases the rate of drug release decreases. By reducing the amount of chitosan, a less stable matrix appears to be formed and the drug is released faster. Thus chitosan concentration appears to affect the rate of drug release.

3.2.4 DISCUSSION

This initial study showed that the concentration of chitosan in the formulation does have a direct effect on the dissolution profile of the drug. On comparing the pellet containing no chitosan with the standard mix, the pellet not containing any chitosan was the slowest releasing. The pellets containing increasing amounts of chitosan showed graded dissolution profiles; with the pellet containing the highest concentration of chitosan showing the slowest rate of drug release. Incorporating chitosan as a dry powder in a pellet of this type therefore, does not appear to be of benefit in slowing the release rate of the drug but there does appear to be a concentration effect with chitosan in the pellets.

3.3 CHITOSAN GEL FORMULATIONS

3.3.1 INTRODUCTION

As was seen from the initial study, incorporating chitosan as a dry powder does not produce a controlled release pellet. Therefore it was decided to incorporate a solvent for chitosan. Chitosan is soluble in a number of dilute acids, including acetic acid. When chitosan is in the presence of a multivalent cation it forms gels. Chitosan at a

concentration of 8% w/w dissolved in 0.1M acetic acid forms a gel and the gel is mixed with the dry powders in the formulations.

3.3.2 FORMULATIONS

The composition of the formulations expressed as a percentage is shown overleaf in Table 3.3. The dry powder mixes are prepared as before and the chitosan is now incorporated as a gel in the mix and further water is added as the granulating fluid to ensure a suitable mix for extrusion. The mixes are extruded through either a 1 x 4, 1.5 x 3 or a 2 x 2 die using the ram extruder (Section 2.1.2) and the resultant extrudate spheronized. The die size used for each formulation is indicated at the bottom of Table 3.3. The pellets were dried overnight in an open air oven at 37°C. Each formulation is extruded through a 1mm and a 2mm die to investigate the effect of pellet size on the release rate. Barium sulphate which is insoluble in water is incorporated into the mixes to investigate if this has an effect on the release rate. The lactose (moderately water soluble) and barium sulphate content are balanced against each other, a reduction in one being compensated for by an increase in the concentration of the other. The amount of drug is kept constant throughout, as is the concentration of Avicel.

3.3.3 ANALYSIS OF THE PELLETS

The formulations shown in Table 3.3 produced spherical pellets when analysed visually. The amount of water added as a granulating liquid is manipulated to give a spherical product with a good steady state region on the force - compression curve. The extrusion force required is small. Table 3.4 gives the density of the pellets, the extrusion forces and the percentage weight loss on drying.

The extrusion forces for the mixes are all low values typically ~ 3kN and the force - compression profiles showed a steady state region over three quarters of their length for all the extrusions. The mixes are required to be sufficiently brittle to spheronize.

FORMULA NUMBER	AVICEL %	LACTOSE %	DRUG %	% BARIUM SULPHATE	CHITOSAN (8 %) GEL%	WATER (g)
BS052**	36	28	2	5	29	40
BS047*	36	28	2	5	29	40
KQ001**	37	32	2	-	29	40
KQ002**	37	-	2	32	29	30
KQ003**	32	24	2	4	38	-
KQ004*	37	32	2	-	29	40
KQ005*	37	-	2	32	29	30
KQ006*	32	24	2	4	38	-
KQ007***	36	28	2	5	29	40
KQ008*	32	-	4	32	32	25
KQ009**	32	-	4	32	32	25

* = 1 x 4 DIE ** = 2 x 2 DIE *** = 1.5 x 3 DIE

TABLE 3.3 LIST OF FORMULATIONS PRESENTED AS PERCENTAGE OF DRY MASS BEFORE ADDING EXCESS WATER.

FORMULATION	% Wt LOSS ON DRYING	EXTRUSION FORCE (kN)	DENSITY (g/cm^3)
KQOO1	30.2	2.7	1.38
KQOO2	23.5	3.1	1.63
KQOO3	29.9	3.0	1.34
KQOO4	14.6	2.7	1.36
KQOO5	24.2	3.1	1.67
KQOO6	24.4	3.7	1.42
KQOO7	19.5	3.8	1.41
KQOO8	25.3	2.1	1.87
KQOO9	23.2	2.9	1.99
BSO52	26.5	3.6	1.46
BSO47	25.7	2.4	1.47

Table 3.4 Density, Extrusion Force and % Weight Loss on Drying for the Pellets.

The sieve analysis is presented in Table 3.5 as the cumulative percent overweight for each of the formulations divided into those extruded through each die size; 1.5 x 3, 1 x 4 and 2 x 2 dies. The formulations show quite narrow size distributions.

3.3.4 DISSOLUTION STUDY

The in - vitro dissolution studies were performed in triplicate according to the USP paddle method in phosphate buffer (pH 7.4), at a rotational speed of 100rpm, as described in Section 2.1.5. The beads all remain intact in the dissolution medium. The dissolution profiles for the formulations are presented below.

SIEVE SIZE	KQ007 - % OVERSIZE
2.8mm	0
2.0mm	69.4
1.7mm	29.4
1.4mm	1.0
1.0mm	0.2
710mm	0
Base	0

1.5 MM DIE

SIEVE SIZE	KQOO1	KQOO2	KQOO3	KQOO9	BSO52
4.0mm	0	0	0	0	0
2.8mm	36.3	36.7	35.0	16.1	33.9
2.0mm	62.6	62.4	64.2	79.7	64.9
1.7mm	0.9	0.4	0.6	2.2	0.8
1.4mm	0.2	0.4	0.2	1.9	0.4
1.0mm	0	0.1	0	0.1	0
710mm	0	0	0	0	0
Base	0	0	0	0	0

2MM DIES

SIEVE SIZE	BS047	KQOO4	KQOO5	KQOO6	KQOO8
2.8mm	0	0	0	0	0
2.0mm	0.6	0.4	0.9	1.6	0.7
1.7mm	5.2	3.1	1.2	2.9	3.6
1.4mm	61.2	64.3	33.6	57.6	66.4
1.0mm	33.1	32.2	62.2	37.9	29.3
710mm	0	0	0	0	0
Base	0	0	0	0	0

1MM DIES

Table 3.5 Sieve Analysis of the Pellets.

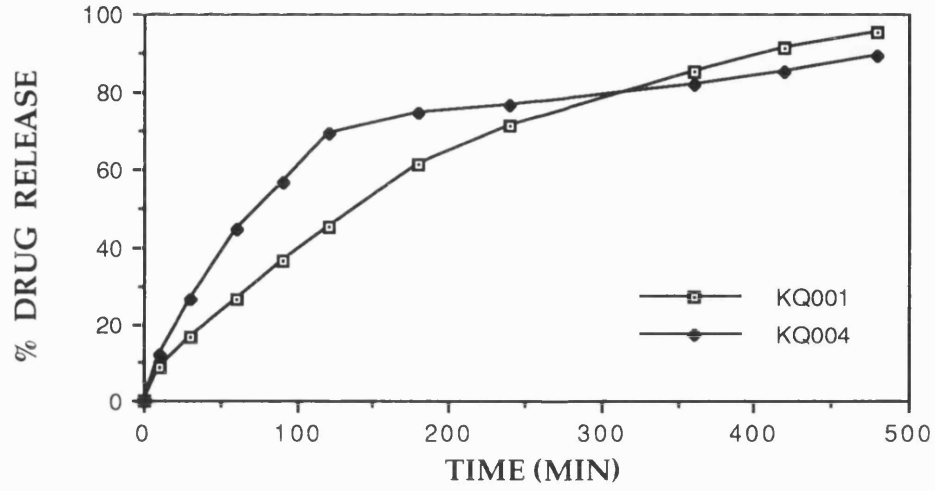


Figure 3.5 Dissolution Profiles of 2mm (KQ001) and 1mm (KQ004) Pellets containing the same concentration of Chitosan, Avicel and Lactose as a percentage of the dry mass.

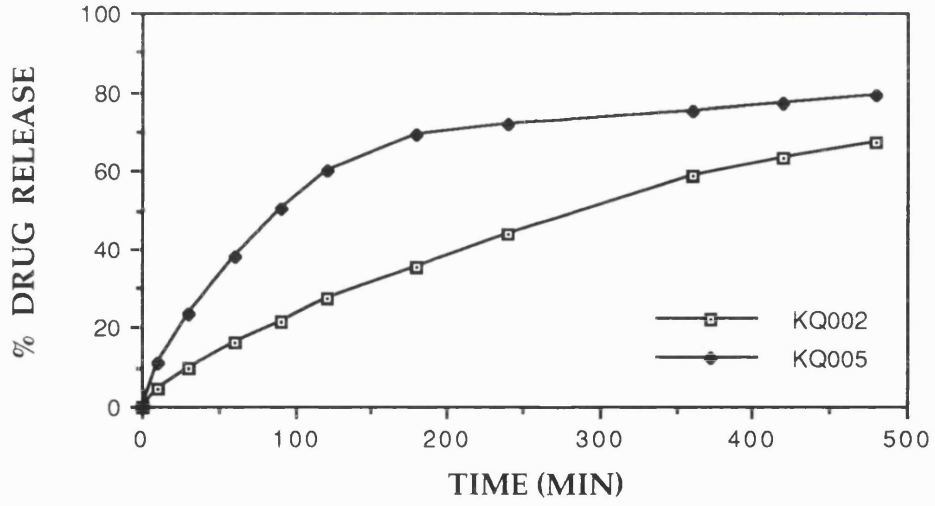


Figure 3.6 Dissolution Profiles of 2mm (KQ002) and 1mm (KQ005) Pellets containing the same Chitosan, Barium Sulphate and Avicel concentration (no Lactose), as a percentage of the dry mass.

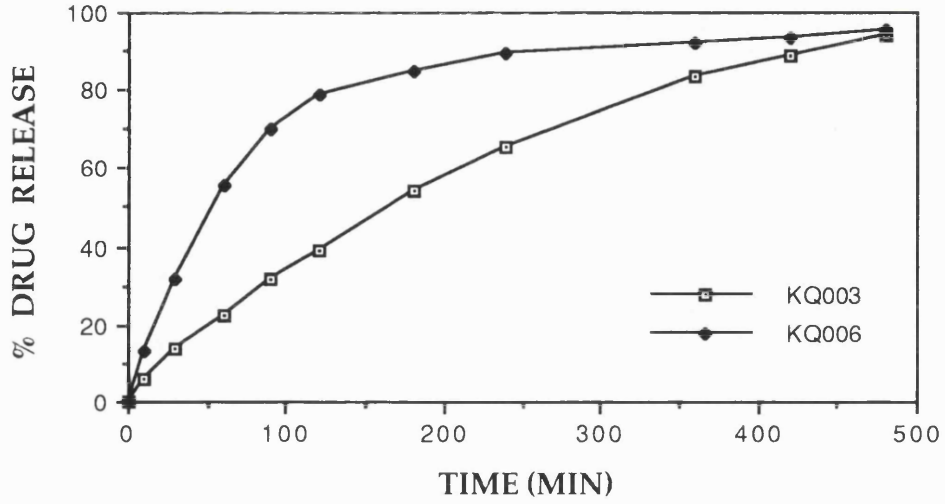


Figure 3.7 Dissolution Profiles of 2mm (KQ003) and 1mm (KQ006) Pellets containing the same Chitosan, Avicel, Lactose and Barium Sulphate concentration, as a percentage of the dry mass, with no extra Water added to the mix.

Figure 3.5 shows the dissolution profiles for KQ001 (2 x 2 die) and KQ004 (1 x 4 die) in which there is no barium sulphate content and the lactose content is 32% of the dry mass. This formulation was extruded through 1mm and 2mm dies in order to compare the release rates for the different sized pellets and this procedure is repeated for each of the formulations. The release rate is considerably faster in both the pellet sizes than that shown for the formulation where barium sulphate replaces the lactose in Figure 3.6. The time for 50% release is shown as ~140 min. - KQ001 (2mm), and ~ 80 min. - KQ004 (1mm). This 2mm pellet formulation shows a slower release profile than the smaller 1.4mm pellet size for the same formulation.

In Figure 3.6 the % drug release from KQ002 (2mm) and KQ005 (1mm) is shown. In this product, the lactose content of the pellets is replaced by barium sulphate entirely and the formulation extruded through a 2 x 2 die and a 1 x 4 die. The concentration of chitosan gel

remains constant. The effect of increasing the barium sulphate content appears to delay the drug release even further. The inclusion of barium sulphate increases the density of the spheres, which remain intact on dissolution. The time to reach 50 % release is ~ 290 min. - KQ002 (2mm) and ~ 90 min. - KQ005 (1mm). The 1 mm pellet size shows a slower levelling off in the release rate after the first 2 hours. This may be due to the porosity of the pellet and the longer time required for the drug to diffuse through the pores in the matrix to the dissolution medium. After 8 hours, 67 % of the drug is released from KQ002, and 79 % from KQ005. The profile again demonstrates the effect, the size of the pellets has on the drug release rate.

Figure 3.7 shows the dissolution profiles for KQ003 and KQ006 in which the chitosan gel content is increased and no extra water is added to the mix. This formulation was sufficiently plastic to extrude and spheronize without adding extra water. The 1mm pellet (KQ006) shows a faster release profile than the 2mm pellet and 50 % release is seen after ~ 170 min. - KQ003 and after ~ 55 min. - KQ006. Compared to the formulation shown in Figure 3.5 increasing the amount of chitosan gel in the formulation does not appear to delay the release properties and the effect is more pronounced in the 2mm pellet size.

Figure 3.8 shows the % drug release vs time profile for KQ008 (1mm) and KQ009 (2mm), in which the lactose content is removed and the drug concentration is doubled. Both the 1mm and the 2mm pellet size show delayed release profiles and 50 % release is seen after ~ 210 min. - KQ008, and after ~ 420 min. - KQ009. The amount of Avicel is reduced slightly in this formulation and the barium sulphate content is increased to equal the Avicel concentration. This formulation produced the slowest release profile. From these results the concentration of barium sulphate in the spheres appears to be critical, and even in drug loading the matrix, delays the drug release rate. No swelling was observed in any of the pellets, which remain intact throughout the dissolution test.

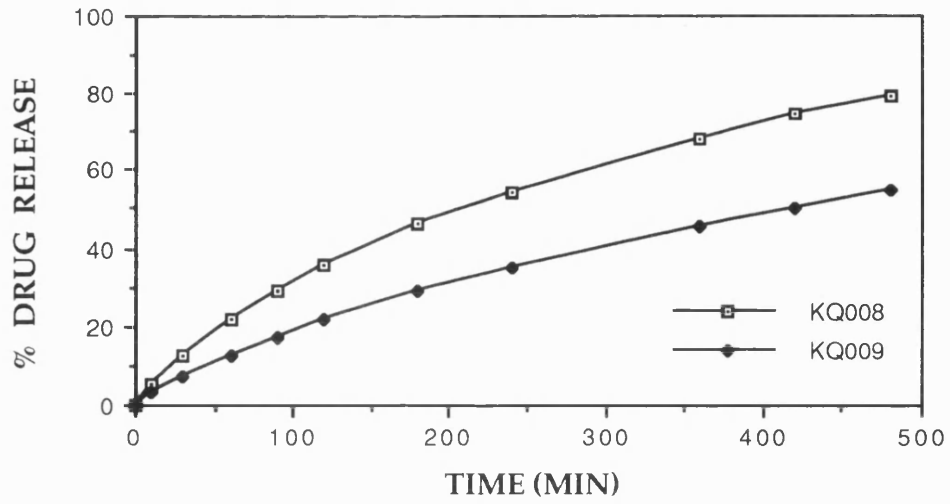


Figure 3.8 Dissolution Profiles of 1mm (KQ008) and 2mm (KQ009) Pellets containing the same concentration of Chitosan, Avicel and Barium Sulphate as a percentage of the dry mass, and the drug concentration is doubled.

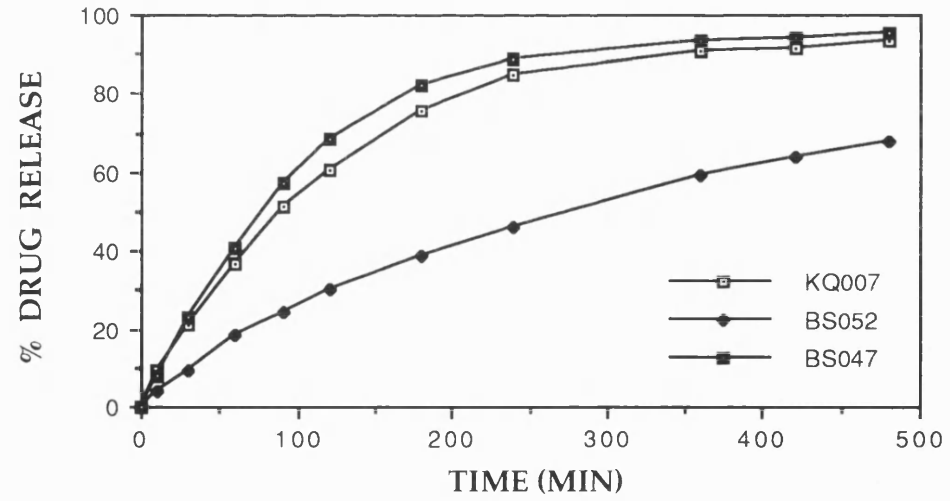


Figure 3.9 Dissolution Profiles of 2mm (BS052), 1.5mm (KQ007) and 1mm (BS047) Pellets containing the same formulation as a percentage of the dry mass.

The graph (Figure 3.9) shows that BS052 gives the slowest release profile, with BS047 giving the fastest release and KQ007 showing an intermediate release rate between these two formulations. Thus it appears that the size of the pellets is a factor in controlling the release rate of the drug from the matrix. All of the pellets remain intact throughout the dissolution process. From these graphs the mechanism of release appears to follow first order kinetics. A release rate of 50% is seen after ~ 280 minutes - BS052, ~ 90 minutes - KQ007, and ~ 80 minutes - BS047.

In order to determine the order of a reaction occuring on dissolution of the drug, various kinetic models can be applied to the data. The plots of the percentage drug released vs time show a curvilinear shape in general. The release does not appear to be zero order. A graph of the log % remaining vs time (first order model) and a linear correlation coefficient is shown. If the plot is linear this implies that the reaction follows first order kinetics. The first order rate constant may then be determined from the slope of the line. From the graphs shown ; KQ001, KQ002, KQ003, KQ008, KQ009, and BS052 all appear to be first order reactions and most of the others are in quite close agreement. The median values for the release over eight hours are calculated from the semi-interquartile range and this calculated value compared with the actual release at the particular time point. Any standard deviation greater than 5% from the calculated value was considered outside the fit of the model. This measurement gives an indication of how close the data fits to the straight line. The correlation coefficient only shows that the parameters are related and does not give any indication of the goodness of fit. The first order model includes most of the 2mm sized pellets while the 1mm pellets do not correlate quite as closely.

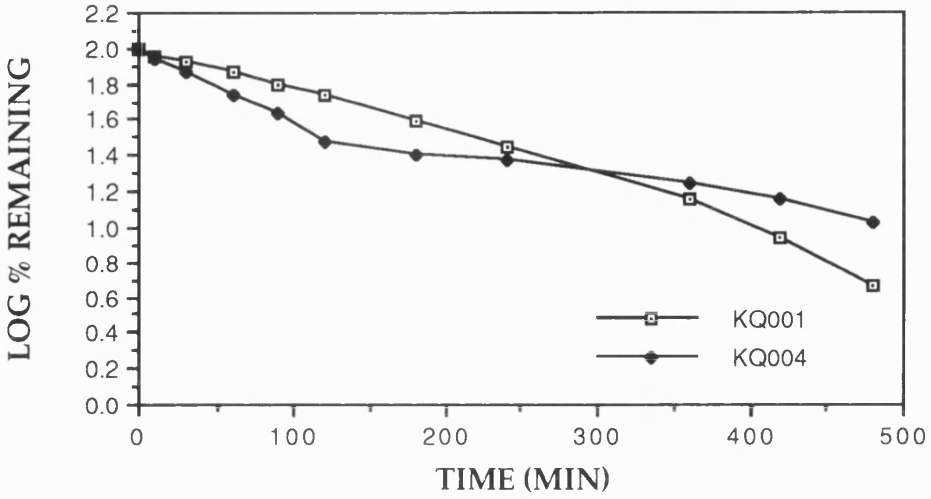


Figure 3.10 The Log % Remaining plotted for a 1mm (KQ004) and 2mm (KQ001) pellets of the same formulation.
KQ001 r = 0.988 KQ004 r = 0.917

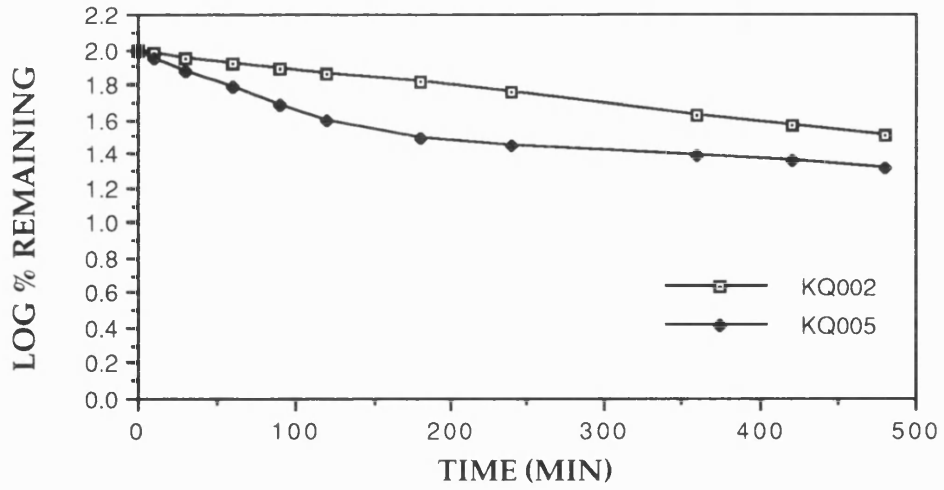


Figure 3.11 The Log % Remaining plotted for a 1mm (KQ005) and 2mm (KQ002) pellets of the same formulation.
KQ002 r = 0.998 KQ005 r = 0.862

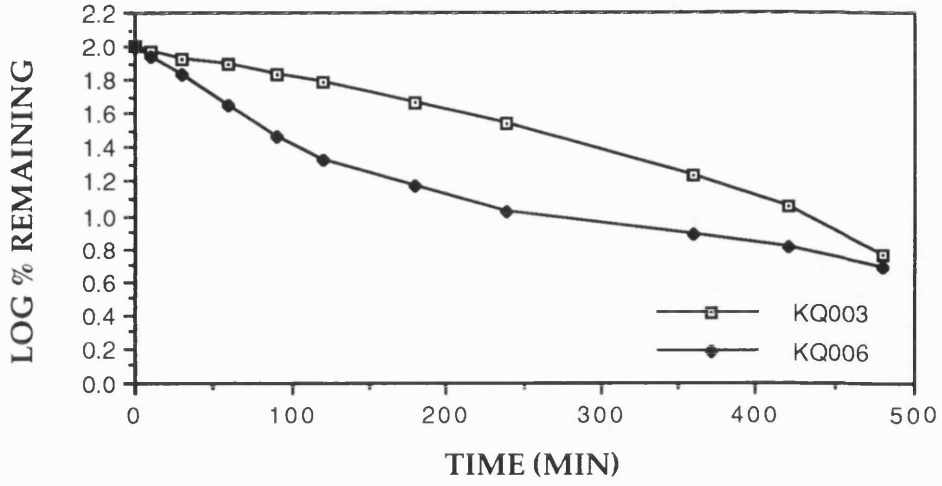


Figure 3.12 The Log % Remaining plotted for a 1mm (KQ006) and 2mm (KQ003) pellets of the same formulation.
KQ003 r = 0.980 KQ006 r = 0.917

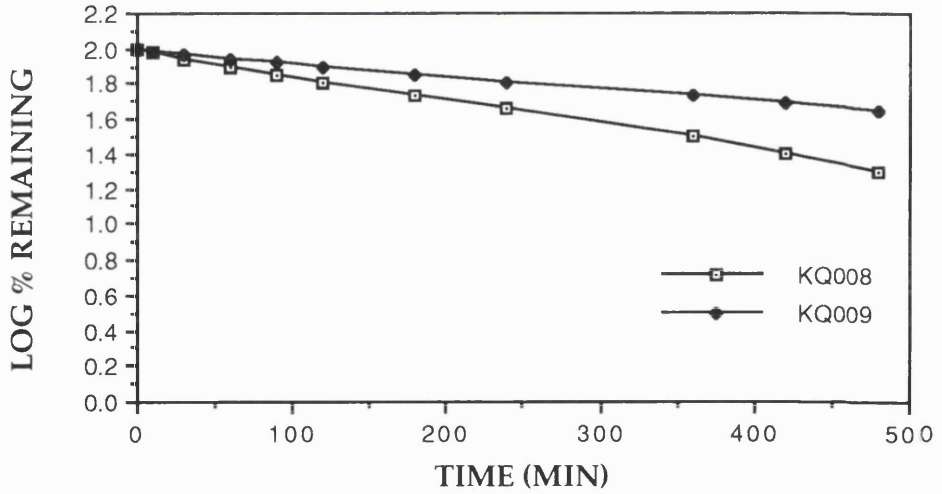


Figure 3.13 The Log % Remaining plotted for a 1mm (KQ008) and 2mm (KQ009) pellets of the same formulation.
KQ008 r = 0.998 KQ009 r = 0.997

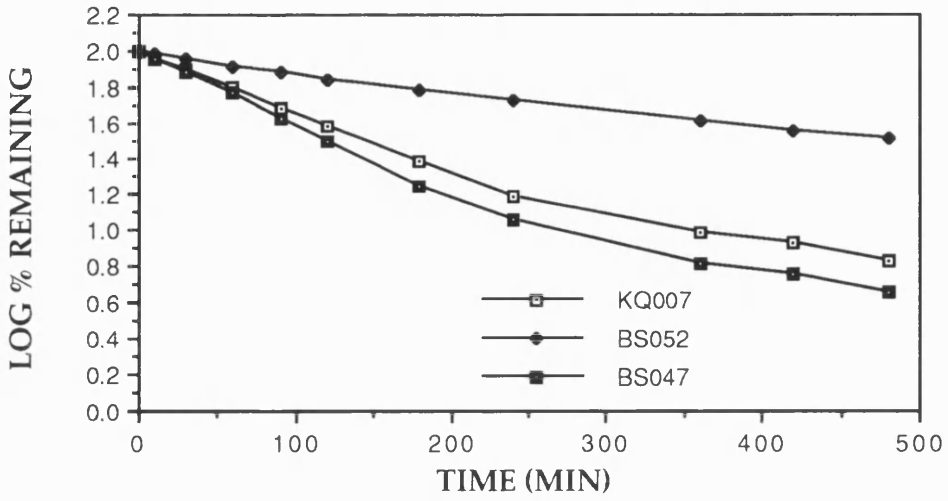


Figure 3.14 The Log % Remaining plotted for 1mm (BS047), 1.5mm (KQ007) and 2mm (BS052) pellets of the same formulation.
BS047 r = 0.962 KQ007 r = 0.971 BS052 r = 0.995

In order to define the mechanism of drug release in the matrix formulation, various kinetic models are used. Higuchi's (1963) square root equation for fused matrix systems is used to evaluate if the release is diffusion controlled. A plot of % Released vs square root of time is shown for each formulation and KQ001, KQ002, KQ003, KQ008, KQ009, and BS052 follow this model. Thus it appears that all the 2mm pellets show diffusion controlled release and KQ008 is the only 1mm pellet to do so. The 1.5mm (KQ007) shows an intermediate release rate. From both the log % remaining plots and these Higuchi ones it would seem that there are two different release mechanisms for the 1mm and 2mm pellets. With drug loading one would assume faster initial release as a larger surface area is available for dissolution in the 2mm pellet and the drug is dispersed throughout the matrix. Diffusion takes place initially from the the surface of the pellet and eventually the drug is leached from the matrix into the

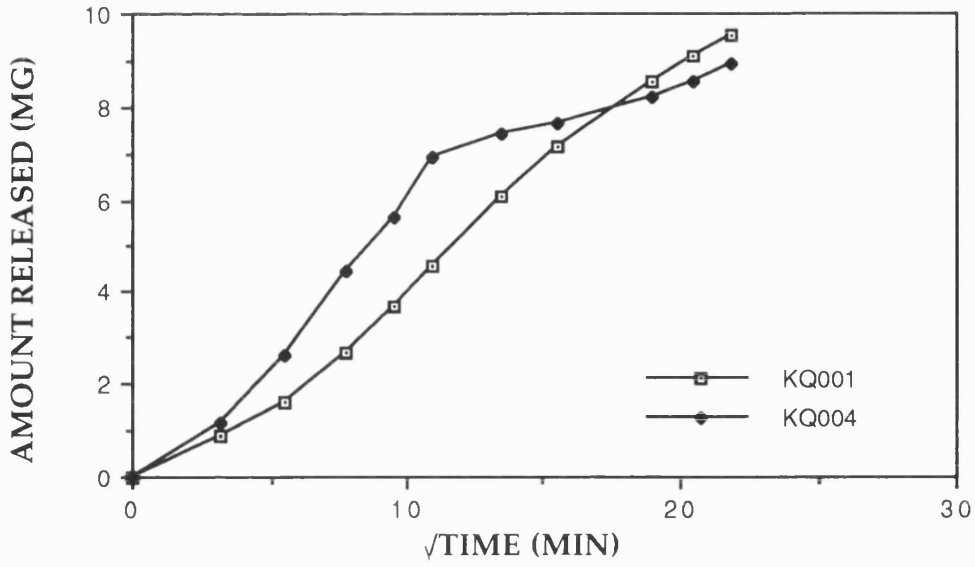


Figure 3.15 Higuchi model for KQ001(2mm) and KQ004(1mm) formulations containing the same concentration of Chitosan, Avicel and Lactose.

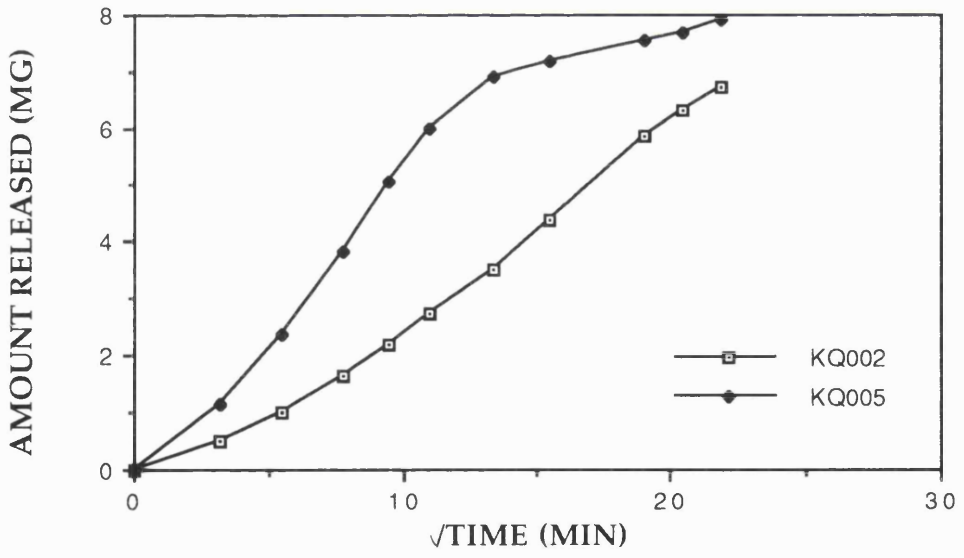


Figure 3.16 Higuchi model for KQ002 (2mm) and KQ005 (1mm) containing the same Chitosan, Barium Sulphate and Avicel concentration (no Lactose).

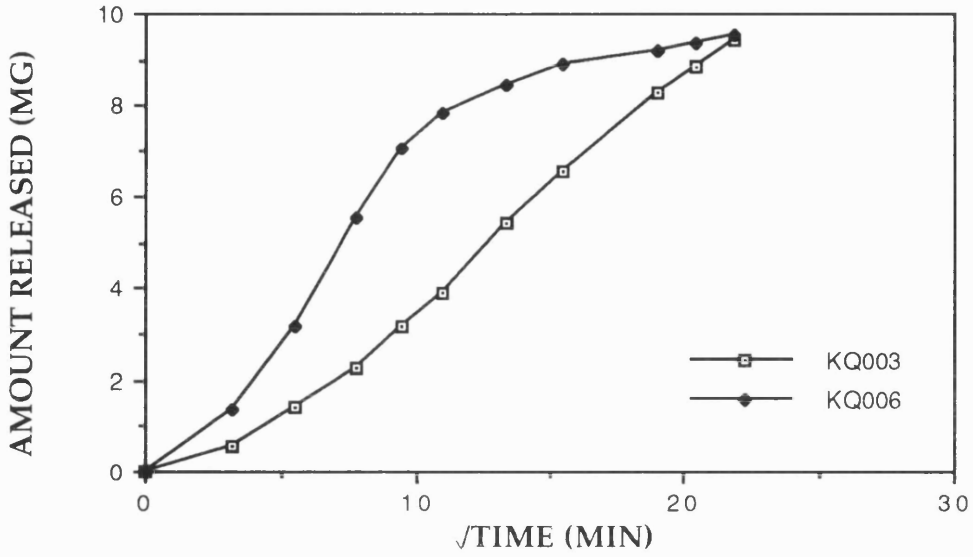


Figure 3.17 Higuchi model for KQ003 (2mm) and KQ006 (1mm) pellets containing the same Chitosan, Avicel, Lactose and Barium Sulphate concentration.

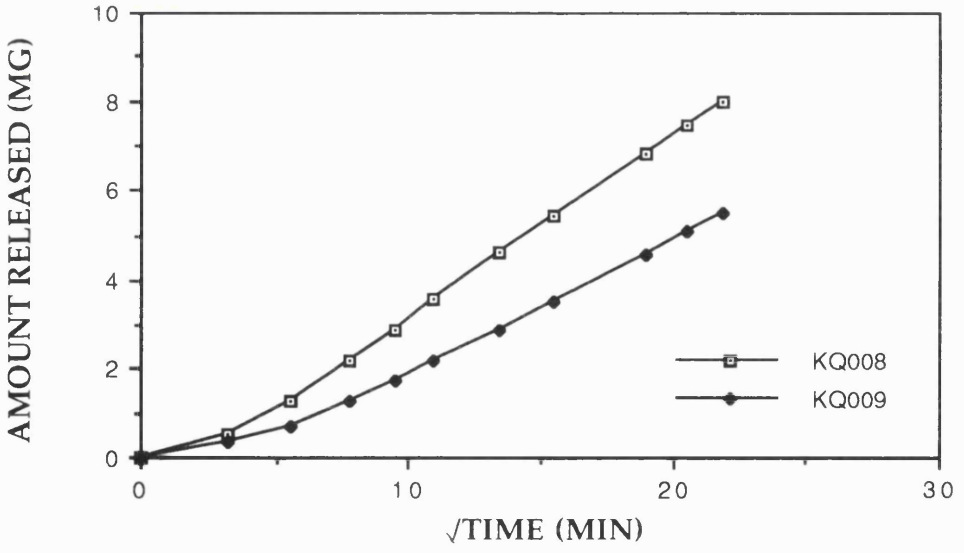


Figure 3.18 Higuchi model for KQ008 (1mm) and KQ009 (2mm) pellets containing the same concentration of Chitosan, Avicel and Barium Sulphate as a percentage of the dry mass, and the drug concentration is doubled.

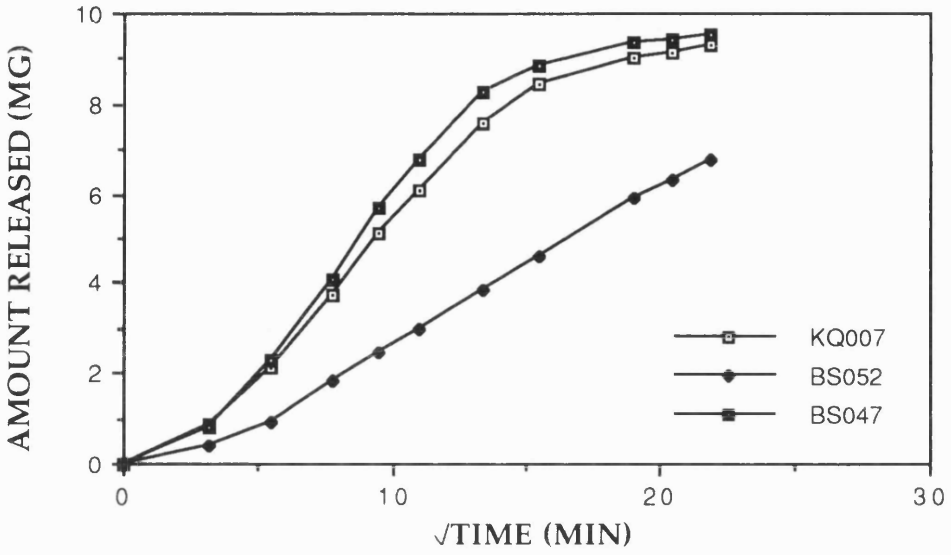


Figure 3.19 Higuchi model for BS052 (2mm), KQ007 (1.5mm) and BS047 (1mm) pellets containing the same formulation as a percentage of the dry mass.

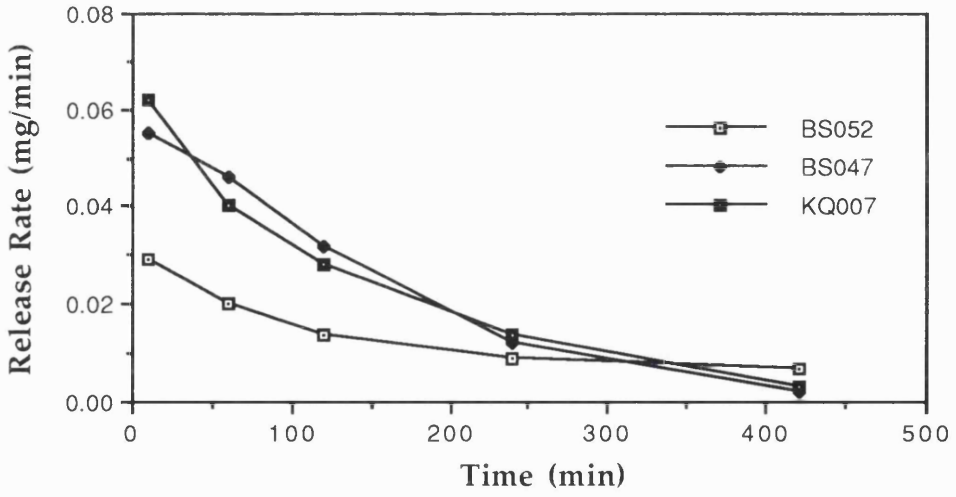


Figure 3.20 The rate of Drug Released in mg/min for a 1mm (BS047), 1.5mm (KQ007) and 2mm (BS052) pellet of the same formulation.

dissolution medium. In the 2mm pellets there is a deeper core and hence the drug has a longer distance to travel to the surface and therefore a slower dissolution rate would be expected.

The release rates calculated at the midpoint range value from the same formulation extruded through three different dies are shown in Figure 3.20. The release rate is faster initially for the 1mm pellet and from 100-400 minutes the release appears to be constant reaching a zero order profile. The 2mm pellet seems to give the most constant release profile. The release rate from the 1.5mm pellet is plotted and this shows a gradual tailing off in the amount of drug released. Release is fastest initially from the surface of the sphere and the drug is then leached from the core at a slower rate. The release rate tapers off as the core becomes depleted of drug. The 1mm pellet shows the most rapid release and least controlled release, with the release rate halved in ~ 150 minutes but there does not appear to be a burst effect with the release rate decreasing in an almost linear fashion.

Figure 3.21 is a plot of the amount of drug released in mg divided by the surface area of each pellet, assuming the surface area remains constant for each pellet throughout the dissolution process. The pellets remain intact on dissolution and do not erode to any extent. Again the 2mm pellet shows the slowest release rate with the 1mm showing the fastest. The surface areas are 10.18 cm^2 - BS047, 15.21 cm^2 - KQ007, and 24.63 cm^2 - BS052 assuming the pellets are perfect spheres. This graph indicates that the 2mm pellet is releasing drug at a much slower rate per unit surface area than either the 1mm or the 1.5mm pellet. The 1.5mm pellet shows an initial burst effect and the release rate then levels off gradually. Thus the amount of drug released from each pellet matrix is not simply controlled by the difference in unit size, as they do not appear to have a linear relationship between the amount of drug released per unit surface area. This difference in release rate is seen through each of the variations in the formulations between the 1mm and 2mm pellets.

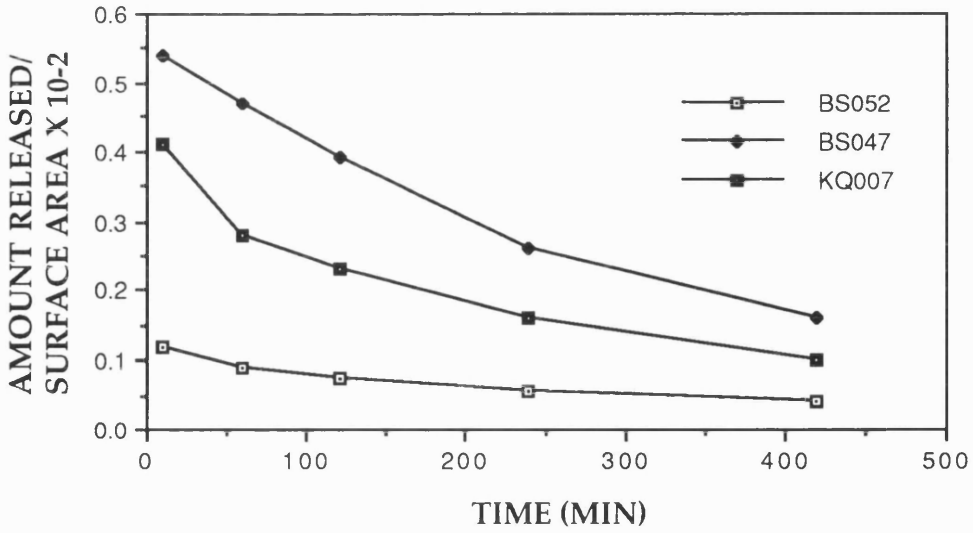


Figure 3.21 The amount of drug released divided by the surface area of a 1mm (BS047), 1.5mm (KQ007) and a 2mm (BS052) pellet of the same formulation.

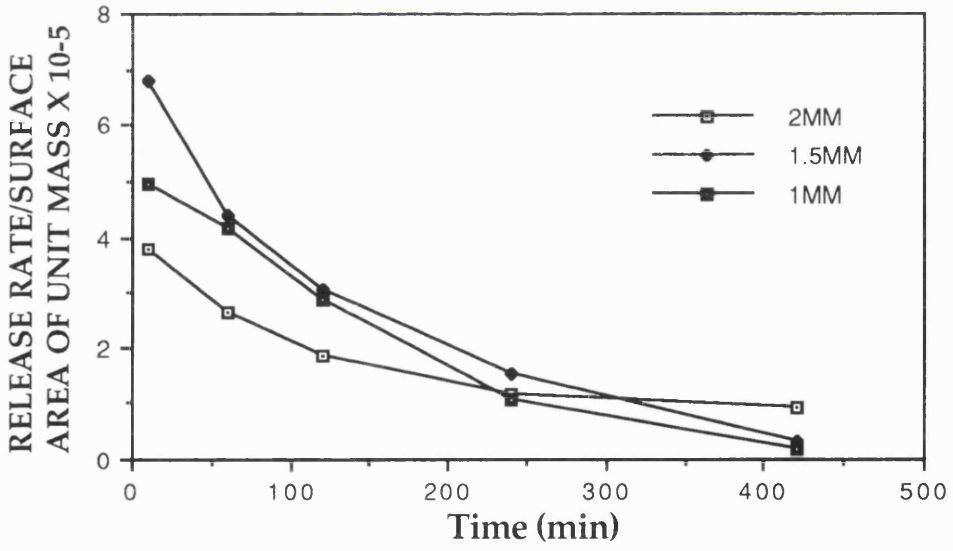


Figure 3.22 The amount of drug released divided by the surface area of a unit mass of pellets of equivalent weight; for 2mm (BS052), 1mm (BS047) and 1.5mm (KQ007) pellets of the same formulation.

The 1.5mm pellet shows an intermediate profile between these two pellets.

Figure 3.22 shows the amount of drug released divided by the surface area of the unit mass of pellets of equivalent weight in the dissolution medium (0.5g). These graphs indicate the amount of drug released per unit mass surface area, and the 2mm pellet shows a faster initial release and then a levelling off to zero order release. The 1mm pellet takes longer to reach this slowing in the release rate and then tails off more gradually. The 1.5mm pellets show the largest initial release per unit surface area and then a gradual levelling off. This shows that the size of the pellets and the total surface area available to the dissolution medium, is a controlling factor in the rate of drug release.

3.3.5 DISSOLUTION STUDY AT GASTRIC pH

The solubility of diclofenac sodium is markedly dependent on pH as it is a weakly acidic drug with a pKa value of 3.8. The rate of dissolution will therefore depend on the pH of the dissolution medium. Three of the pellets formulated; BS052, BS047 and KQ005 were subjected to a dissolution test at gastric pH. The dissolution medium was 1000ml of 0.1M HCL with a measured pH of 1.2 and the sample size was 0.5g of pellets. The test was performed in triplicate as detailed in Section 2.1.5. The beads all remained intact after eighteen hours in the dissolution medium but KQ005 showed slight swelling; measured by an increase in diameter from 1mm to 1.4mm after dissolution.

Figure 3.23 shows the dissolution profiles obtained from the three pellets in an acidic medium. The maximum amount of drug released after eighteen hours never exceeds 60%. There appears to be a slight initial burst and the drug is then slowly released, as diclofenac is a weakly acidic drug. BS047 and BS052 are the same

formulation extruded through 1mm and 2mm dies while KQ005(1mm) contains a six - fold increase in barium sulphate concentration and no lactose. The percentage of chitosan in the dry mass is the same for all three pellets. KQ005 shows the slowest release, 25% after six hours while BS047 shows 41% release and BS052, 42% release at the same time point. In phosphate buffer, KQ005 showed 50% release in under four hours, BS052 shows 50% release in just over four hours and BS047 shows 50% release in ~ 80 minutes.

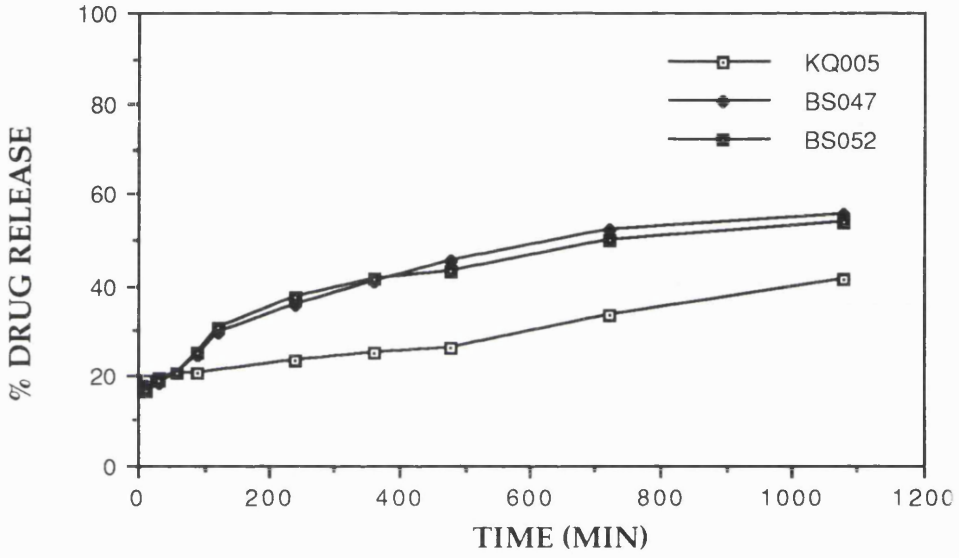


Figure 3.23 Dissolution of three pellets, BS047 (1mm), BS052 (2mm) and KQ005 (1mm) in an acidic medium at pH 1.2, where BS047 and BS052 are identical formulations and in KQ005 the lactose concentration is replaced by Barium Sulphate.

3.3.6 SCANNING ELECTRON MICROGRAPHS

The scanning electron micrographs for a selection of pellets are shown overleaf in Figure 3.24 (a) KQ001 (2mm), (b) KQ004 (1mm), (c) KQ007 (1.5mm), (d) BS052 (2mm). The samples of pellets are prepared

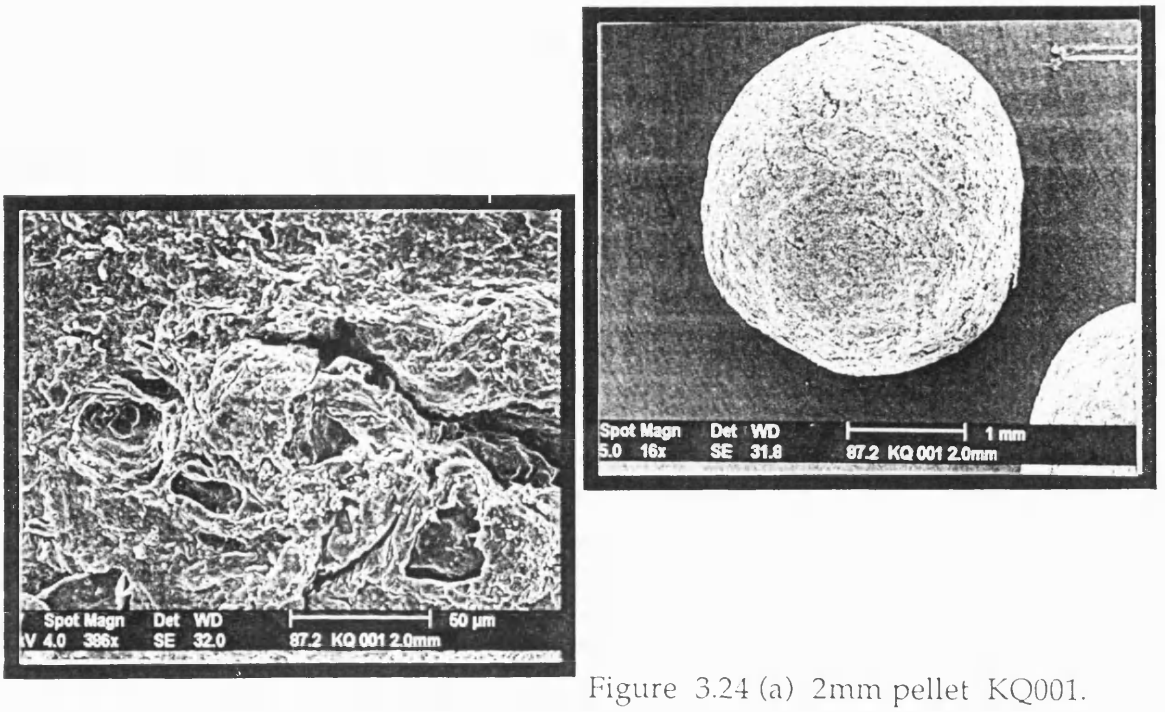


Figure 3.24 (a) 2mm pellet KQ001.

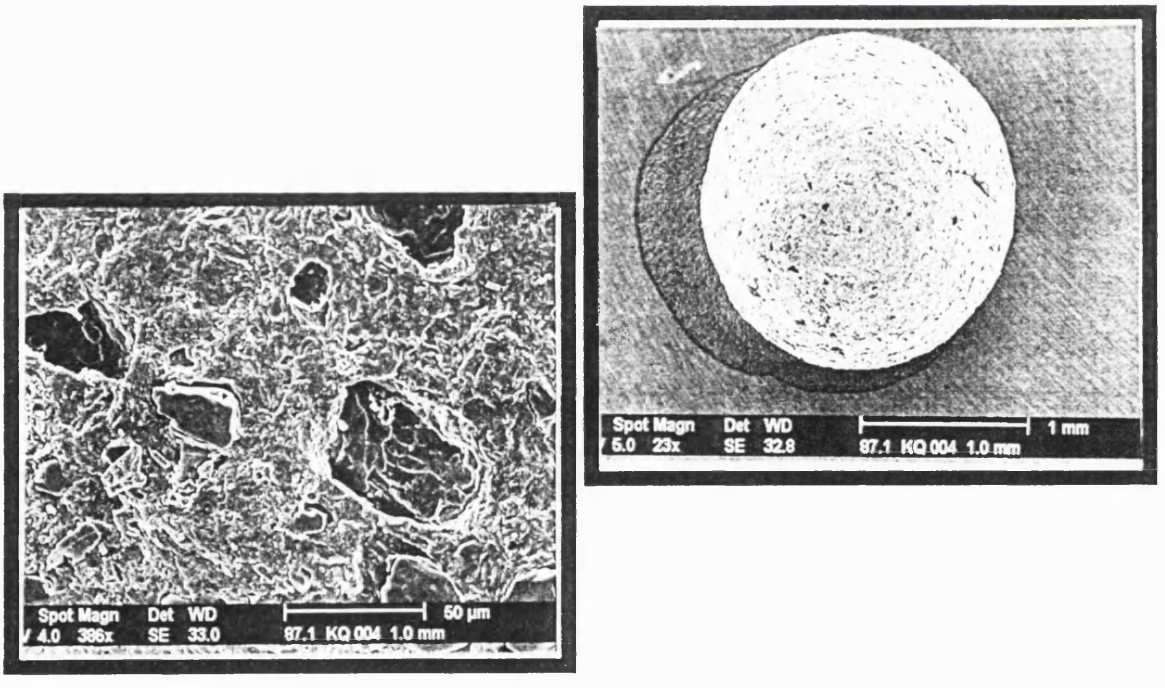


Figure 3.24 (b) 1mm pellet KQ004.

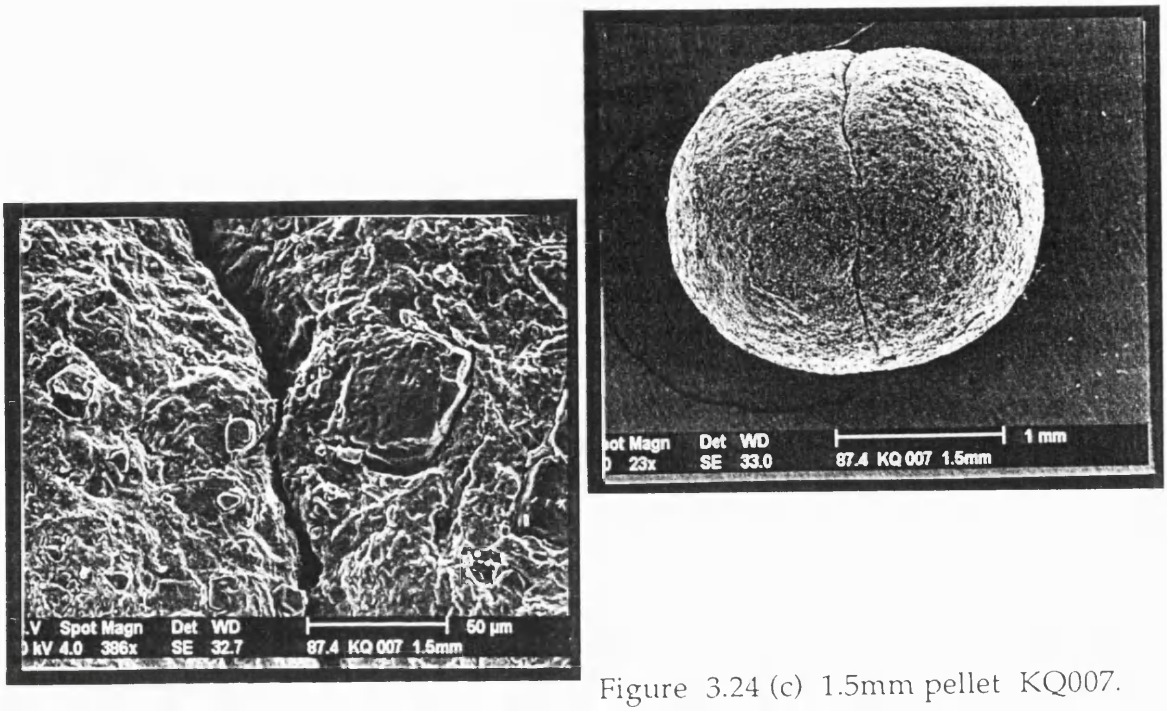


Figure 3.24 (c) 1.5mm pellet KQ007.

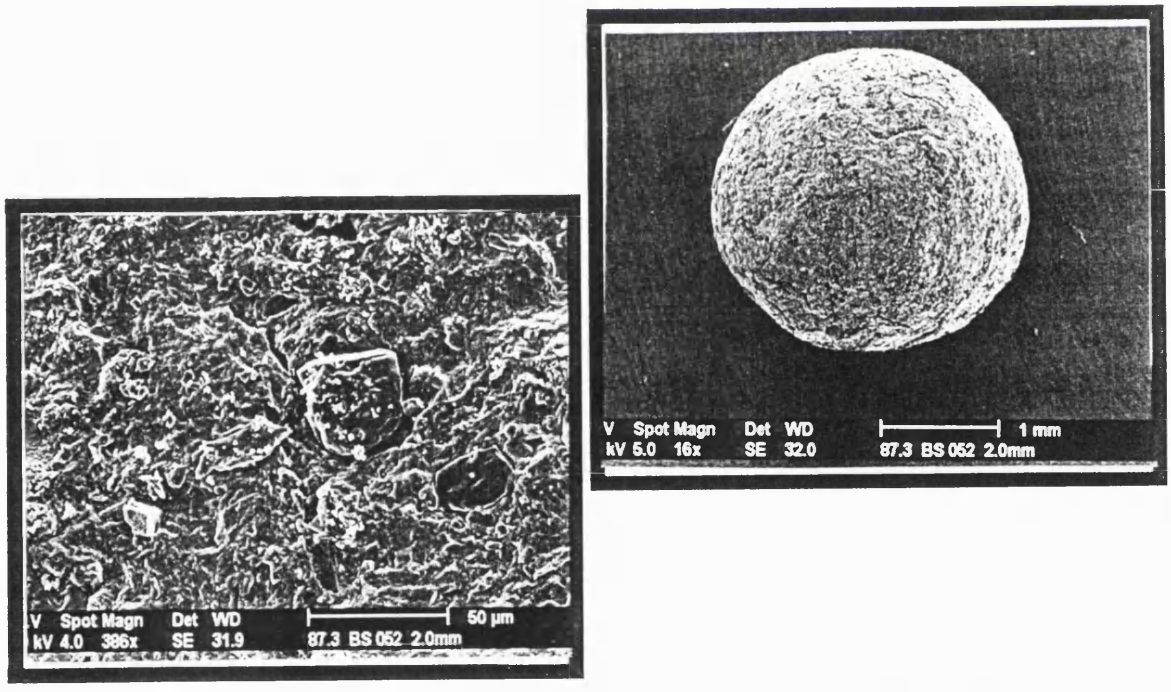


Figure 3.24 (d) 2mm pellet BS052.

and scanned as described in Section 2.1.6.3. The surface morphology of the pellet can be viewed in the first micrograph for each sample and the second micrograph is a cross-sectional view. This sample includes a pellet extruded through a 1mm, 1.5mm and a 2mm die.

3.4 DISCUSSION

Chitosan, a polycationic polysaccharide forms gels with suitable multivalent cations. The ionic interaction occurs between the positively charged amino groups and the negatively charged counterion. The protonation of the amino groups enables the dissolution of chitosan by a number of strong and weak acids. The effect of increasing the acetic acid concentration does not improve the retardant effect, as chitosan appears to be just as soluble in the dilute solutions of acetic acid (10% w/w). Increasing the acetic acid concentration from 10% w/w to 40% w/w does not have any advantage for the product but dramatically increases the pungent odour and wear on the dies during extrusion.

The initial formulations presented included chitosan in dry powder form and the chitosan concentration was shown to influence the rate of drug release from the pellets. Chitosan has been shown to act as a disintegrant at a concentration of 10% in tablets when incorporated in dry powder form, Nigalaye et al, (1990). The pellets remained intact but were fast releasing in these formulations. Microcrystalline cellulose is thought to modify the rheological properties of the other ingredients in the mix so that a degree of plasticity is attained which facilitates the extrusion of the mixture. It appears to control water movement through the plastic mass and this interaction with water is due to the ability of microcrystalline cellulose to retain a large quantity of moisture, Fielden et al. (1988). The formulations presented all readily extrude and spheronize at low extrusion forces but it was found that mixes without a sufficient percentage of microcrystalline cellulose present did not form spheres.

The chitosan gel content remains constant in the following batch of formulations, except in formulations KQ003 and KQ006 where it is increased and the water content correspondingly decreased. This resulted in a slightly faster release rate and this effect is more evident with the 2mm pellet. Conversely increasing the barium sulphate content in KQ002 and KQ005 reduced the release rate especially in the 1mm pellet. The effect of removing the lactose and replacing it with barium sulphate appears to be important in controlling the release rate. The barium sulphate concentration influences the density of the pellets and is itself insoluble in water. In KQ008 and KQ009 the lactose content was removed and the Avicel content reduced and hence, the barium sulphate concentration and the chitosan gel concentration in the overall formulation is higher and this resulted in the slowest release rate despite the drug loading in the matrix. Both profiles are approaching zero order release rates, and in the 1mm pellet the initially faster release rate is curtailed.

From the log % remaining plots, all of the 2mm pellets appear to follow first order kinetics, while the 1mm pellets deviate from this model slightly but are still reasonable fits to the model. The Higuchi plots demonstrate that the 2mm pellets show diffusion controlled release and the drug loaded 1mm pellet (KQ008) is the only one of the 1mm pellets to do so. The 2mm pellets have a larger area for the drug to pass through from the core and the dissolution medium leaches the drug from the outer layers initially and then from the core itself via the pores in the matrix. This reduction in the release rate is seen without a coating layer on the pellets to modify the release properties. The release rate profiles show the 2mm pellet approaches zero order while the 1.5mm and the 1mm show a faster initial release rate and then levels off as the matrix becomes depleted of drug. The drug release from the chitosan pellets depends on the penetration of the dissolution medium into the pellet. The pellets when wetted did not swell or dissolve. The dissolution of the chitosan matrix is thought to occur via the formation of a hydrogel matrix. Bodmeier et al, (1989) showed that the swelling of chitosan is

dependent on the pH of the dissolution medium. The pellets when wetted by an acidic dissolution medium appear to swell extensively. However no swelling was evident in this dissolution medium at pH7.4. The pellets in the acidic medium did not appear to swell, apart from KQ005 which showed a slight increase in diameter after eighteen hours in the medium. Van Wilder et al. (1991) investigated the release of diclofenac sodium from two oral sustained release formulations in four different release media. They conclude that the release from the formulations is strongly medium dependent and faster dissolution is obtained in media without an acid present or with higher pH values. The coated pellets were found to be more sensitive to changes in the dissolution parameters than the matrix tablet formulation.

Nernst and Brunner, (1904) have developed an equation to describe the diffusive layer model. Diffusion is controlled by the the difference between the saturated solubility in the diffusive layer surrounding the dissolving particle and the concentration of drug in the bulk fluid. For these experiments sink conditions apply as the drug concentration is less than 10% of the dissolution medium. Changes in the diffusion layer around the dosage form, due to the degree of agitation, generally do not have a major effect on the rate of drug release. A constant rate of agitation (100rpm) was employed for this study. Tapia et al, (1993) have shown that the release is independent of the stirring speed. In general, matrix systems are not as dependent on the degree of agitation as diffusion from the non-eroding matrix is the rate controlling factor. The drug may have to diffuse initially through the core itself. Drug particles, dispersed in the deeper matrix layers have a longer diffusional path to the surface.

Scott and Hollenbeck, (1991) have shown that when a comparison is made between pellets in which a drug is uniformly dispersed and pellets in which it is randomly dispersed, longer release times for pellets containing non-uniformly dispersed drug are predicted. The drug would appear to be randomly dispersed in these formulations as it is incorporated in a mixing process and some of

the drug crystals are visible in the scanning electron micrographs. The scanning electron micrographs show that the pellets have a smooth outer surface with some pores visible and appear to be quite densely packed structures.

This insoluble non-erosion type matrix system was found to be effective in sustaining the release of diclofenac from the pellets. Increasing the barium sulphate concentration retarded the diffusion of the drug from the matrix and yielded slower release profiles at constant concentrations of polymer. The size of the pellet also has a marked effect on drug release and increasing the size from 1mm to 2mm showed a marked sustained release effect which appears unrelated to the increased surface area of a unit mass of equivalent weight. The release from the 2mm sphere is via a diffusion process and both size of pellets appear to follow first order kinetics but is approaching zero order for the 2mm sphere as is seen from the release rate vs time profile. Increasing the drug concentration in the formulation containing barium sulphate further reduced the drug release rate. These results suggest that chitosan has potential as a method of drug delivery and increasing the size of the pellet results in a marked delay in drug release not apparently related to the changes in surface area.

CHAPTER 4

THE INFLUENCE OF ELECTROLYTES ON THE FORMATION & RELEASE PROPERTIES OF PELLETS CONTAINING CHITOSAN/DICLOFENAC

4.1 INTRODUCTION

Chitosan, incorporated as a dry powder, or when dissolved in a suitable solvent as a gel, produces a controlled release effect but not to any significant extent as shown in the previous chapter. Increasing the concentration of chitosan in the formulation has been shown to affect the release rate, but this has proved difficult in this matrix pellet, produced by extrusion - spheronization, as the increase in viscosity of the gel, produced an extrudate that does not readily form spheres. An alternative method of increasing the concentration of chitosan was then investigated. Chitosan, a polycationic polysaccharide forms gels with suitable multivalent cations. The ionic interaction occurs between the positively charged amino groups and the negatively charged counterion. The protonation of the amino groups enables the dissolution of chitosan by a number of strong and weak acids. The viscosity of chitosan in the presence of an external salt is considerably reduced and this could allow the incorporation of increased quantities of the polymer. Four different salts; magnesium chloride, sodium chloride, potassium chloride and potassium hydrogen carbonate at two values of ionic strength are included in the chitosan gels incorporated into the matrix pellets. The concentration of Avicel, lactose and the drug, diclofenac sodium is kept constant throughout the formulations, while chitosan is incorporated at two levels (one double the other) and each salt is incorporated at two levels also. The salts are included at equivalent molar concentrations. The water content of each formulation varies depending on the amount required to produce an extrudate that is sufficiently plastic to deform in the spheronizer. The percentage weight loss on drying and the density of each of the spheres is presented along with their dissolution profiles. Scanning electron micrographs of the pellets are shown; one of each type of salt included in the study.

The general rheological properties of the chitosan gels is investigated using a continuous shear method to examine if there is any relationship between the viscosity of the various gels and the dissolution profiles of the pellets. The presence of the salt affects the viscosity of the chitosan gel in the matrix pellet. A simple flow

curve of shear stress vs shear rate is presented for each of the salts at the two different molar concentrations used in the formulation of the pellets with the two different levels of chitosan in the gels.

4.2 POTASSIUM CHLORIDE

A number of different cations are examined in this study including the monovalent potassium ion. The electrolyte is added to the chitosan powder in a 10%w/w glacial acetic acid solution. The formulations included 1.6M and 0.8M concentrations of the monovalent ions.

4.2.1 FORMULATIONS

The dry powders are mixed as described in Section 2.1.1 including the gel, consisting of the chitosan powder, salt and acetic acid solution. The quantity of water required to produce a spherical product is determined by trial and error. The mixture is extruded immediately through a 2 x 2 die attached to a barrel of the LLoyds MX50 physical testing apparatus at a speed of 300mm/min. The various formulations that produced spherical products are shown below in Table 4.1. The content of Avicel, lactose, and the drug, diclofenac sodium is not shown in the table as these were kept constant for all the electrolyte formulations; Avicel - 34%w/w, lactose - 27%w/w and diclofenac sodium - 5%w/w respectively. The gel mix in which the concentration of both salt and chitosan can vary and the amount of water added, is detailed in the tabulated formulations. The chitosan concentration is given as the percentage dry powder but the chitosan was incorporated as a gel.

	K-03-002	K-03-003	K-03-005	K-03-007	K-03-008
CHITOSAN	5%	7%	3.5%	7%	3.5%
KCL	1.6M	1.6M	1.6M	0.8M	0.8M
WATER	45G	30G	58G	38G	36G
PRODUCT	SPHERES	SPHERES	SPHERES	SPHERES	SPHERES

Table 4.1 Formulations of Mixtures containing Potassium Chloride.

4.2.2 ANALYSIS OF THE PELLETS

The resulting pellets are dried in an open air oven to constant temperature and their density measured as described in Section 2.1.6.2. The results are shown in Table 4.2.

	K-03-002	K-03-003	K-03-005	K-03-007	K-03-008
DENSITY (g/cm^3)	1.46	1.38	1.42	1.32	1.31
% WT LOSS on DRYING	18.8	14.3	12.5	10.9	10.5

Table 4.2 Density and % Weight Loss on Drying for the formulations containing Potassium Chloride.

The pellets are then sieved and the percentage oversize is shown in Table 4.3.

SIEVE SIZE	K-03-002	K-03-003	K-03-005	K-03-007	K-03-008
2.8MM	2.1	0.3	1.6	0.7	5.3
2MM	33.9	21.6	24.4	16.8	28.9
1.4MM	57.4	73.1	63.2	66.7	53.5
1.0MM	6.6	5.0	10.8	15.8	12.3
BASE	0	0	0	0	0

Table 4.3 Sieve fractions of the pellets from the formulations containing Potassium Chloride.

4.2.3 DISSOLUTION PROFILES

The dissolution behaviour of the pellets is determined as detailed in Section 2.1.5. Each experiment is conducted in triplicate and the average result taken. The weight of the sample is kept constant in each case. The dissolution medium is phosphate buffer pH 7.4 and the pellets for dissolution are taken from the sieve fraction 1.4 - 2mm.

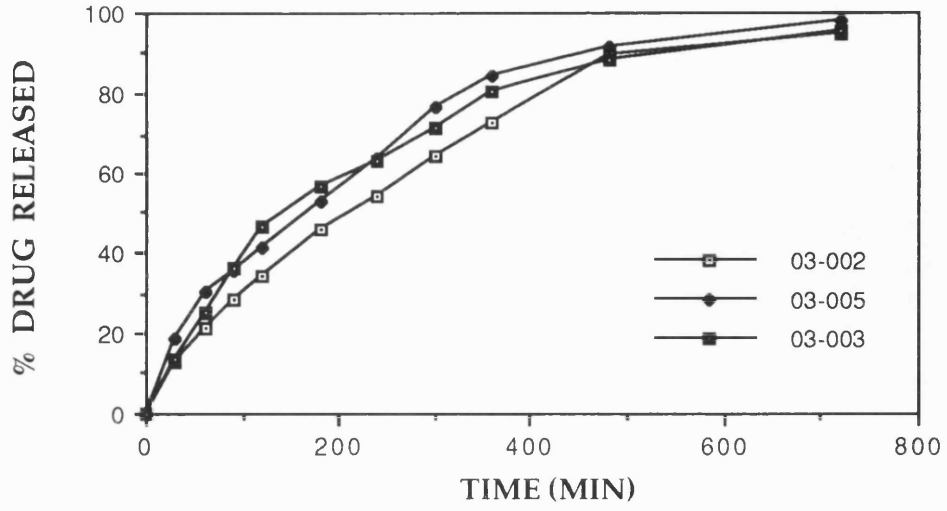


Figure 4.1 Dissolution profiles of pellets containing differing concentrations of chitosan; (03-002) 5%, (03-005) 3.5%, and (03-003) 7% and a 1.6M concentration of Potassium Chloride.

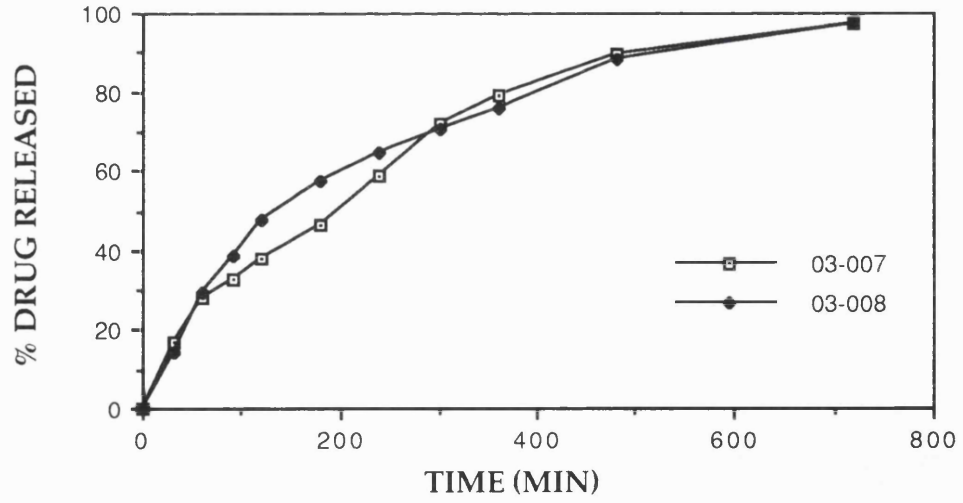


Figure 4.2 Dissolution profiles of pellets containing differing concentrations of chitosan; (03-007) 7% and (03-008) 3.5% and a 0.8M concentration of Potassium Chloride.

4.2.4 DISCUSSION

The formulations include potassium chloride at two molar concentrations (0.8 and 1.6M) with two different concentrations of chitosan. A formulation containing an intermediate concentration of chitosan with the higher salt concentration is also shown. The water content is critical for each of the formulations and determines whether or not the mass will form spheres. The extrusion force is kept low in these experiments also, typically 2 - 4 kN. The analysis of the pellets shows that the formulations containing the higher molar concentration of salt had higher values for density than those containing the lower concentration. These denser pellets show a greater water loss on drying which would increase the density also. All the formulations were extruded through a 2 x 2 die at a constant speed of 200mm/min. and on sieving show a narrow size distribution. The majority of the pellets are in the range from 1.4mm to 2mm.

The in-vitro release profiles of the pellets are presented in Figures 4.1 and 4.2. The sample weight and drug loading are constant throughout, as is the volume of dissolution medium - 1000ml phosphate buffer pH 7.4. Figure 4.1 shows the plot of three different concentrations of chitosan with a 1.6M concentration of the salt. The intermediate concentration (5%) of chitosan gives the slowest release profile while the lowest chitosan concentration shows the fastest release. The curves all follow the same general shape; a steady release of the drug over the first eight hours and then a gradual tailing off. There is no initial burst effect. Fifty percent of the drug is released within four hours for all the formulations, and within three hours for the formulation containing the lowest concentration of chitosan. Figure 4.2 shows the dissolution profiles for the lower salt concentration (0.8M) at two levels of chitosan. The formulation containing the higher chitosan concentration (03-007) shows a slower release of the drug initially and then tails off in the same fashion as the other formulation. Fifty percent release is seen after three hours for the formulation containing the least amount of chitosan and after three and a half hours for the formulation containing increased

amounts of chitosan. Thus the chitosan concentration does appear to affect the release of the drug at both concentrations of the salt.

4.3 MAGNESIUM CHLORIDE

Magnesium chloride is a divalent salt and is incorporated into the formulations at an equivalent ionic strength to the potassium chloride salt.

4.3.1 FORMULATIONS

The formulations are prepared in exactly the same fashion as those of the potassium chloride salt. The concentration of Avicel, lactose and the drug, diclofenac sodium remain unchanged at 34%w/w, 27% w/w and 5% w/w respectively. The mixtures are packed into the barrel of the LLoyds MX50 and extruded through a 2 x 2 die at 300mm/min. (Section 2.1.2). The formulation mixtures that were sufficiently plastic to form pellets on spheronization are shown in Table 4.4(a) and 4.4(b). The electrolyte is included at two molar concentrations 1.6M and 0.8M as previously.

	04-003	04-006	04-010	04-011	04-013
CHITOSAN	5.5%	7%	7%	3.5%	3.5%
$MGCL_2$	1.6M	1.6M	0.8M	0.8M	1.6M
WATER	66G	55G	35G	31G	50G
PRODUCT	SPHERES	SPHERES	SPHERES	SPHERES	SPHERES

Table 4.4(a)

	04-014	04-015	04-017	04-019
CHITOSAN	0	0	5%	5%
$MGCL_2$	1.6M	1.6M	1.6M	0.8M
WATER	70G	85G	64G	44G
PRODUCT	SPHERES	SPHERES	SPHERES	SPHERES

Table 4.4 (b) Formulations of mixtures containing Magnesium Chloride as the electrolyte.

4.3.2 ANALYSIS OF THE PELLETS

The pellets are dried overnight in an open air oven and the percentage weight loss on drying and the density (Section 2.1.6.2) of the spheres is shown in Table 4.5. The sieve fractions presented as the percentage oversize are shown in Table 4.6.

	% WT LOSS ON DRYING	DENSITY (g/cm^3)
04-003	18.6	1.55
04-006	23.1	1.47
04-010	15.7	1.37
04-011	12.1	1.51
04-013	13.3	1.34
04-014	18.1	1.51
04-015	18.9	1.50
04-017	28.3	1.34
04-019	14.4	1.42

Table 4.5 Density and percentage weight loss on drying for formulations containing Magnesium Chloride.

	2.8MM	2.0MM	1.4MM	1.0MM	BASE
04-003	2.4	89.5	8.1	0	0
04-006	0	69.8	19.6	10.6	0
04-010	2.5	88.4	9.1	0	0
04-011	13.8	83.6	1.4	1.2	0
04-013	0	2.3	93.4	4.3	0
04-014	0	1.8	33.5	64.7	0
04-015	0	4.7	56.8	38.5	0
04-017	9.8	62.6	24.9	2.7	0
04-019	2.6	78.4	18.4	0.6	0

Table 4.6 Sieve fractions for formulations containing Magnesium Chloride.

4.3.3 DISSOLUTION PROFILES

The dissolution profiles for the pellets containing magnesium chloride are shown in Figures 4.3, 4.4 and 4.5. The in - vitro analysis is carried out in phosphate buffer pH7.4 as described in Section 2.1.5. The pellets taken from the sieve fraction 1.4 - 2 mm all remain intact at the end of the dissolution run. No swelling was observed in the pellets and none had a diameter greater than 2mm at the end of the dissolution run.

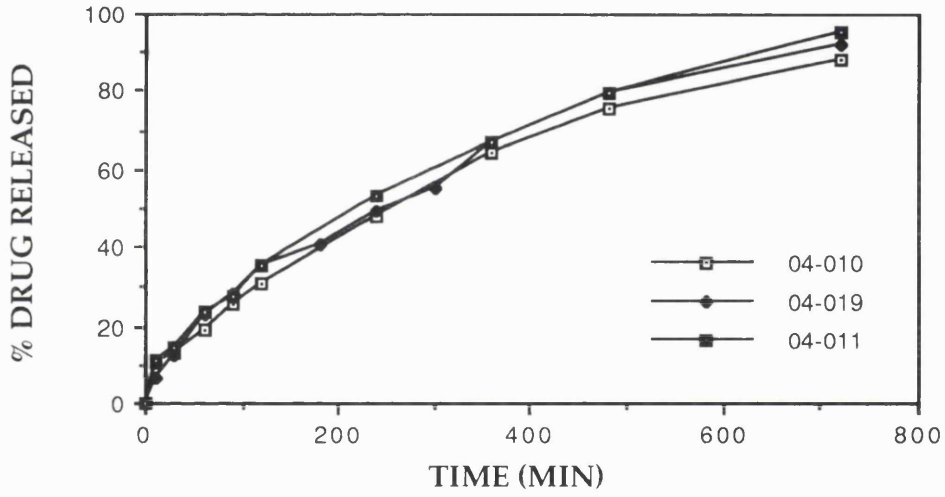


Figure 4.3 Dissolution profiles of formulations containing differing concentrations of chitosan; (04-010) 7%, (04-011) 3.5%, (04-019) 5% and a 0.8M concentration of Magnesium Chloride.

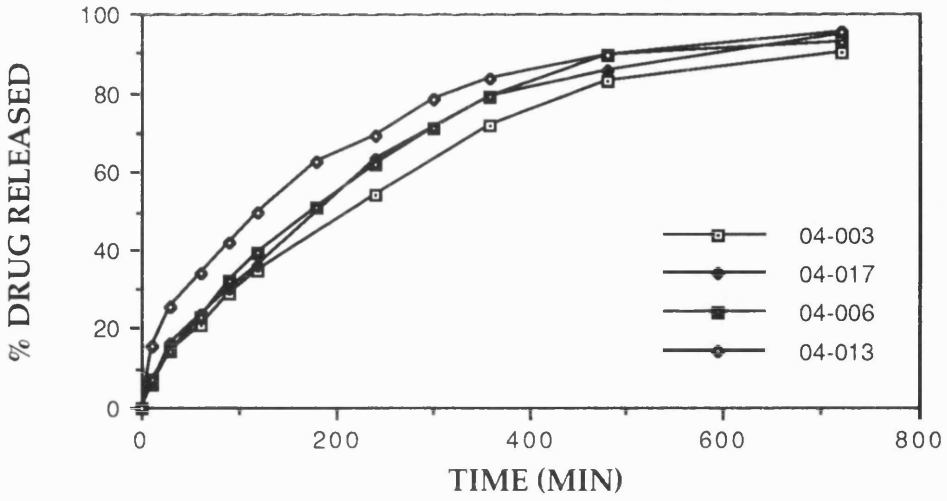


Figure 4.4 Dissolution profiles of pellets containing differing concentrations of chitosan; (04-003) 5.5%, (04-017) 5%, (04-006) 7%, (04-013) 3.5% and 1.6M concentration of Magnesium Chloride.

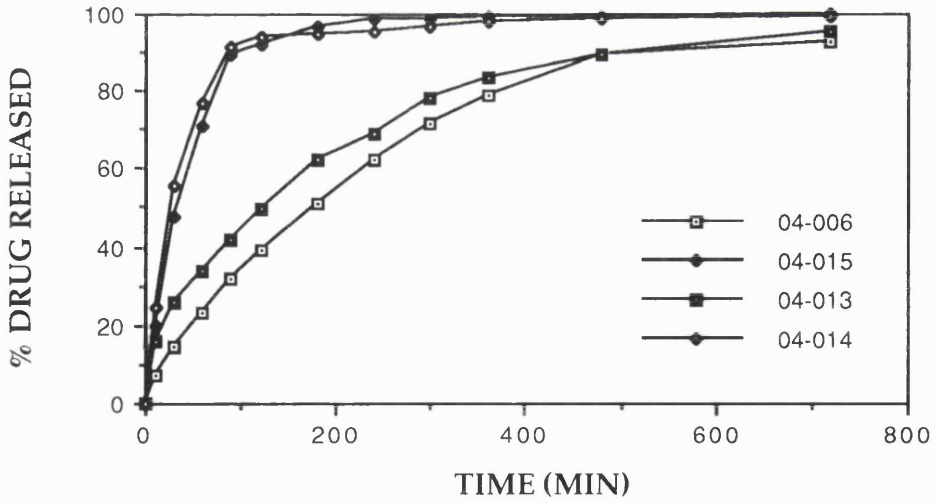


Figure 4.5 Dissolution profiles of pellets containing differing concentrations of chitosan; (04-006) 7%, (04-015) 0%, (04-013) 3.5% and (04-014) 0% and a 1.6M concentration of Magnesium Chloride.

4.3.4 DISCUSSION

These formulations contain magnesium chloride at the same molar concentrations (1.6 and 0.8M) as the previous salt and the concentration of chitosan varies. Two formulations that contain no chitosan, just the 1.6M salt concentration are included as these form spheres with two different levels of added water. The formulations are very sensitive to the water concentration added. The extrusion forces are low (2-4kN) and this produces better quality extrudate. The analysis of the pellets shows that the density appears to be related to the weight loss on drying. The less water that is lost on drying, the lower the density values for the pellet. The formulations containing the lower salt concentration, 04-010, 04-011 and 04-019 all have less added water initially and lose less water on drying. The density of the pellets with no added chitosan but different amounts of water is similar, as is their weight loss on drying. The pellets showed a narrow size distribution with the majority over the 2mm band except for the formulations with no added chitosan which had a smaller pellet size.

The dissolution profiles of the formulations with the 0.8M concentration of electrolyte are shown in Figure 4.3. The concentration of chitosan appears to influence the release, as the higher the concentration of chitosan in the pellet, the slower the release. The plots all show gradual release of the drug over eight hours. Fifty percent release occurs between three and a half and four hours for the formulations. Figure 4.4 shows the profiles for the formulations containing the higher salt concentration (1.6M) and four levels of chitosan. The formulation containing the least amount of chitosan (04-006) shows a faster release profile than the other formulations with the formulation containing the second highest chitosan concentration showing the slowest release of drug. This could suggest that there is an optimum level of interaction between the polymer and the salt. Figure 4.5 shows the release profiles of formulations containing 1.6M salt concentration, the highest and lowest level of chitosan and formulations with no chitosan, which showed fastest release of the drug; 50% within 50 minutes. The

formulation with highest chitosan concentration is again the slowest releasing pellet.

4.4 SODIUM CHLORIDE

Sodium chloride is another monovalent electrolyte added to the chitosan powder in 10% acetic acid solution to produce a less viscous gel, to incorporate into the matrix pellet.

4.4.1 FORMULATIONS

The formulations containing sodium chloride are mixed in an identical fashion to that of the other salts. The dry powders and the chitosan gel are initially mixed and the quantity of water required to form a sufficiently plastic mix to extrude added. The mixtures are extruded as described in Section 2.1.2 and subsequently spheronized. The mixes are extruded through a 2 x 2 die. The Avicel, lactose and drug (dicofenac sodium) content are kept constant at 34% w/w, 27% w/w and 5% w/w respectively for each formulation. Table 4.7 shows the variables in the formulations and the different concentrations of chitosan with the salt and the quantity of water needed to produce good quality extrudate.

	05-001	05-017	05-018	05-021	05-023	05-024
CHITOSAN	3.5%	3.5%	5%	7%	7%	7%
NACL	0.8M	1.6M	1.6M	0.8M	1.6M	1.6M
WATER	40G	60G	70G	85G	77G	74G
PRODUCT	SPHERES	SPHERES	SPHERES	SPHERES	SPHERES	SPHERES

Table 4.7 Formulations of mixtures containing Sodium Chloride.

4.4.2 ANALYSIS OF THE PELLETS

The pellets are analysed as described in Section 2.1.6. After drying to constant weight in an open air oven, the percentage

weight loss on drying is noted. The density of the different pellets measured (Section 2.1.6.2) is shown in Table 4.8.

	05-001	05-017	05-018	05-021	05-023	05-024
% WT LOSS ON DRYING	11.5	24.3	21.7	22.8	15.1	14.2
DENSITY (g/cm^3)	1.41	1.52	1.34	1.42	1.37	1.35

Table 4.8 Density and percentage weight loss on drying for formulations containing Sodium Chloride.

Table 4.9 below shows the range of pellet sizes in the different sieve fractions. All six formulations show a fairly narrow size distribution with most of the pellets concentrated in one sieve fraction.

	05-001	05-017	05-018	05-021	05-023	05-024
2.8MM	0.3	0	8.3	2.6	6.5	3.2
2.0MM	67.7	12.9	70.5	20.9	72.4	74.2
1.4MM	30.3	78.8	14.1	73.2	18.8	19.4
1.0MM	1.7	7.5	7.1	3.3	2.3	3.2
710uM	0	0.8	0	0	0	0
BASE	0	0	0	0	0	0

Table 4.9 Sieve Distribution for Sodium Chloride formulations.

4.4.3 DISSOLUTION PROFILES

The in - vitro study is carried out using the apparatus described in Section 2.1.5. A constant weight sample of pellets is placed in the dissolution medium and samples taken at predetermined intervals. The pellets all remain intact throughout the study and do not float in the medium. The drug loading and sample weight are constant

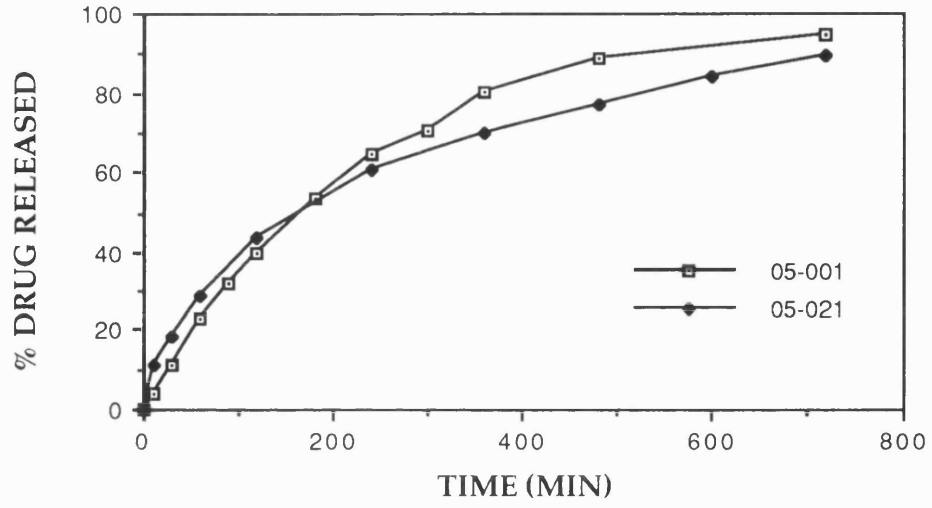


Figure 4.6 Dissolution profiles of pellets containing differing concentrations of chitosan; (05-001) 3.5%, (05-021) 7% and a 0.8M concentration of Sodium Chloride.

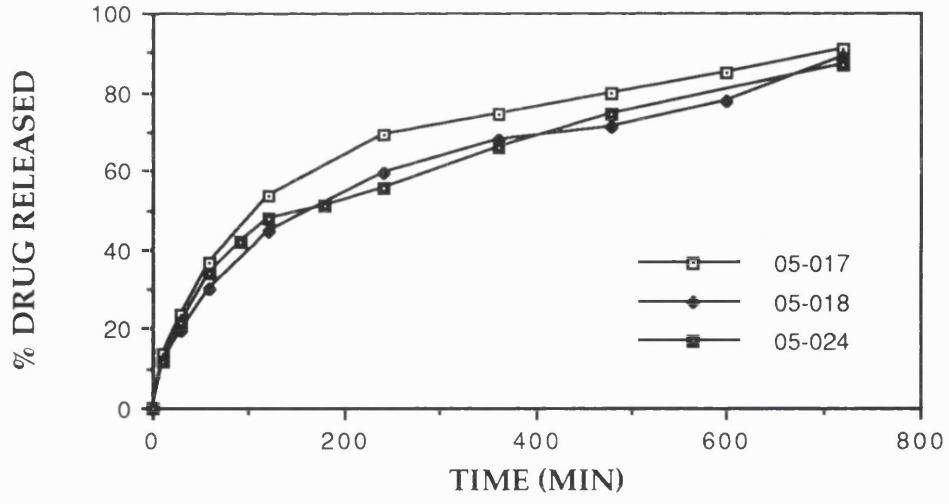


Figure 4.7 Dissolution profiles of pellets containing differing concentrations of chitosan; (05-017) 3.5%, (05-018) 5%, (05-024) 7% and a 1.6M concentration of Sodium Chloride.

and this allows comparisons between pellets release rates to be made. The release profiles are presented as constant electrolyte concentrations, at two different levels, with varying chitosan concentrations at each of these electrolyte levels.

4.4.4 DISCUSSION

The sodium chloride formulations contain the electrolyte at 0.8 and 1.6M concentrations with different concentrations of chitosan. The formulations depend on the water added to form a plastic mass that will deform into pellets. The formulations 05-023 and 05-024 are identical except that they form pellets with different amounts of added water. The density of the spheres is similar, and the formulation with the increased water added (05-024) loses slightly more water on drying to constant weight. The formulations with an increased amount of added water 05-017, 05-018 and 05-021 appear to lose more water on drying. The density of the pellets remains similar to the pellets of the other electrolytes. The sieving of the pellets shows 05-023 and 05-024 have similar size distribution patterns as expected, as only the water content varies. The other formulations show narrow size distribution ranges, 05-017 and 05-021 both show over 70% of the pellets less than 2mm in size.

The dissolution profiles for the formulations containing 0.8M electrolyte concentration are shown in Figure 4.6. The formulation with the higher concentration of chitosan (05-021) has a faster initial release for the first three hours and then tapers off much more slowly. Fifty percent release is seen after three hours, comparable to the formulation with the lower chitosan concentration which subsequently shows a much faster release of the drug. Figure 4.7 shows the release profiles for the formulations containing 1.6M electrolyte concentrations with varying chitosan concentrations. The release rate is faster for the first four hours and then tapers off in a zero order manner. The formulation containing the least amount of chitosan (05-017) releases the drug fastest from the matrix while the formulation with the highest concentration of chitosan (05-024) is the slowest releasing. The dissolution profile for 05-023 (7% chitosan) is not shown as it is identical to that of 05-024. Fifty percent release is

seen after two hours with the formulation containing the least amount of chitosan and the formulation with the highest concentration of chitosan takes in excess of three hours to release fifty percent of the drug. Both concentrations of sodium chloride show the concentration effect of chitosan on the release profile.

4.5 POTASSIUM HYDROGEN CARBONATE

Potassium hydrogen carbonate is the fourth electrolyte investigated in this series of experiments. The salt is incorporated at the same molar concentrations as the previous electrolytes.

4.5.1 FORMULATIONS

The formulations consist of a constant concentration of Avicel, lactose and drug (diclofenac sodium); 34%, 27% and 5% w/w respectively and the chitosan content varies with the electrolyte concentration as shown in Table 4.10. The formulations are mixed as described for the other salts with the water added to form a plastic mass that will readily deform in the spheronizer to form spheres. The extrudate is formed using the LLoyds ram extruder (Section 2.1.2). The die size is kept constant (2 x 2 die), as is the extruder speed at 200mm/min. The water content is critical in deciding which formulations will form spheres as with the other electrolytes.

	06-007	06-011	06-016	06-017	06-021	06-023
CHITOSAN	5%	3.5%	3.5%	5%	7%	7%
KHCO3	0.8M	0.8M	1.6M	1.6M	1.6M	0.8M
WATER	50G	36G	80G	70G	84G	98G
PRODUCT	SPHERES	SPHERES	SPHERES	SPHERES	SPHERES	SPHERES

Table 4.10 Formulations of mixtures containing Potassium Hydrogen Carbonate.

4.5.2 ANALYSIS OF THE PELLETS

The pellets are analysed in a number of different ways (Section 2.1.6) and the percentage weight loss on drying and the density presented in Table 4.11 for the spheres containing potassium hydrogen carbonate.

	06-007	06-011	06-016	06-017	06-021	06-023
% WT LOSS ON DRYING	10.8	19.2	12.8	22.4	17.4	18.7
DENSITY (g/cm^3)	1.32	1.41	1.34	1.52	1.46	1.44

Table 4.11 Density and percentage weight loss on drying for formulations containing Potassium Hydrogen Carbonate.

The distribution of the pellets in the different sieve fractions is shown in Table 4.12 for each of the formulations. Approximately two thirds of the pellets appears to be concentrated in a given sieve fraction for each of the formulations.

	06-007	06-011	06-016	06-017	06-021	06-023
2.8MM	9.8	17.6	7.4	3.5	0.6	4.4
2.0MM	63.2	72.2	65.1	63.3	35.4	57.9
1.4MM	26.4	10.2	27.5	33.2	57.8	28.6
1.0MM	0.6	0	0	0	6.2	9.1
BASE	0	0	0	0	0	0

Table 4.12 Sieve fractions for the pellets containing Potassium Hydrogen Carbonate.

4.5.3 DISSOLUTION PROFILES

The dissolution profiles of the pellets are presented in Figures 4.8 and 4.9. The graphs show a constant molar concentration of the

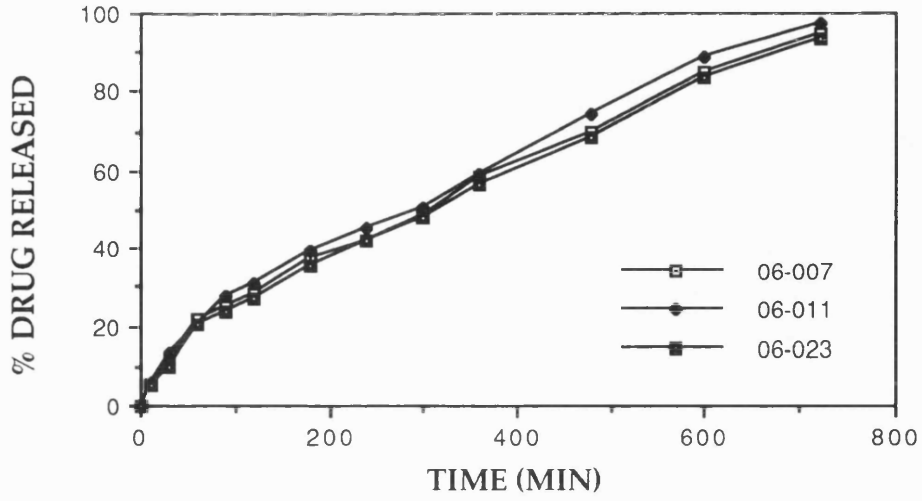


Figure 4.8 Dissolution profiles of pellets containing differing concentrations of chitosan; (06-007) 5%, (06-011) 3.5%, (06-023) 7% and a 0.8M concentration of Potassium Hydrogen Carbonate.

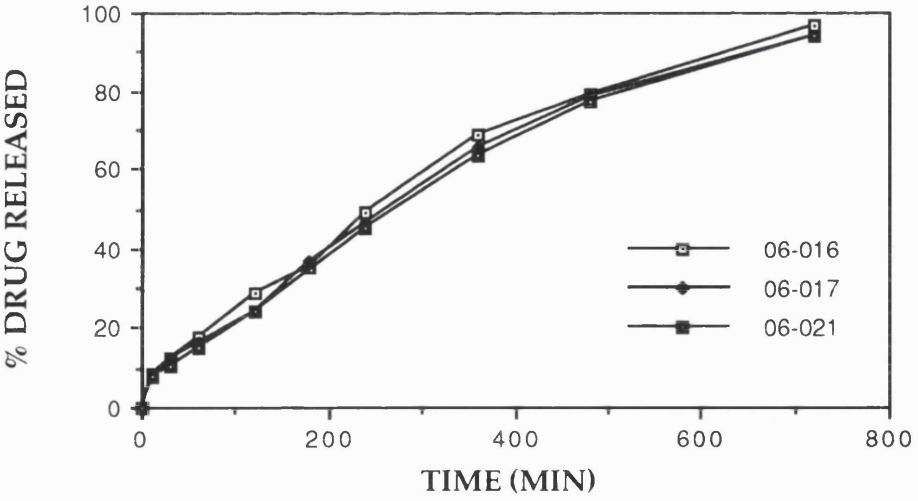


Figure 4.9 Dissolution profiles of pellets containing differing concentrations of chitosan; (06-016) 3.5%, (06-017) 5%, (06-021) 7%, and a 1.6M concentration of Potassium Hydrogen Carbonate.

electrolyte at two different levels and the chitosan concentration varies. The drug loading and sample weight are constant. The USP paddle apparatus is set up as described in Section 2.1.5 with a phosphate buffer dissolution medium. The pellets remain intact throughout the dissolution process and the samples withdrawn at various intervals are assayed using a UV spectrophotometer at an appropriate wavelength. The volume of the dissolution medium is 1000ml in all cases.

4.5.4 DISCUSSION

Six different formulations are presented containing the salt potassium hydrogen carbonate, with three levels of chitosan and two molar concentrations of electrolyte, 0.8 and 1.6M. Water is added to the mix to produce an extrudate that will spheronize into pellets. The quantity of water required varies depending on the mix and the salt concentration. Some of the pellets can lose up to one fifth of their weight on drying. The density of these pellets is similar to that reported for the other pellets containing different electrolytes. The sieving of the pellets shows that the majority are in the sieve fraction 2.0 - 2.8mm, except for the formulation 06-011 which contains slightly larger pellets and the formulation 06-021 which has 64% less than 2mm in size. In general, the pellets have narrow size distribution ranges.

The plots in Figure 4.8 show the release profiles for the pellets containing 0.8M concentration of electrolyte and three levels of chitosan. The formulation containing the least amount of chitosan, 06-011 has the fastest release, the other two formulations show similar release rates with the formulation containing the highest concentration of chitosan being the slowest releasing. The release is steady over the time period and approaches zero order. Figure 4.9 shows the higher concentration of electrolyte 1.6M and the same three levels of chitosan. The plots are similar in shape and approach zero order release. Fifty percent release is seen after ~ four hours, with the formulation containing the highest amount of chitosan, 06-021(7%) showing the slowest release rate of the drug.

The formulation containing the least amount of chitosan (3.5%), 06-016 shows the fastest release of the three levels of chitosan examined.

4.6 SCANNING ELECTRON MICROGRAPHS

Surface and internal morphology of the chitosan pellets are examined using a scanning electron microscope as described in Section 2.2.6.3. Samples of pellets, Figure 4.10 (a) - Potassium chloride, (b) - Magnesium chloride, (c) - Sodium chloride, (d) - Potassium hydrogen carbonate are examined containing each of the four electrolytes in the study.

4.7 RHEOLOGY

The rheological properties of the four electrolytes used to form gels with chitosan are examined using the Carri-med rheometer as described in Section 2.1.10.1. The gels tested were prepared by incorporating chitosan at the same two levels (10% and 20%w/w) as used for the preparation of the pellets and the electrolytes at a 0.8M and 1.6M concentration. A plot of shear rate against shear stress is then shown for each ion at the various concentrations.

4.7.1 FLOW CURVES

The temperature is kept constant at 20°C throughout the experiment. The experiments are conducted as described in Section 2.1.10.2. The samples are loaded and left to equilibrate and the flow curves measured at time zero. The sample size is kept small to avoid edge effects. The up curves are shown only, but the up curves, peak hold and down curves were measured. The less viscous gels tended to go off - scale and hence this made it more difficult to measure the down curves. The ascent time, peak hold time and descent time are set for one minute each, for each sample. The sample is subjected to a shear stress sweep over a set range of

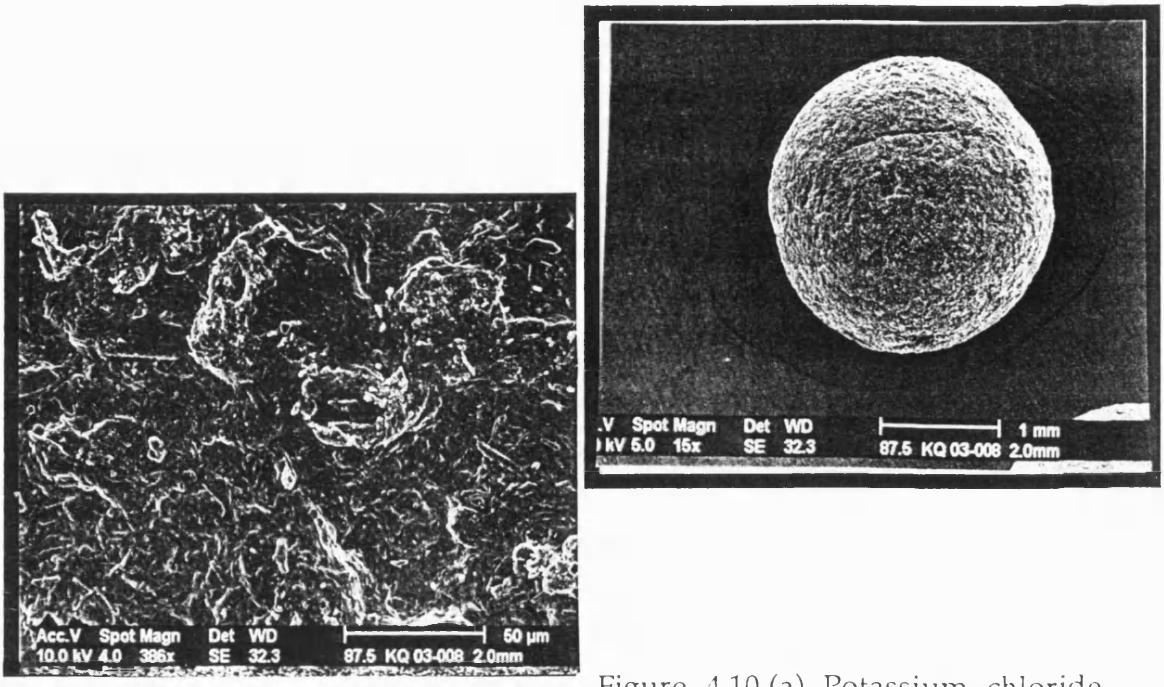


Figure 4.10 (a) Potassium chloride.

Figure 4.10 (a) shows the rounded pellet containing Potassium chloride and a view of its porous internal structure.

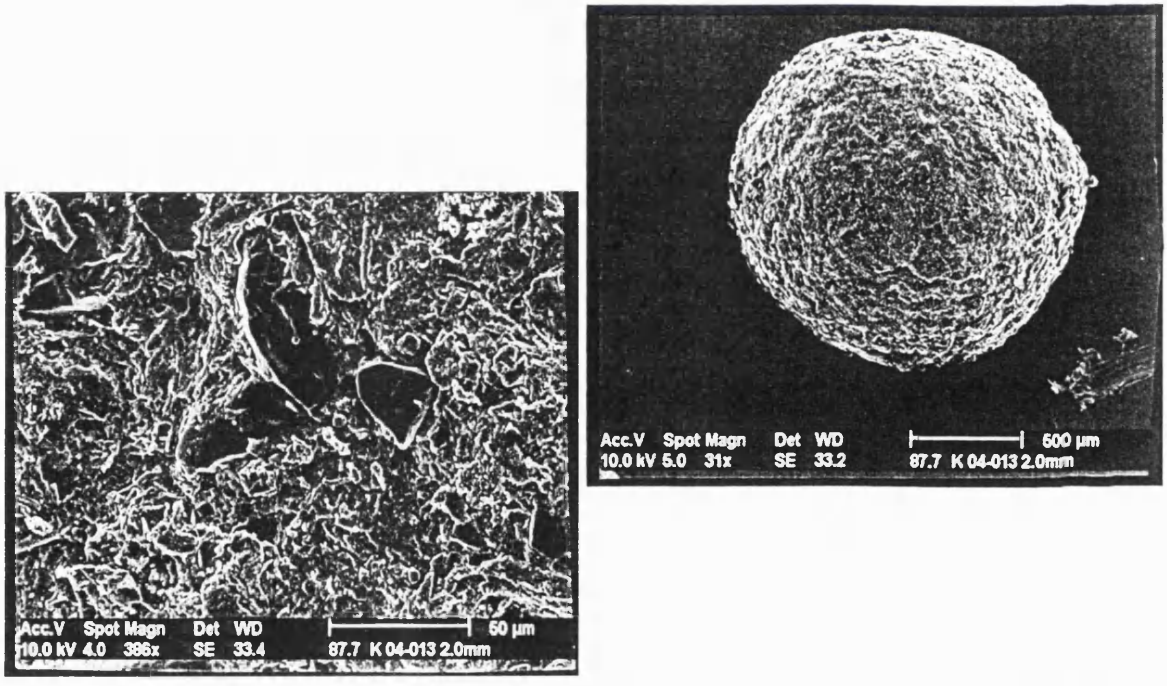
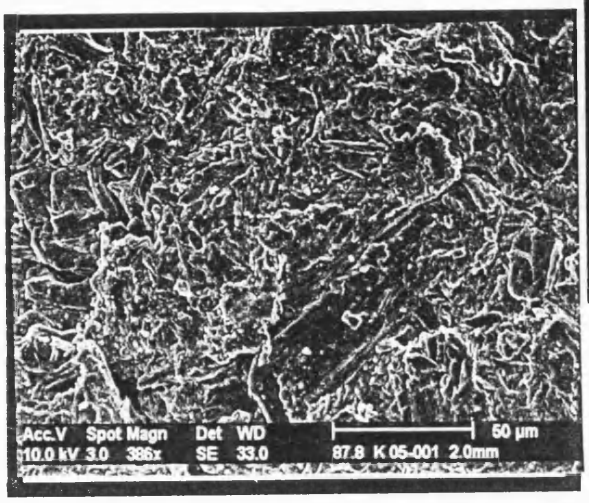


Figure 4.10 (b) Magnesium chloride.

Figure 4.10 (b) shows a view of the 2mm pellet containing Magnesium chloride with a porous surface and larger drug particles scattered throughout the matrix visible in the cross-sectional view.



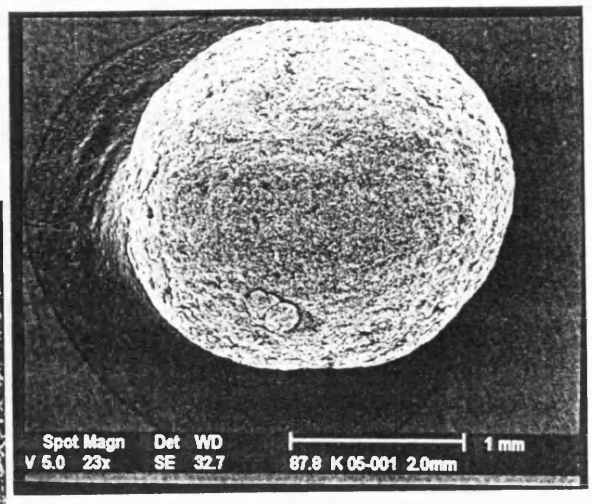
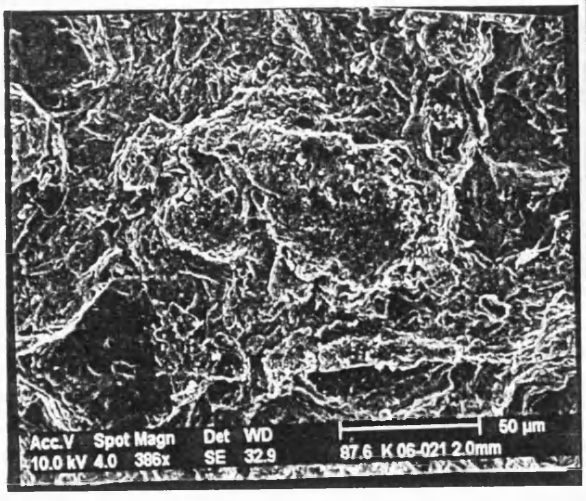


Figure 4.10 (c) Sodium chloride.

Figure 4.10 (c) shows a slightly less porous surface for the pellet containing Sodium chloride.



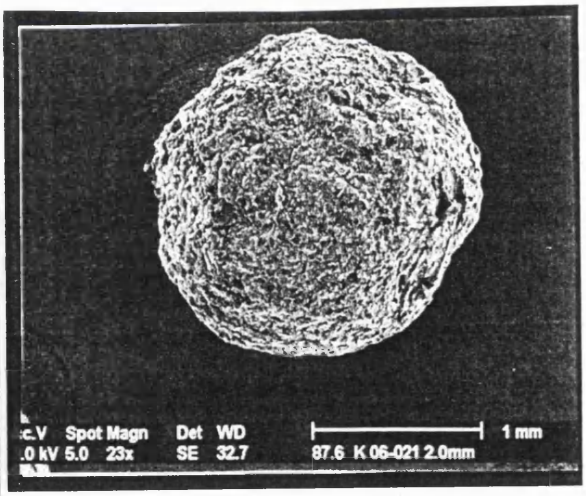


Figure 4.10 (d) Potassium Hydrogen Carbonate.

Figure 4.10 (d) shows a porous irregular surface for the pellet containing Potassium Hydrogen Carbonate and a cross-sectional view of the porous matrix.

values (determined by trial and error) and the corresponding shear rate is noted along with the viscosity of the sample. The instrument has limited application at very low values of shear stress and very high values. This can be overcome to some extent by employing a bigger gap or cone angle at low strain values and a smaller angle/gap at high shear values. Figures 4.11 and 4.12 show the flow curves for the gels containing chitosan 10%w/w and the lower and higher concentration of electrolytes respectively. Figures 4.13 and 4.14 show the plots for the gels with the higher chitosan concentration (20%w/w) and again the two levels of the various electrolytes. Figure 4.15 shows the flow curves for the two concentrations of chitosan examined dissolved in a 10% solution of acetic acid, in the absence of any electrolyte. The plots are non-Newtonian indicating pseudoplastic flow. The lower chitosan concentration is considerably less viscous as would be expected.

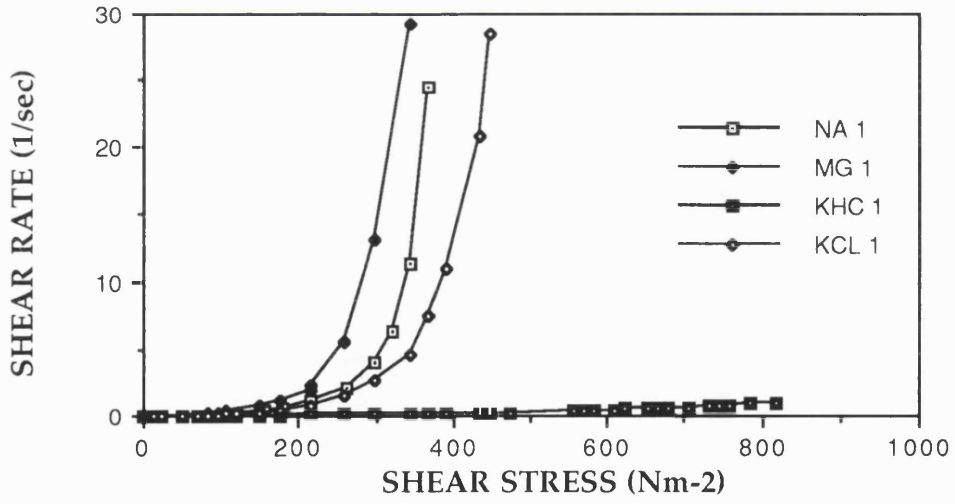


Figure 4.11 Flow curve of gels containing 10%w/w Chitosan and 0.8M concentration of the electrolyte.

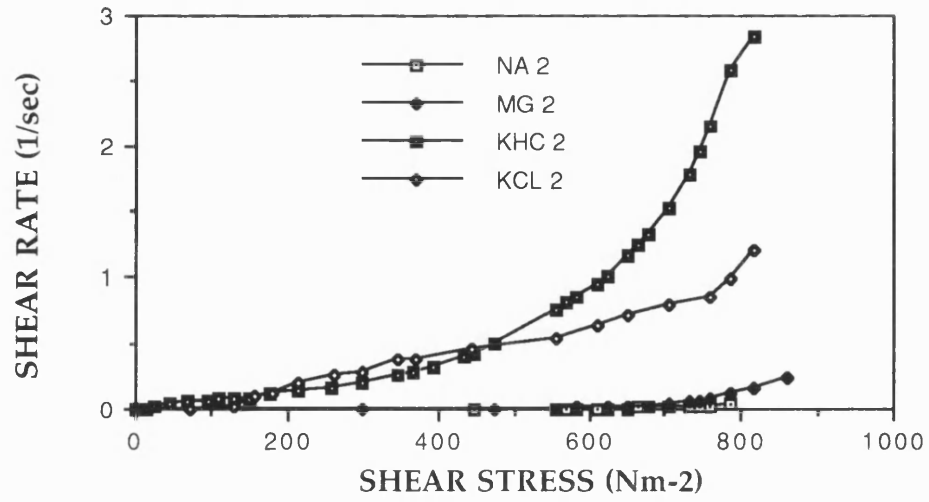


Figure 4.12 Flow curve of gels containing 10%w/w Chitosan and 1.6M concentration of the electrolyte.

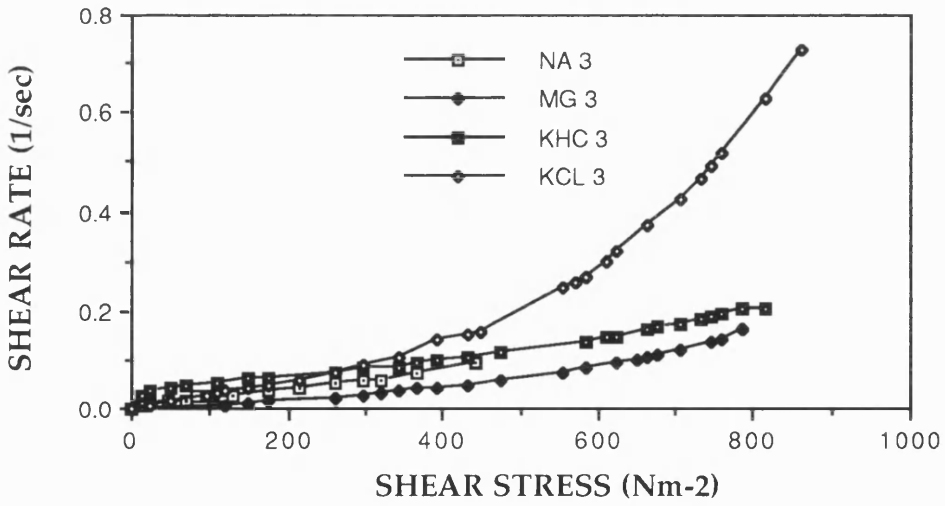


Figure 4.13 Flow curve of gels containing 20%w/w Chitosan and 0.8M concentration of the electrolyte.

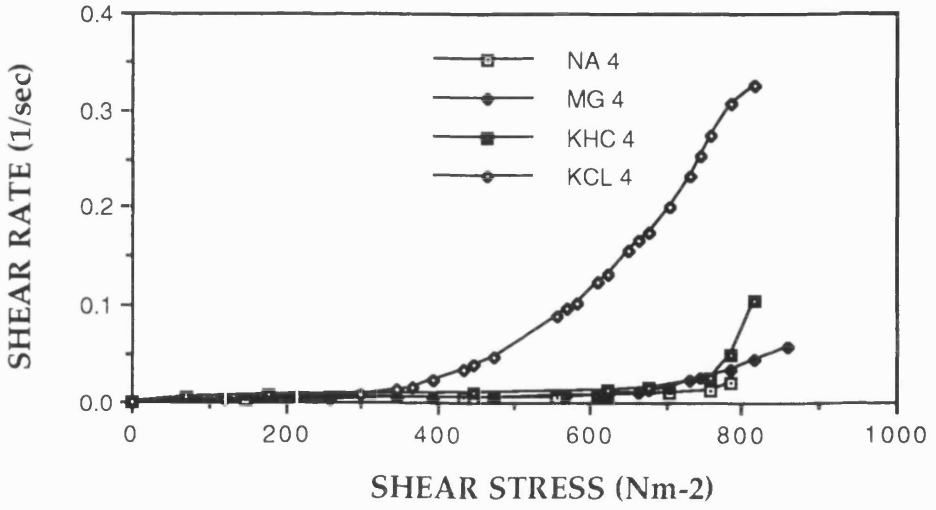


Figure 4.14 Flow curve of gels containing 20%w/w Chitosan and 1.6M concentration of the electrolyte.

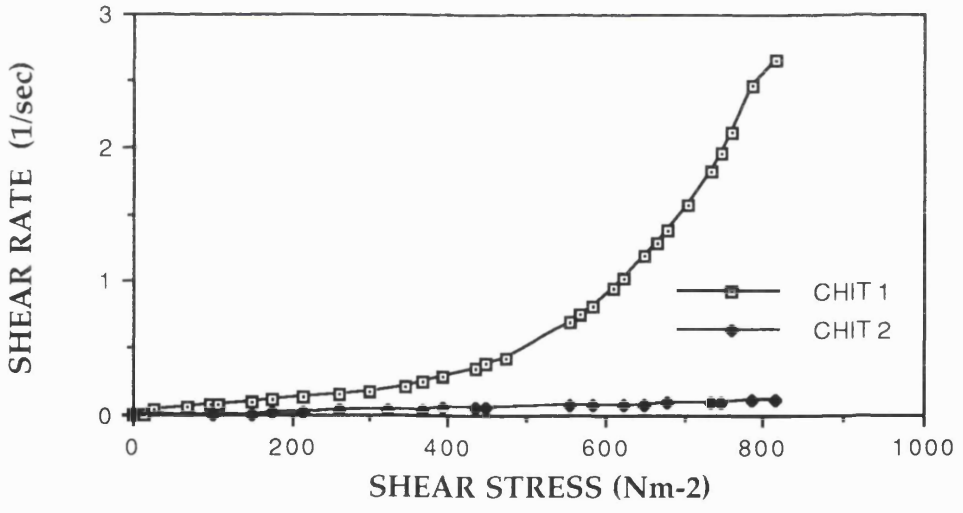


Figure 4.15 Flow curves of 10% (Chit 1) and 20% (Chit 2) w/w Chitosan in the absence of any electrolyte.

4.7.2 DISCUSSION

The flow curves shown in Figure 4.11 indicate that the viscosity of the gels appears to undergo a change at a shear stress value of ~ 200Nm-2. At lower values of shear the gels are fairly consistant. The dramatic increase in the shear rate value above the shear stress value of 200Nm-2 for the magnesium chloride, sodium chloride and potassium chloride electrolytes seems to indicate that the intermolecular associations between the electrolytes and chitosan are destroyed at high rates of shear. The curves all show pseudoplastic flow and the existance of an apparent yield value before flow begins can be seen. Figure 4.12 shows the flow curves for the gels containing the same concentration of chitosan as before but the molar concentration of electrolyte is doubled and this dramatically affects the viscosity. The four different chitosan gels containing electrolytes at the higher concentration are all much more viscous and the shear rates for the corresponding shear stresses are considerably reduced when compared to the lower electrolyte concentration. The magnesium and sodium salts show similar profiles while the potassium chloride and potassium hydrogen carbonate salt also show similar profiles and are slightly less viscous.

Figure 4.13 and 4.14 show the plots of the gels containing the higher concentration of chitosan (20%w/w) and the lower and higher electrolyte concentrations respectively. The viscosity of this set of gels is increased in comparison with the gels containing just 10% chitosan. The gels with the lower salt concentrations show similar profiles, until at 600Nm-2 the potassium chloride gel shows an increased shear rate compared to the others due to a breakdown in its structure. Magnesium, the divalent ion, forms the most viscous gel and this implies that it forms a stronger association with the chitosan polymer. As the shear stress increases these intermolecular bonds tend to dissociate and the gels become less viscous. Figure 4.14 shows the flow curves for the higher salt concentration and here the shear rate for the corresponding shear stress is the lowest of all for the various gels examined. Above a shear stress value of 400Nm-2 the shear rate increases dramatically for the potassium chloride salt

as the viscosity of the gel decreases. The other electrolytes all show similar profiles.

4.8 CONCLUSION

In conclusion, these flow curves for the gels containing chitosan and an electrolyte at different concentrations show that the higher the concentration of chitosan in the gel, the more viscous it is. The concentration of electrolyte also has an effect and doubling the electrolyte concentration results in an increase in the shear rate at a fixed stress. The potassium chloride salt appears to form the least viscous gel overall and this may be as it forms the weakest interactions with the chitosan polymer and at high rates of shear, these interactions are easier to breakdown than those formed between chitosan and the other electrolytes. The magnesium ion appears to form the strongest bonds of the salts studied. The chitosan gel, in the absence of any salt is shown to be slightly more viscous than the corresponding gel containing an electrolyte. The analysis of the flow curves shows that the pseudoplastic model is the best fit for the chitosan gels. The shear thinning character is suggestive of the breakdown of a weak network or gel structure and this is generally associated with the existance of a rigid chain conformation of a polymer.

Therefore the higher electrolyte concentrations at both of the chitosan concentrations show that sodium and magnesium form the two more viscous gels while potassium hydrogen carbonate and potassium chloride form the less viscous gels. At the higher chitosan concentrations the order of viscosity of the gels remains unchanged despite the change in electrolyte concentration and Magnesium chloride > Sodium chloride > Potassium hydrogen carbonate > Potassium chloride.

CHAPTER 5

THE INFLUENCE OF ACIDS ON THE FORMATION & RELEASE PROPERTIES OF PELLETS CONTAINING CHITOSAN/DICLOFENAC

5.1 INTRODUCTION

Chitosan has been shown to be insoluble in water, alkali and organic solvents, but soluble in most organic acids when the pH of the solution is less than six (Muzzarelli, 1973). Standard grades of chitosan require the addition of an acid to solubilise in water. Acetic and formic acids are two of the most widely used acids to dissolve chitosan. Some dilute inorganic acids, such as nitric acid and perchloric acid have also been used to prepare a chitosan solution, but only after prolonged stirring and warming. Compared with the more common organic acids, the solubility in inorganic acids seems to be limited with regard to the concentration ratio of chitosan/acid. The concentration ratio between chitosan and the acid is very important, particularly in the case of mineral and multi-protonic acids.

Sulphuric acid does not dissolve chitosan because it forms chitosan sulphate which is a white crystalline solid. Precipitated chitosan (as opposed to the powdered form) can be more easily dissolved by hydrochloric and nitric acids. Concentrated hydrochloric acid at high temperatures has been shown to produce degradation of the polymer. Skaugrud, (1989) lists viscosity readings for 1% chitosan in various organic acids at different concentrations, including oxalic, citric, formic, proprionic and succinic acids at low values of pH. On studying the solubility of chitosan in organic acids, concentrations as high as 50% have been shown to produce workable solutions, as seen with acetic, lactic and propionic acid. In the case of formic acid it is possible to dissolve chitosan in the pure acid.

This study involves using various organic acids (at equimolar concentrations) to dissolve chitosan and subsequently incorporate the chitosan gel in the formulation of a matrix pellet by extrusion-spheronization. Acetic acid has been used previously to dissolve chitosan powder and forms a gel that is incorporated into the matrix pellet. Acetic acid, however possesses a number of disadvantages; a pungent odour and the ability to corrode the stainless steel barrel and dies, if left in contact for any length of time. The in-vitro release profiles of these pellets, formed with the various acids are

then determined. The rheological properties of these gels are also investigated to see if a relationship exists between the viscosity of the given gels and the dissolution profile for the pellets. A simple flow curve is presented for each of the gels formed with the various acids.

Chitosan has a high molecular weight and consists of a long linear chain of molecules; (1 - 4) linked glycans with the number of glucosamine units dependent on the degree of deacetylation. On reaction with an acid a certain minimum amount is needed to make the glucosamine units into the soluble form. The repelling effect of these positively charged units on each other will result in an extended conformation of the polymer in solution and hence differences in their rheological profiles. The differences in the acids studied to reach their viscosity peak should reflect the buffering capacities of the various acids.

5.2 FORMULATIONS

These formulations are prepared as described in Section 2.2.1, where the dry powders are mixed initially with the chitosan gel and finally enough water is added to produce a plastic mass that will extrude and deform in the spheronizer to form pellets. The mixes are left to stand for one hour and then extruded. The chitosan gels are formed by dissolving the chitosan powder in the organic acid while stirring continuously. The amount of water is found to be critical in these formulations and some formulations appear to form pellets with different amounts of water. The formulations of the various mixes is shown in Table 5.1. The quantity of Avicel, lactose and drug (diclofenac sodium) is kept constant throughout this study and the molarity of the acid solution is consistant to enable comparisons to be made between the different formulations. The formulations all contain 35% Avicel, 28% lactose, 5% diclofenac sodium and 32% of the chitosan gel presented as a percentage of the dry weight. Additional water is added to the formulations but the quantity required to produce spheres varies. The chitosan gel consists of 8% chitosan dissolved in a 1.6M concentration of the particular

acid. The acids in the study are maleic, ascorbic, lactic, hydrochloric, tartaric, citric and acetic acids.

FORMULA	F-MAL-03	F-MAL-09	F-ASC-05	F-LAC-03	F-LAC-05
ACID	1.6M	1.6M	1.6M	1.6M	1.6M
WATER	10G	12G	12G	18G	17G
RESULT	SPHERES	SPHERES	SPHERES	SPHERES	SPHERES
EXTRUSION FORCE (kN)	3.45	3.36	2.59	2.59	3.26

MAL - Maleic ASC - Ascorbic LAC - Lactic

FORMULA	F-HCL-06	F-HCL-07	F-HCL-08	F-HCL-09	F-HCL-05
ACID	1.6M	1.6M	1.6M	1.6M	1.6M
WATER	50G	55G	40G	45G	58G
RESULT	SPHERES	SPHERES	SMALL SPHERES	SPHERES	LARGE SPHERES
EXTRUSION FORCE (kN)	2.04	1.73	1.28	1.65	2.02

HCL - Hydrochloric

FORMULA	F-TAR-03	F-TAR-07	F-CIT-03	F-CIT-04	F-ACE-02
ACID	1.6M	1.6M	1.6M	1.6M	1.6M
WATER	8G	24G	38G	36G	35G
RESULT	SPHERES	SPHERES	WET	DUMBELL	SPHERES
EXTRUSION FORCE (kN)	3.85	2.89	2.83	3.53	2.55

TAR - Tartaric CIT - Citric ACE - Acetic

Table 5.1 Formulations of extruded mixtures containing Avicel, Lactose, Drug and Chitosan dissolved in various Acids, that produce Pellets and their Extrusion Forces.

The mixes to be extruded are packed into the barrel of the LLoyds MX50 physical testing machine (Section 2.2.2). The mixes are all extruded through a 2 x 2 die at a speed of 200mm/min. The extrusion forces are low as seen in Table 5.1. The extrudate is then

spheronized at a speed of 1000rpm for 15 minutes and the resulting pellets are dried in an open air oven to constant weight. The formulations that produce spheres were determined by trial and error. Any formulation that produces dumbells had increasing quantities of water added until a rounded pellet resulted or large pellets that agglomerated were produced. Citric acid formulations did not produce spheres and did not readily deform in the spheronizer. Two formulations one too wet and the other too dry are included in the table for comparison purposes. Hydrochloric acid on the other hand readily produced spheres with various quantities of water.

The extrudate produced in most cases is of good quality, smooth and deforms readily. However, some sharkskinned extrudate is produced by different formulations at low extrusion forces. The occurrence of sharkskinning is thought to depend on the length of the die and the properties of the material mixes. The length of time for mixing is kept constant in all cases as is the die length, so the answer appears to lie in the properties of the individual mixes. The strength and elastic properties of the various gels and the quantity of water required, are critical factors in producing a formulation that forms a smooth extrudate and that will deform in the spheronizer.

5.3 ANALYSIS OF THE PELLETS

The percentage weight loss on drying for the pellets produced is shown in Table 5.2 along with the density of the pellets measured using the Beckmann pycnometer (Section 2.2.6.2).

The average weight loss on drying appears to be between 20 and 30% with the citric acid dumbells/pellets showing the lowest water loss and producing quite dense pellets. The citric acid formulations did not produce the rounded pellet shapes similar to the other formulations but produced dumbell shapes and on addition of extra water produced agglomeration of pellets. The pellets in general show a narrow distribution in terms of density.

PRODUCT	% WT LOSS ON DRYING	DENSITY (g/cm^3)
F-MAL- 03	30.6	1.37
F-MAL-09	31.1	1.41
F-ASC- 05	24.8	1.42
F-LAC-03	25.7	1.35
F-LAC-05	24.5	1.33
F-HCL-05	34.6	1.28
F-HCL-08	38.2	1.34
F-TAR-03	22.3	1.46
F-TAR-07	29.2	1.44
F-CIT-03	12.6	1.56
F-CIT-04	11.2	1.58
F-ACE-02	18.7	1.38

Table 5.2 Density and % Weight Loss on Drying for the Pellets.

5.3.1 SIEVE ANALYSIS

The pellets were sieved and the results for the hydrochloric acid pellets are shown in Table 5.3 below. The other pellets all showed > 65% within the range 1.4 -2.0 mm and this was used in the dissolution tests.

Sieve	2.8mm	2.0mm	1.4mm	1.0mm	710um	Base
F-HCL-05	16.9	68.5	14.6	0	0	0
F-HCL-06	0	6.5	73.7	16.1	3.7	0
F-HCL-08	0	0	33.4	59.5	7.1	0

Table 5.3 Sieve Analysis for Hydrochloric Acid Pellets presented as cumulative percent oversize.

5.4 DISSOLUTION STUDY

The formulations that produced pellets were tested in a dissolution apparatus employing the USP paddle method. A constant weight of pellets (0.5g) is placed in the dissolution apparatus as described in Section 2.2.5. None of the pellets floated in the medium (phosphate buffer pH 7.4) and all remained intact after the dissolution run. Each run is performed in triplicate and the mean result is shown here.

Figure 5.1 shows the release profiles for the pellets containing either lactic or ascorbic acid over a time period of eighteen hours. The release is steadily increasing for the first eight hours and then begins to level off. The lactic acid formulations are identical apart from the fact that the formulation F-LAC -03 contains slightly more added water initially but also lost more water on drying and was extruded at a lower force. The pellets tested in the dissolution medium were all in the sieve size range of 1.4 - 2.0mm where the largest percentage of the pellets lay apart from some of the hydrochloric acid formulations. The two formulations have similar release profiles with the ascorbic acid pellets showing fractionally slower release initially and then marginally faster after eight hours. Fifty percent release is seen after ~ four hours for the ascorbic acid pellet and after ~ three and a half hours for the lactic acid pellet. The weight of sample and size range is kept constant, so the surface area should be equivalent and the differences in dissolution profiles appear to be due to the acids and their interaction with the polymer. A plot of the release rate against time for these formulations is shown in Figure 5.6/7. The release rate is calculated from the slopes obtained at certain time intervals for each of the formulations from a plot of the amount of drug released in milligrams vs time.

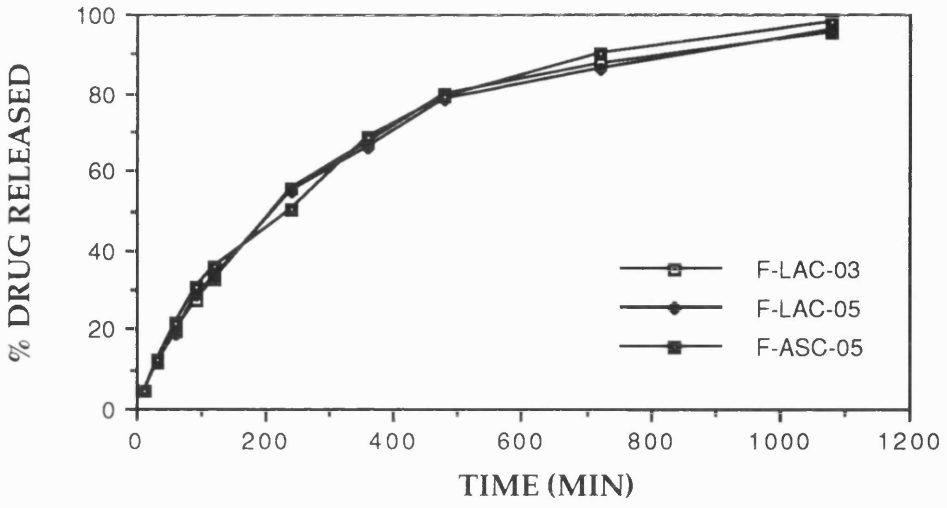


Figure 5.1 Dissolution Profiles for Pellets containing either Lactic or Ascorbic Acid.

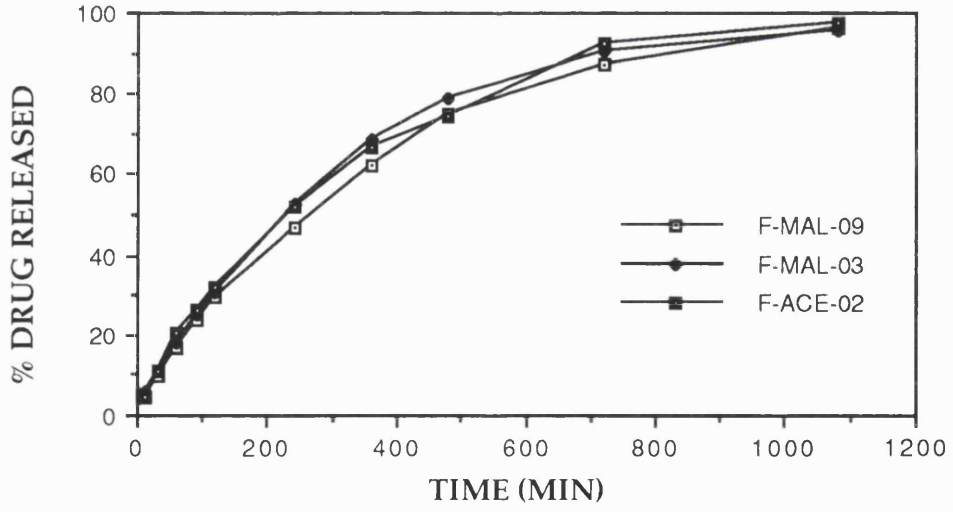


Figure 5.2 Dissolution Profiles for Pellets containing either Maleic or Acetic Acid.

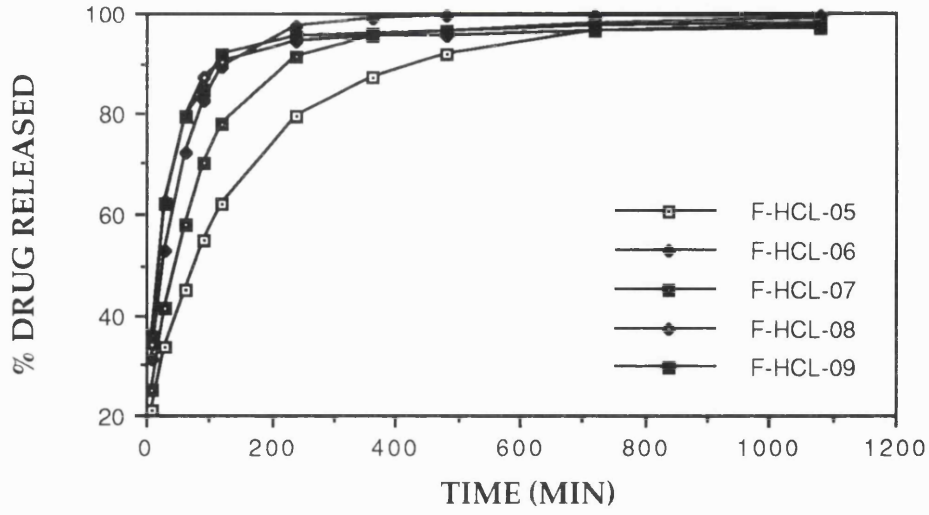


Figure 5.3 Dissolution Profiles for Pellets containing Hydrochloric Acid.

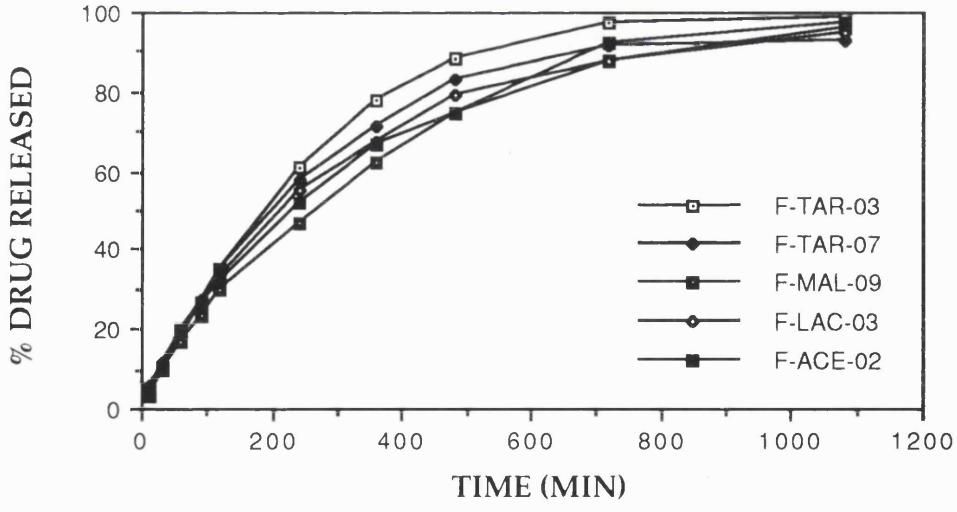


Figure 5.4 Dissolution Profiles for Pellets containing Lactic, Tartaric, Maleic or Acetic Acid.

Figure 5.2 shows the dissolution profiles for the formulations containing either maleic or acetic acids. All three formulations have similar shaped curves with F-MAL-09 being the slowest releasing formulation. The time to reach fifty percent release is ~ three and a half hours for F-MAL-03 and F-ACE-02, with F-MAL-09 taking ~ four and a half hours. The formulation F-MAL-09 contains slightly more added water than the other maleic acid formulation although both form spheres and had similar extrusion force profiles. The pellet samples are once again taken from the same size range as before, which contains the highest percentage of pellets as determined by sieve analysis. Maleic acid has two pKa values and is a polyprotic acid as is tartaric acid and citric acid. The ionisation of these acids probably takes place in stages.

Figure 5.3 shows the dissolution profiles for the hydrochloric acid formulations. Hydrochloric acid is the strongest acid in this series and therefore has the lowest pKa value. This formulation produces pellets with varying amounts of added water, over a range of values. Some of the pellets produced are of a larger size than others and all of the formulations have very low extrusion force values. The density of these pellets is seen to be lower than the other pellet formulations. These pellets are faster releasing than the other formulations and fifty percent release is seen after just 90 min. for the slowest releasing; which is the largest pellet, and after 30 - 40 min. for the other pellets. The shape of these dissolution curves is slightly different to the other formulations as the release is quite fast for the first three hours and then levels off. The effect of the size of the pellet is seen again here with the larger pellets releasing the drug fastest and the smaller pellets F-HCL-07 being slower releasing as they have less surface area and a smaller matrix size available to the dissolution medium.

The dissolution profile of the tartaric acid pellets is shown in Figure 5.4 along with the profiles of lactic, maleic and acetic acid pellets for comparison purposes. They all share the same shape profile and the maleic acid pellets are the slowest releasing and the tartaric acid pellets are the faster releasing of this selection. Fifty percent release for the tartaric acid pellets is seen after three hours

compared to four and a half hours for the maleic acid F-MAL-09 pellets. F-TAR-07 contains three times as much added water as F-TAR-03 and both produce pellets with the former being the slower releasing pellet.

The release rate of the drug diclofenac sodium from the acetic and maleic acid pellets is shown in Figure 5.5. The release rate for the acetic acid pellet levels off at 120 min. compared to 240 min. for the maleic acid pellets which are faster initially, but also level off to a steady rate. The graphs have three overlap points and follow the same general shape; faster release for the first 120 min. and then a slowing down in the rate of drug release.

Figure 5.6 shows the release rate vs time for the lactic acid pellets. The release rate is similar initially to the previous two pellets examined but the release rate levels off in a gradual linear fashion. This pellet is the closest to zero order release.

The release rates for the ascorbic and tartaric acid pellets can be seen in Figure 5.7. The ascorbic acid pellet shows a gradual linear release also similar to that of the lactic acid pellet, approaching zero order. Tartaric acid pellets on the other hand, release the drug faster initially and then appears to level off at 60 min. and subsequently the rate of release increases again before levelling off. The tartaric acid pellets show the highest release rates of the pellets examined so far.

The three hydrochloric acid pellets, F-HCL-05, F-HCL-07 and F-HCL-08 are examined in Figure 5.8. The main difference in these pellets is the water content and their size. The F-HCL-05 formulation has the larger size pellets and the F-HCL-08 has the smallest sized pellets. The release rate is fastest here for the first 60 min. and then tails off. These pellets release faster than any of the others examined. The smallest spheres F-HCL-08, are the fastest releasing followed by the medium sized pellets and the larger pellets have the slowest release rates. After 120 minutes the release rates level off for all the

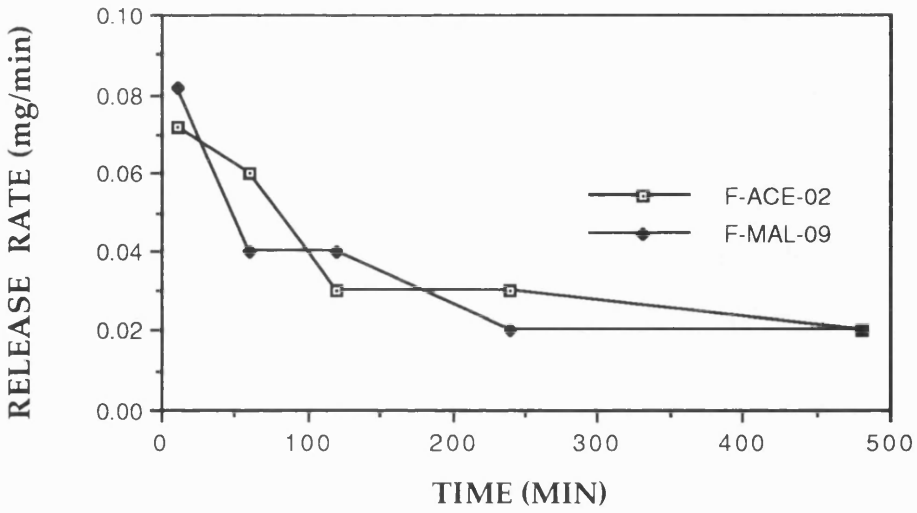


Figure 5.5 Release Rate of Drug (diclofenac) from Acetic and Maleic Acid Pellets.

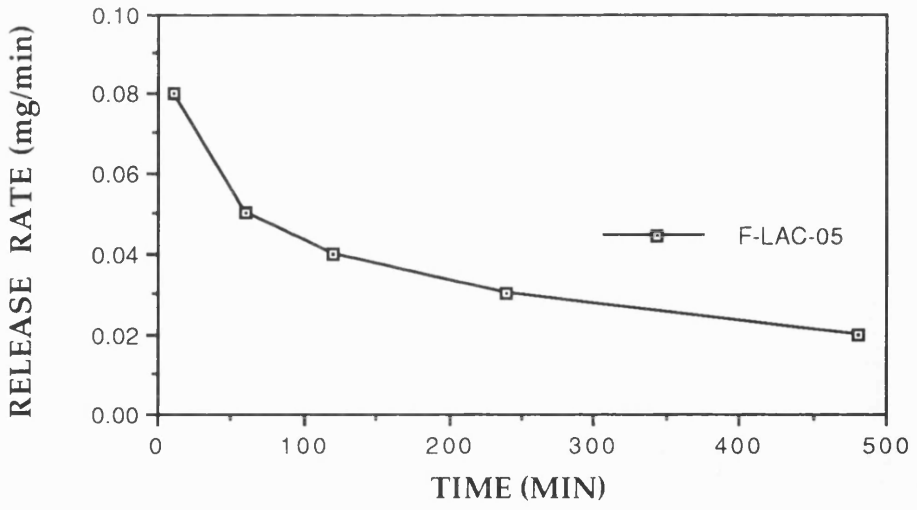


Figure 5.6 Release Rate of Drug (diclofenac) from Lactic Acid Pellets.

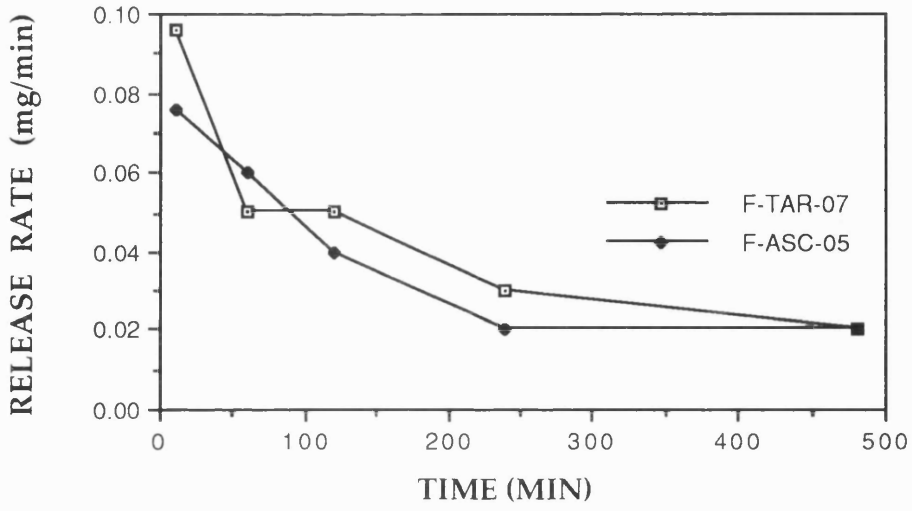


Figure 5.7 Release Rate of Drug (diclofenac) from Tartaric and Ascorbic Acid Pellets.

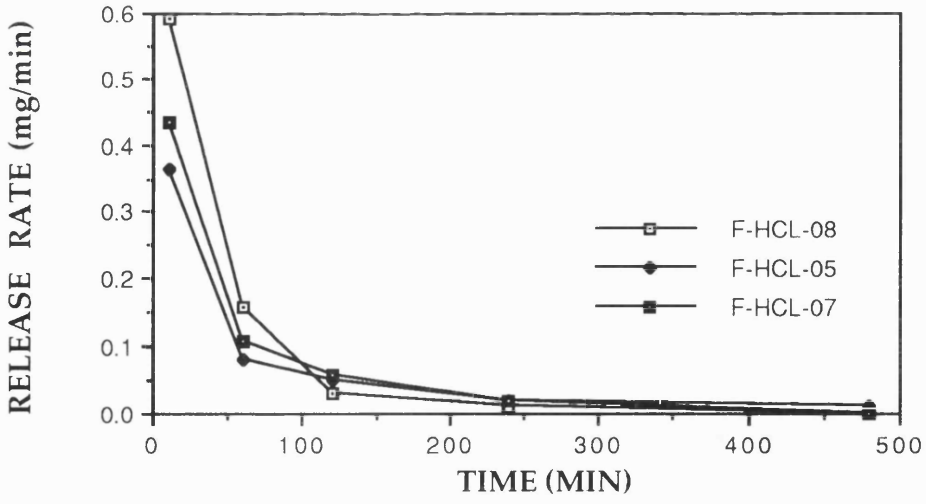


Figure 5.8 Release Rate of Drug (diclofenac) from Hydrochloric Acid Pellets.

pellets. These pellets show differences to the other pellets in that the release rates are much higher for the first 120 min. and then reach a plateau.

5.5 SCANNING ELECTRON MICROGRAPHS

Scanning electron micrographs are shown for the pellets, either the whole or cross - sectional area and also the cross-sectional area viewed at a higher magnification. The pellets were coated for four minutes using an Emitech K550 coater and scanned with a Philips XL20 microscope as described in Section 2.2.6.3. The micrographs for each of the seven acids are shown in Figure 5.9 (a) Acetic acid - (b) Ascorbic acid - (c) Citric acid - (d) Lactic acid - (e) Maleic acid - (f) Tartaric acid - (g) Hydrochloric acid.

5.6 SWELLING INDEX

This swelling index test is adapted from the British Pharmacopeia 1993. The test is performed as described in Section 2.2.9 for each of the acid solutions and chitosan. The swelling index is calculated from the mean of three tests. The results are shown below;

Ascorbic acid - 24.1
Acetic acid - 25.2
Citric acid - 25.0
Hydrochloric acid - 22.4
Lactic acid - 25.2
Maleic acid - 25.2
Tartaric acid - 25.2

The hydrochloric acid solution after standing for three hours with the chitosan, formed the least viscous gel and still had a 'runny' consistency in contrast to the other acids, which all formed much more viscous gels. The ascorbic acid gel also showed a lower

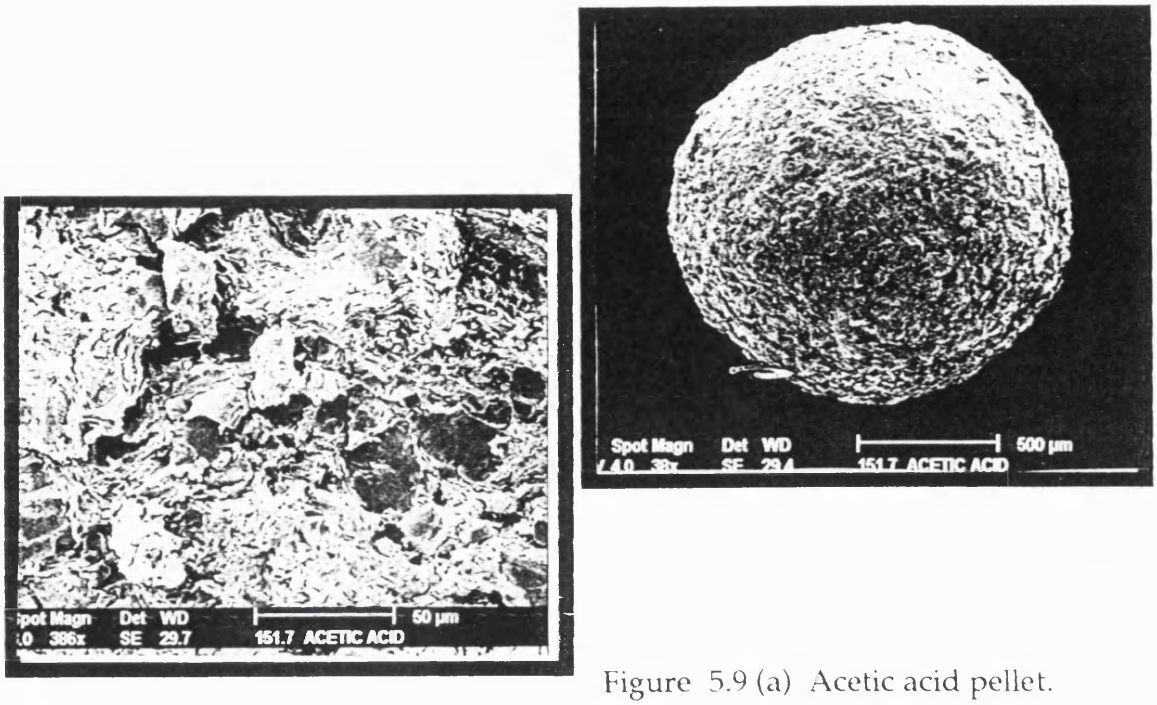


Figure 5.9 (a) Acetic acid pellet.

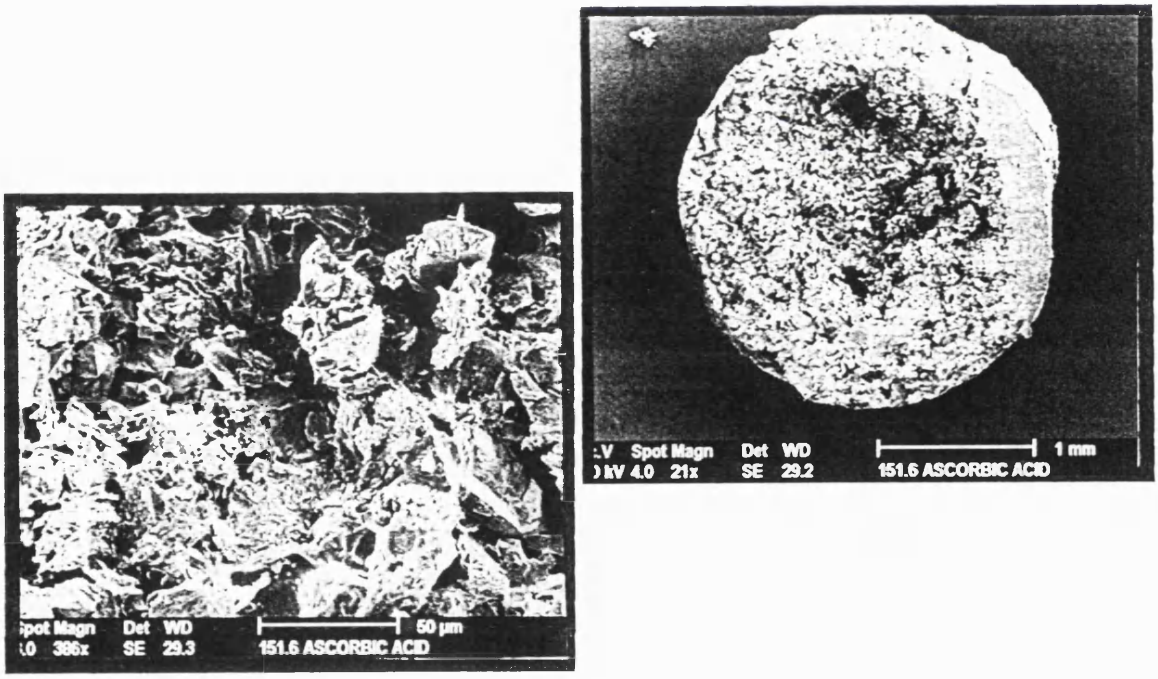
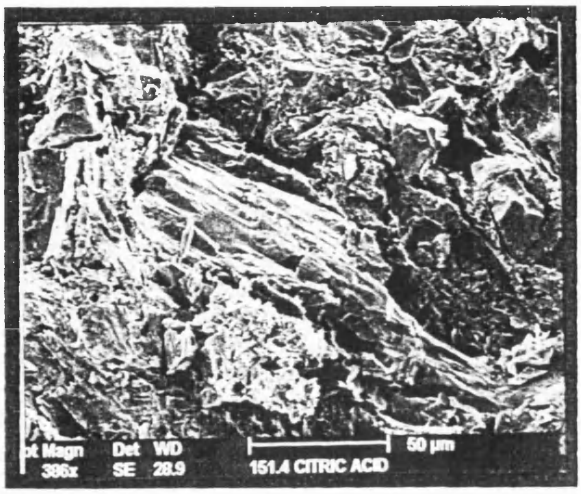


Figure 5.9 (b) Ascorbic acid pellet.



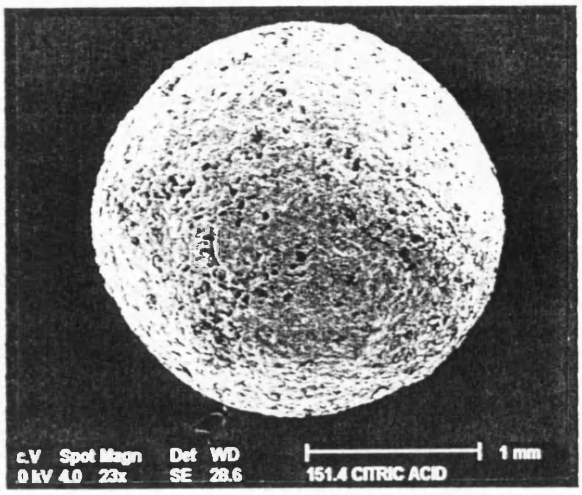
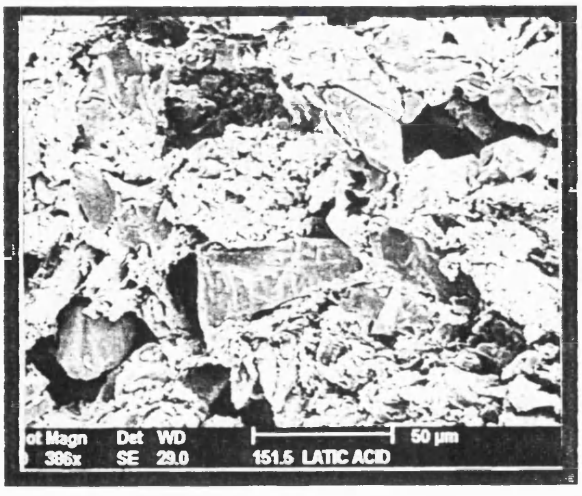


Figure 5.9 (c) Citric acid pellet.



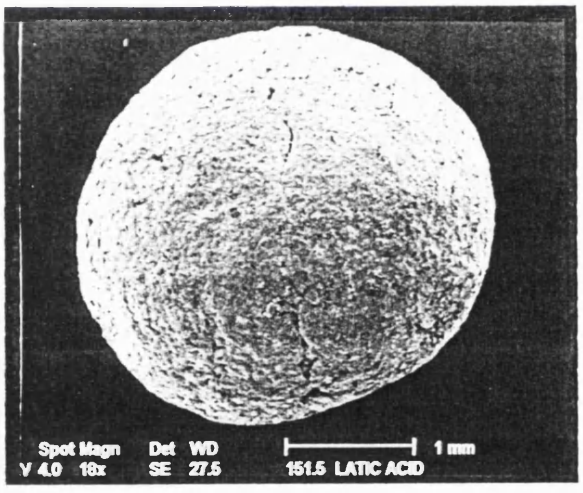


Figure 5.9 (d) Lactic acid pellet.

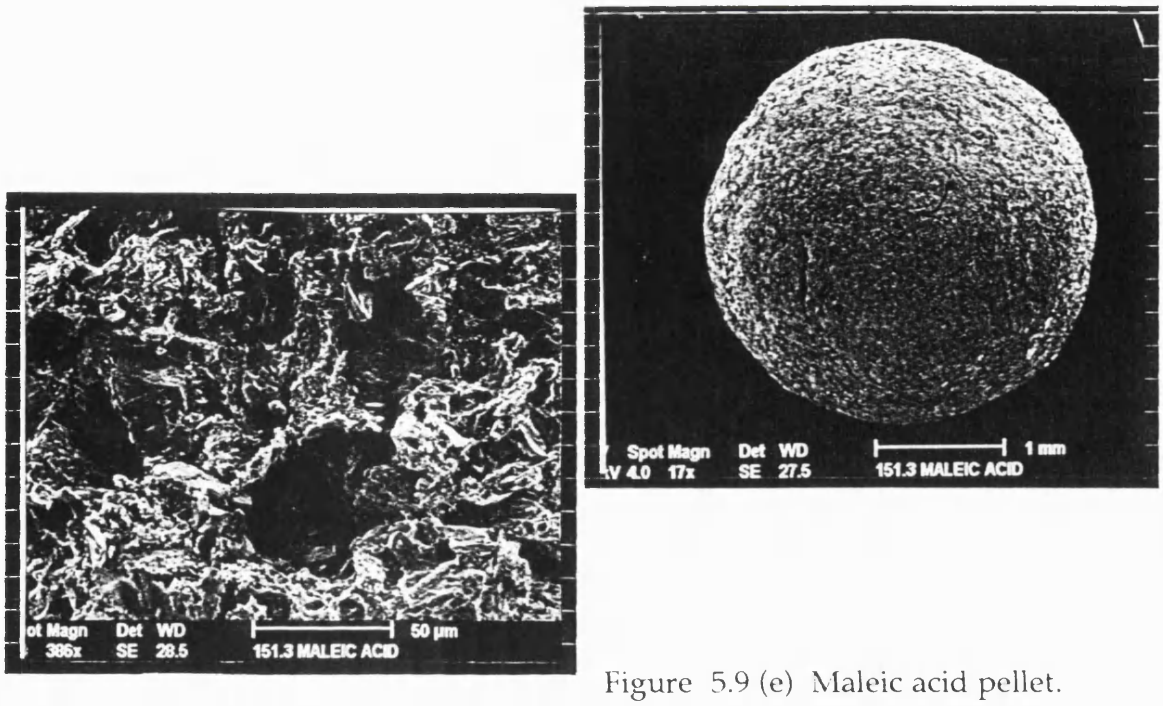


Figure 5.9 (e) Maleic acid pellet.

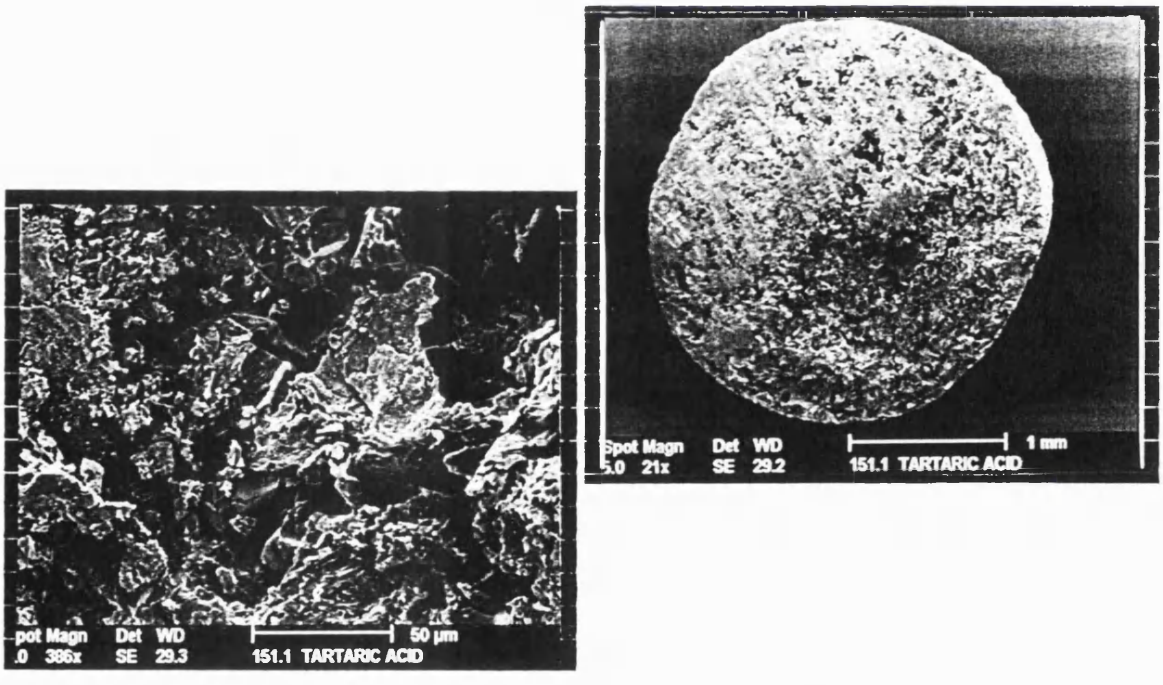


Figure 5.9 (f) Tartaric acid pellet.

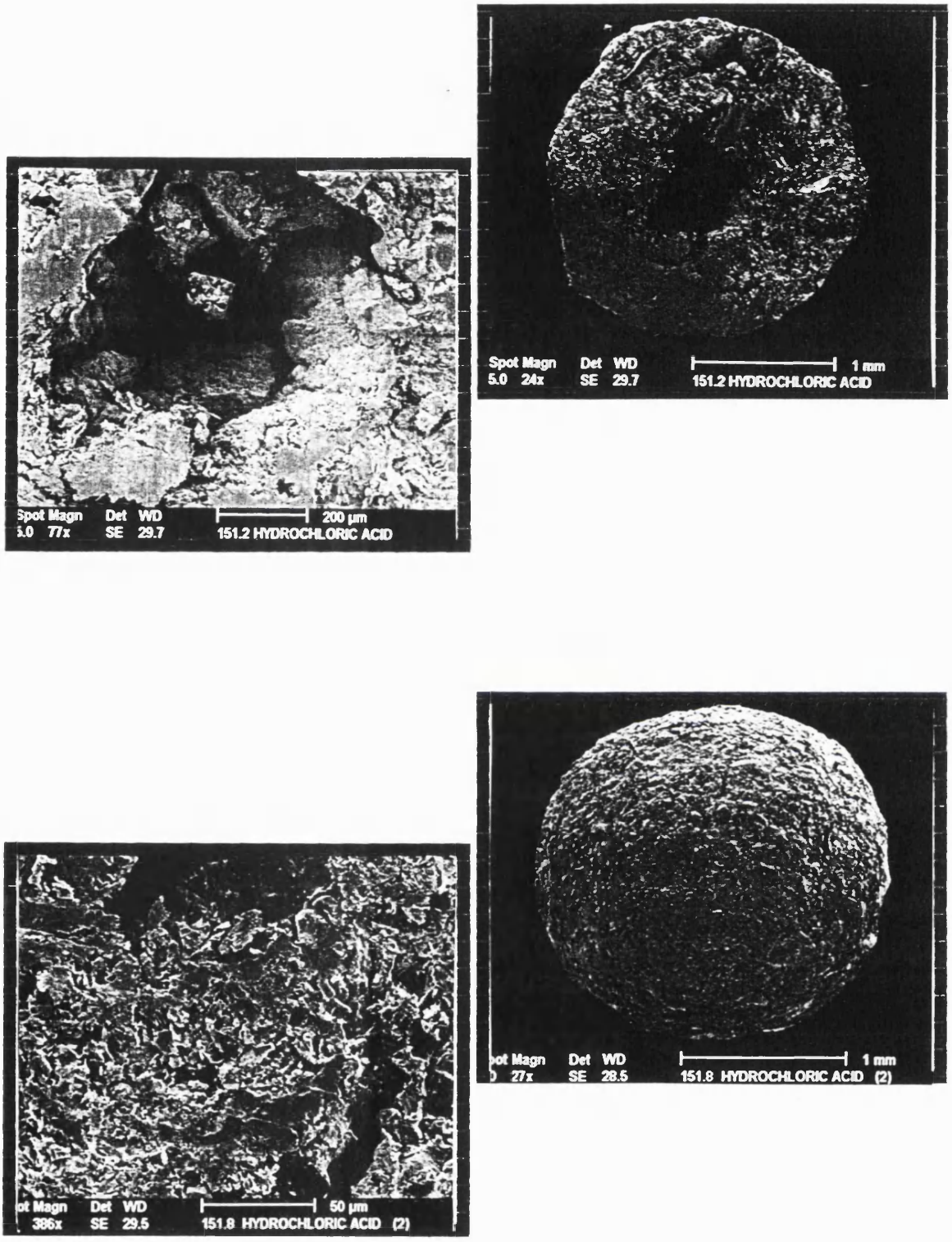


Figure 5.9 (g) Hydrochloric acid pellet.

swelling index than the others which had similar values for their swelling index.

5.7 RHEOLOGY

Chitosan, in the previous chapter has been shown to behave as a pseudoplastic material showing decreasing viscosity at increasing shear. The behaviour of the gels incorporated in the mixes in Section 5.2 above is examined using flow curves obtained from the Carri-med rheometer (see Sections 2.2.10.1/2). The gels all consist of 10%w/v chitosan dissolved in 1.6M solutions of the various acids. The gels are tested by placing a small sample (to avoid edge effects) on the plate of the instrument, allowed to equilibrate and the ram is then raised. The shear stress is then varied and the corresponding shear rate noted. Samples are examined in one continuous process as the stress is applied electronically. The temperature is kept constant throughout these experiments at 20°C.

5.7.1 FLOW CURVES

The Carri-med controlled stress rheometer carries out a measurement of the flow curve for the sample gel and then proceeds to analyse it. As the instrument does not force a sample to flow, yield values can be measured directly. The ascent and descent times for these experiments is set at one minute each and also for the peak hold time. The results for the up curves are shown here as in some cases it was not possible to measure sufficient points on the down curve to present for analysis.

Figure 5.10 shows the up curves for the ascorbic and maleic acid gels. The shear rate increases with increasing shear stress and when the shear stress reaches a value of ~ 500Nm^{-2}, the shear rate begins to increase at a much faster rate. The rheological model that best fits both of these sets of flow curve data is the pseudoplastic model and the quality of fit indicated by the regression coefficient is good. The maleic acid gel appears to be less viscous than the ascorbic

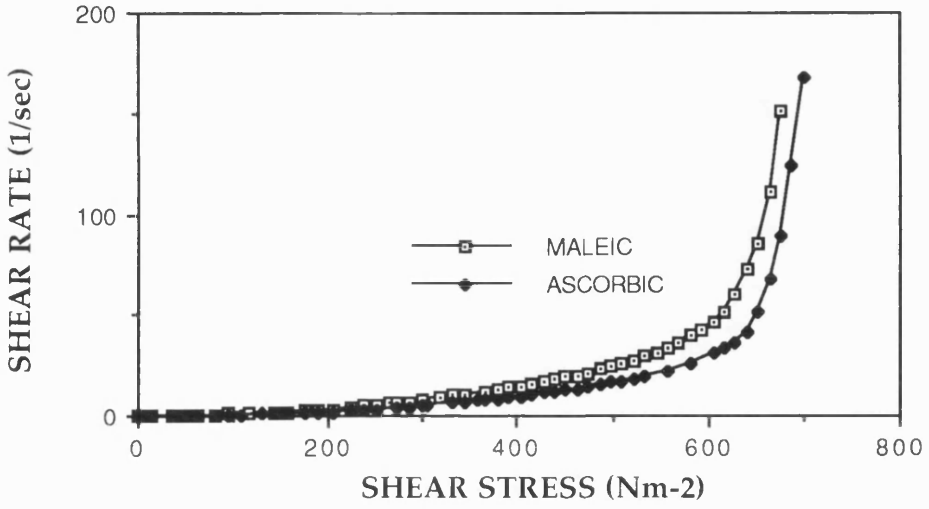


Figure 5.10 Flow curves for the Ascorbic and Maleic Acid Gels.

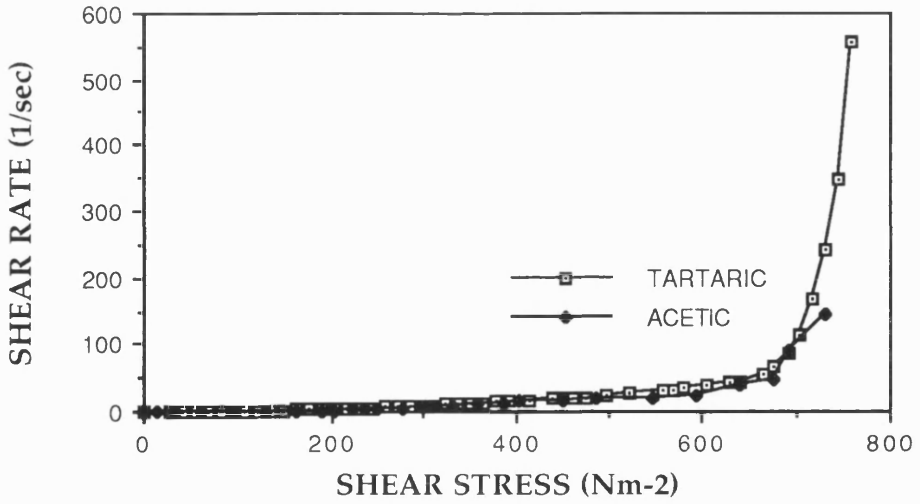


Figure 5.11 Flow curves for the Acetic and Tartaric Acid Gels.

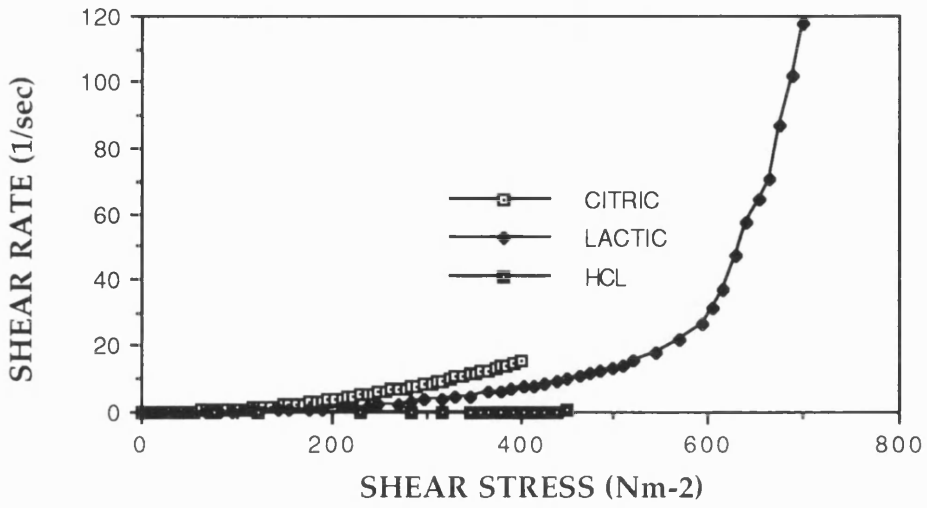


Figure 5.12 Flow curves for the Citric, Lactic and Hydrochloric Acid Gels.

acid gel from these flow curves and maleic acid is the stronger acid of the two, with the lower pKa values and interacts with the chitosan powder to form a less viscous gel.

Figure 5.11 shows the flow curves for the gels containing tartaric acid and acetic acid. Both of the gels have similar flow profiles with the tartaric acid gel being slightly less viscous. The pseudoplastic model is again the best fit for these two gels. The shear rate increases dramatically once the shear stress reaches a value of ~ 680 Nm^{-2} for both of the gels.

Figure 5.12 shows the flow curves for the remaining three gels; containing 10% w/w chitosan and citric, lactic and hydrochloric acids. Citric acid is less viscous than the gel containing lactic acid and once again, the pseudoplastic model is the one that gives the best fit. The hydrochloric acid gel is much less viscous than the other gels judging on appearances and flows easily. A shear stress value of 400Nm^{-2} does not give any appreciable value of shear rate. This gel gives very low readings and another type of instrument would

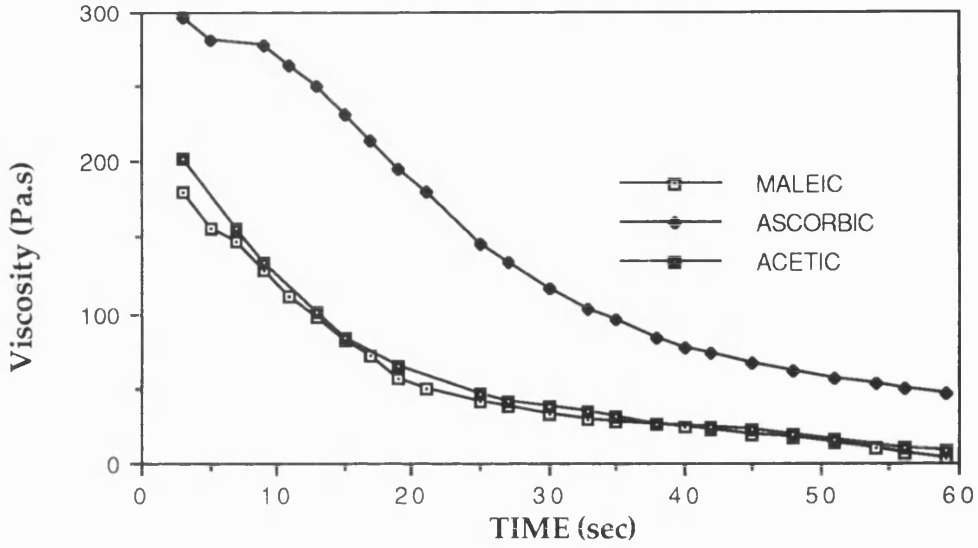


Figure 5.13 Viscosity vs Time curve for Chitosan Gels containing Maleic, Ascorbic or Acetic Acid.

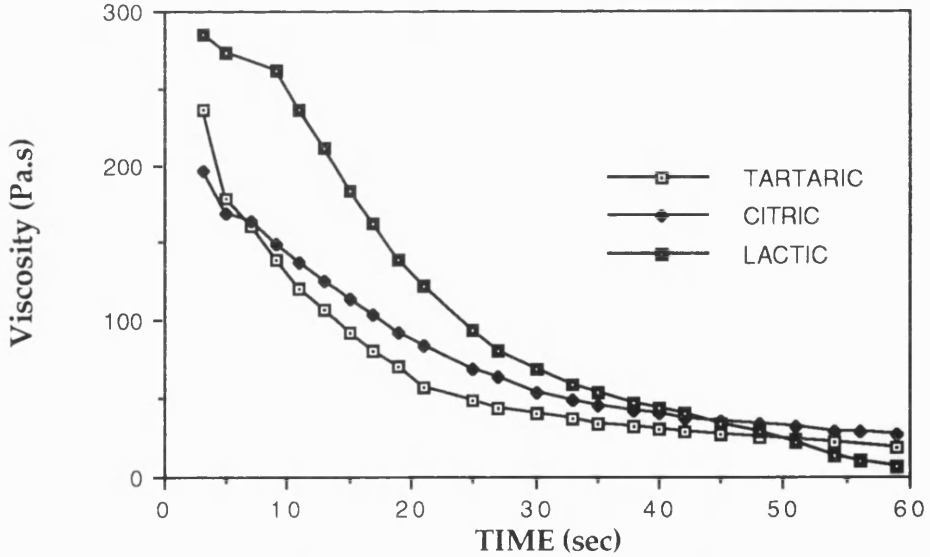


Figure 5.14 Viscosity vs Time curve for Chitosan Gels containing Tartaric, Citric or Lactic Acid.

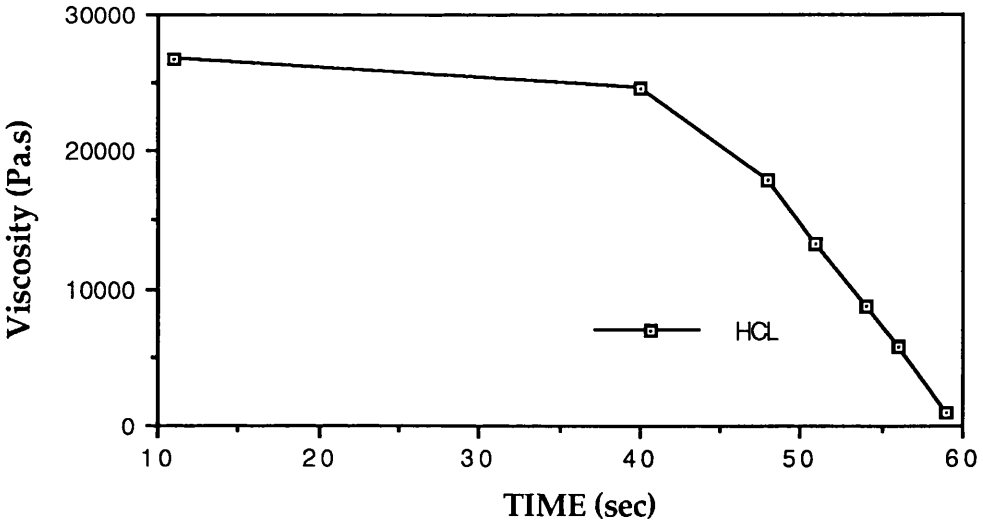


Figure 5.15 Viscosity vs Time curve for Chitosan Gel containing Hydrochloric Acid.

probably be more sensitive to its flow measurement, one with a larger sample size like the traditional cup and bob setup. Citric and lactic acid gels appear to be the more viscous ones overall from the results of these flow measurements.

Figures 5.13 -5.15 show the changes in viscosity over time for the seven gels in the study. The gels apart from the hydrochloric acid gel all share a similar shaped profile; the viscosity decreasing with time, faster initially and then levelling off. The hydrochloric acid gel has higher viscosity values and remains fairly constant for the first 40 seconds and then the viscosity begins to decrease. This high viscosity at low shear rates may be due to inertia of the cone or plate and the properties of the sample.

Maleic, ascorbic and acetic acid gel profiles are shown in Figure 5.13 and of these, maleic acid has the lowest viscosity closely followed by acetic acid. Ascorbic acid has a higher initial viscosity which decreases in a similar fashion to the others. Lactic, citric and ascorbic acid all show a slight plateau region after the initial viscosity drop and then the viscosity continues to fall off. Lactic acid is shown

to have a higher viscosity than citric and tartaric acid which has the lower viscosity of the three gels shown in Figure 5.14. Thus the order of the viscosity of the gels is as follows; hydrochloric > ascorbic > lactic > citric > tartaric > acetic > maleic.

5.8 DISCUSSION

Chitosan has the ability to form gels with a wide number of different acids as can be seen from this study. Pellets are formed by extrusion - spheronization keeping as many of the variables in the formulations as possible constant. The chitosan powder is dissolved in the acid to form a gel and this gel is then incorporated into the mix. The extrusion speed is kept constant (200 mm/min) and these formulations produced pellets, provided varying amounts of additional water is added. Citric acid was an exception to this and did not produce rounded pellets, but dumbells, or on adding further water - agglomeration of pellets resulted. The water content varies depending on the acid in the formulation and no pattern appears. Harrison, (1982) showed that as the extrusion rate is reduced, the water content of the extrudate rises and the water content of the plug of material, remaining in the barrel of the extruder decreases. The difference in moisture content between the plug and the extrudate implies that there is a change in the water distribution of the wet mass during the extrusion process.

The dissolution profiles for the pellets produced showed that the various hydrochloric acid pellets were fast releasing. The larger size pellets were slower releasing and the smaller sized ones were faster, releasing 50% of the drug, diclofenac within 90 minutes. Maleic acid pellets were the slowest releasing (50% after four and a half hours) followed by the acetic acid ones. The tartaric acid and lactic acid were slightly faster releasing with 50% release seen after three, and three and half hours respectively. Overall the pellets had differences in the time to reach 50% release but the hydrochloric acid pellets were much the fastest even though this is the strongest acid.

A plot of the percentage drug release at the two hour time point is shown in Figure 5.16. The graph shows that hydrochloric acid pellet formulations are the fastest releasing, followed by ascorbic and tartaric acid while maleic acid is the slowest. The other pellet formulations apart from the hydrochloric acid ones all show fairly similar release (30 - 40%) at this time point.

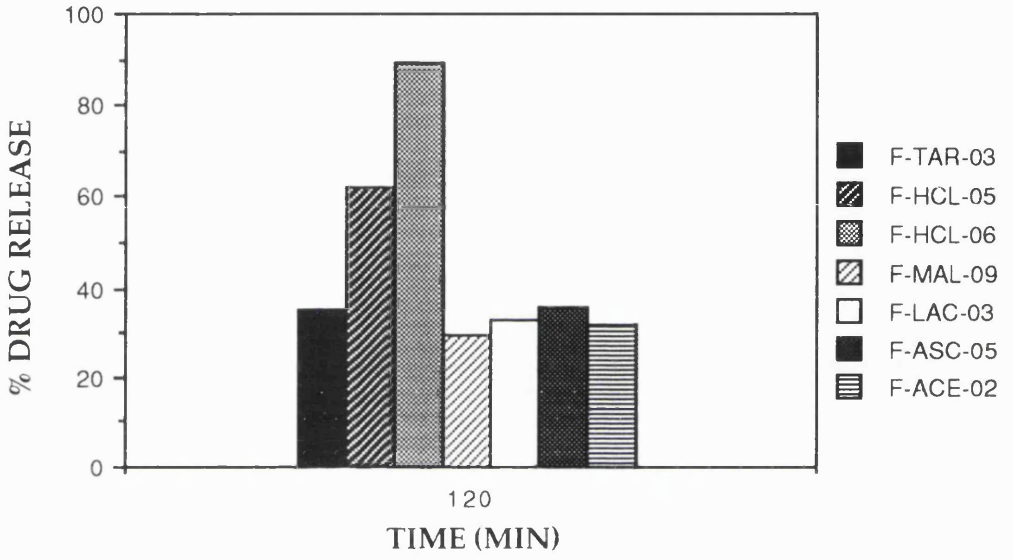


Figure 5.16 Comparison of Drug Release at 120 minutes for the Pellets containing various Acids.

The release rates for the various pellets are shown in Figures 5.5 - 5.8. The release rate profiles in the case of the hydrochloric acid pellets show an initial burst effect and the release rate then appears to be zero order over the time period 100 - 500 minutes. The release rates for the other chitosan gels in various acids show the same zero order profile from ~ 70 min. onwards and have slightly faster release initially but not the burst effect seen with the hydrochloric pellets. Acetic and ascorbic acids have the lowest initial release rates, though all the pellets apart from the hydrochloric acid ones, show similar initial release rates between 0.076 and 0.094mg/min.

The rheological examination of the seven gels showed differences in the flow curves also. The model that gives the best fit from the computer analysis for the data is the pseudoplastic model. The shear rate increases with the increase in shear stress until at a value of ~ 600Nm^{-2} there is a dramatic increase in the shear rate. The hydrochloric acid gel appears to be quite fluid compared to the others and on testing with the cone and plate apparatus gives high viscosity readings. The sample size is small and may be difficult to retain between the parallel plates and the high viscosity reading may be due to the properties of the sample when under test.

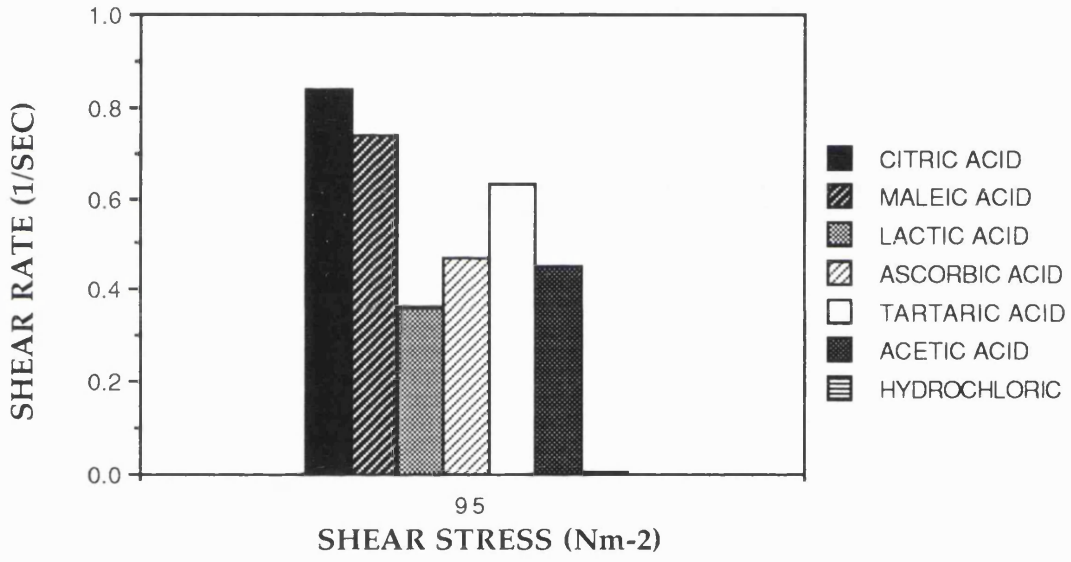


Figure 5.17 Comparison of Shear Rates at a fixed Shear Stress (95 Nm^{-2}) for the Chitosan - Acid Gels.

Figure 5.17 shows the differences in the shear rates of the gels at a fixed value of shear stress. The resultant shear rate is highest for citric acid followed by maleic acid and tartaric acid. The lowest shear rate is for hydrochloric acid which shows hardly any reponse to the applied stress.

The viscosity vs time curves showed that the hydrochloric acid gel was different to the others and the least viscous gel was maleic

acid and ascorbic acid was the most viscous gel. Hydrochloric acid gel started with a very high viscosity plateau and this then dropped sharply after 60 seconds. Maleic acid also produced the slowest releasing pellets and readily formed pellets on extrusion - spheronization. There is no evidence of a yield value for the gels as they begin to flow at a value of $6Nm^{-2}$ the first measurement point in the linear shear stress sweep, even though some of the corresponding shear rate values are low. Once again the hydrochloric acid gel is the exception and this gel appears to have a yield value as it does not flow until the shear stress reaches a value of 120 Nm^{-2} and then gives low readings of shear rate.

The scanning electron micrographs show samples of the pellets, either the whole pellet or a cross-sectional area and a view at a higher magnification. The acetic acid pellet, Figure 5.9 (a) shows a porous surface and is spherical in shape. The higher magnification of a cross-sectional area shows a homogenous matrix with folds and invaginations. A cross-sectional area of the ascorbic acid pellet, Figure 5.9 (b) is shown and the smooth part to the right hand side is due to cutting of the pellet. The smooth Avicel particles are again visible. The citric acid pellet Figure 5.9 (c) has a smooth surface and drug crystals are visible at the higher magnification. The maleic acid, Figure 5.9 (e) and tartaric acid pellets Figure 5.9 (f) also have the smooth spherical surface and their cross-section reveals the same type of matrix as the others. The hydrochloric acid pellet Figure 5.9 (g) reveals a hollow centre on cutting but has the same surface as the other pellets. This centre may explain the faster drug release of these pellets as the drug is not uniformly dispersed and does not have to leach out from the centre of the pellet.

CHAPTER 6

CONTROLLED STRESS RHEOMETRY

6.1 OSCILLATION

Oscillation rheometry involves the application of a stress to a material in a sinusoidal fashion which then generates a strain wave either in or out of phase, depending on the viscous and elastic components of the material. The theory is discussed in Section 1.3.10. Oscillatory measurements can be made at different values of torque and frequency. The material is assumed to show linear behaviour and this usually only occurs at relatively small strains and hence low stresses. In the linear region, G' the storage modulus is constant and as the stress increases the storage modulus (G') value will decrease. The Loss Modulus (G") is equal to the dynamic viscosity η' multiplied by the angular velocity. Plugs of material were prepared containing chitosan dissolved in the same acids (as presented in Chapter 5), in the same formulations as those that were used to investigate the preparation of controlled release pellets. These plugs are then subjected to a range of torque values fron 10 - 5000 microN.m and the storage modulus (G') and the dynamic viscosity (n') or the tan delta value plotted for each plug containing the various acids. A fresh plug containing chitosan and one of the seven acids is subjected to a frequency sweep (0.1 -10 Hz). From the results of these experiments a single value of torque is selected and a single frequency value and this stress applied to the test plugs and the results noted.

6.1.1 PREPARATION OF THE PLUGS

The plugs are prepared as detailed in Section 2.2.10.3 by extruding the mixtures against a blank die. The formulations for the plugs are identical to those used to prepare the pellets reported in Chapter 5. Chitosan is dissolved in one of the seven acids; acetic, ascorbic, citric, hydrochloric, lactic, maleic or tartaric to form a gel and then incorporated into the dry powder mix. The height of the plug is then measured and a value of 0.7mm subtracted (the depth of immersion found to be best, MacRitchie, 1993). The plug height is kept as low as possible to ensure that the oscillation wave reaches the bottom of the sample. A height of 19mm is the maximum for

the gap between the parallel plates of the Carrimed CSL 500, but in practice the height of the plugs was ~ 5 - 7mm in these tests, as plugs smaller then these tended to break on production. Each measurement is repeated and a fresh plug placed between the parallel plates of the Carrimed CSL 500 for measurement.

6.1.2 ANALYSIS OF THE PLUGS - TORQUE & FREQUENCY SWEEPS.

Oscillatory analysis of the plugs of material gives values for the storage modulus (G'), the dynamic viscosity (n') and the tan δ value which gives the relative viscosity - elasticity of the material. Initially the various plugs are all subjected to a standard torque sweep (10-5000 microNm) at a frequency of 1Hz. The plugs of chitosan with the different acids are also subjected to a frequency sweep from 0.1 Hz to 10 Hz at a constant value of torque. The temperature for all these experiments is kept constant at 20°C. The results of the torque and frequency sweeps for each of the acids is shown in Figures 6.1 -6.14.

The results for the plugs containing acetic acid are shown in Figure 6.1 and 6.2. The torque sweep at a frequency of 1Hz shows that as the value of torque increases the storage modulus value is approximately constant over the range of 1000-5000 microN.M but above this, the value of the storage modulus begins to drop off. The dynamic viscosity appears to follow the same linear region. The high G' value suggests that the material is quite elastic in nature and withstands the range of torque values without losing any significant degree of elasticity. At the set torque value of 4000 microN.m, the varying frequency affects the storage modulus G' and the dynamic viscosity. As the frequency increases the storage modulus gradually increases while the dynamic viscosity drops initially and then remains level. At a frequency of below 1Hz the two curves crossover, the storage modulus increasing and the viscosity decreasing. The tan delta values which are not shown in the graph for acetic acid are a measure of the relative elasticity to viscosity for the plugs of material. The tan delta values for the torque sweep were fairly constant, and for the frequency sweep, slowly increased as the

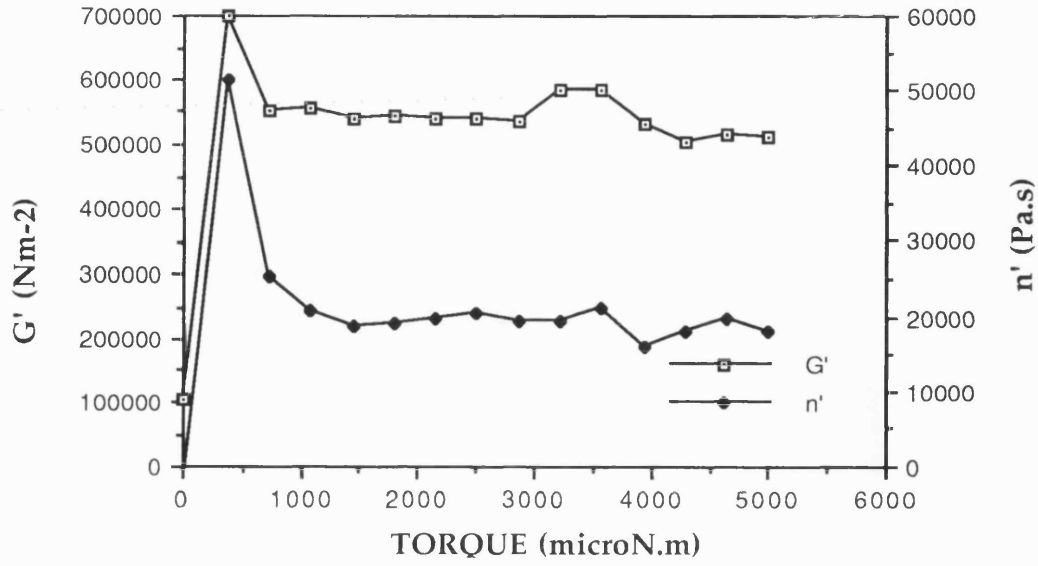


Figure 6.1 The Effect of various values of Torque on the Storage Modulus (G') and the Dynamic Viscosity (n') of the plug containing Chitosan and **Acetic acid** at an Oscillatory Frequency of 1Hz.

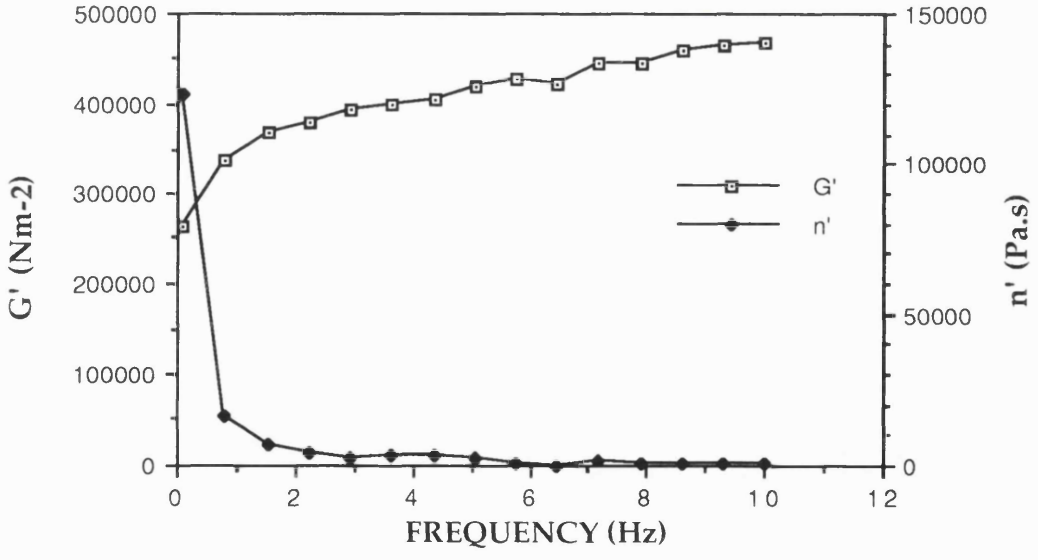


Figure 6.2 Effect of various values of Frequency on the Storage Modulus (G') and the Dynamic Viscosity (n') of the plug containing Chitosan and **Acetic acid** at a Torque of 4000 microN.m.

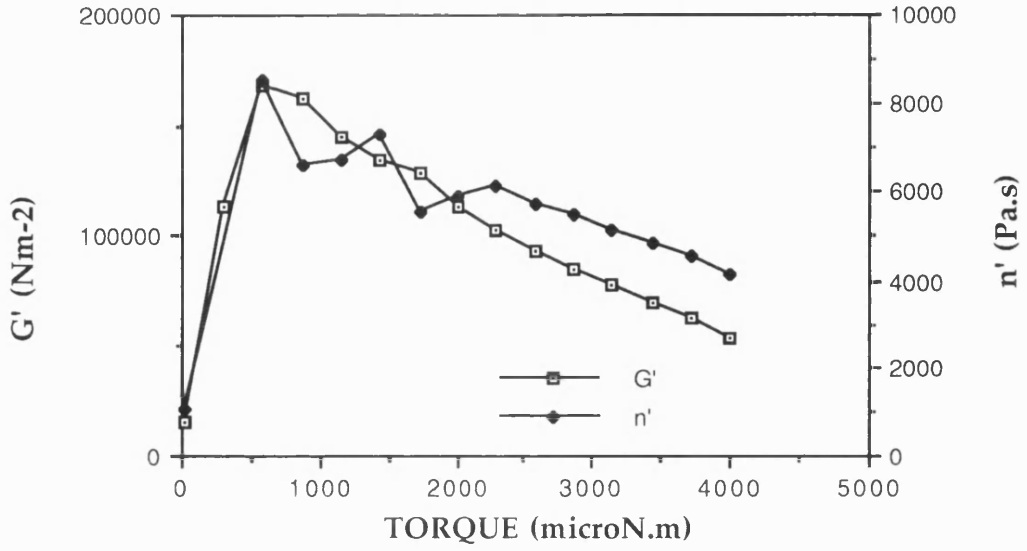


Figure 6.3 The Effect of various values of Torque on the Storage Modulus (G') and the Dynamic Viscosity (n') of the plug containing Chitosan and **Lactic acid** at an Oscillatory Frequency of 1Hz.

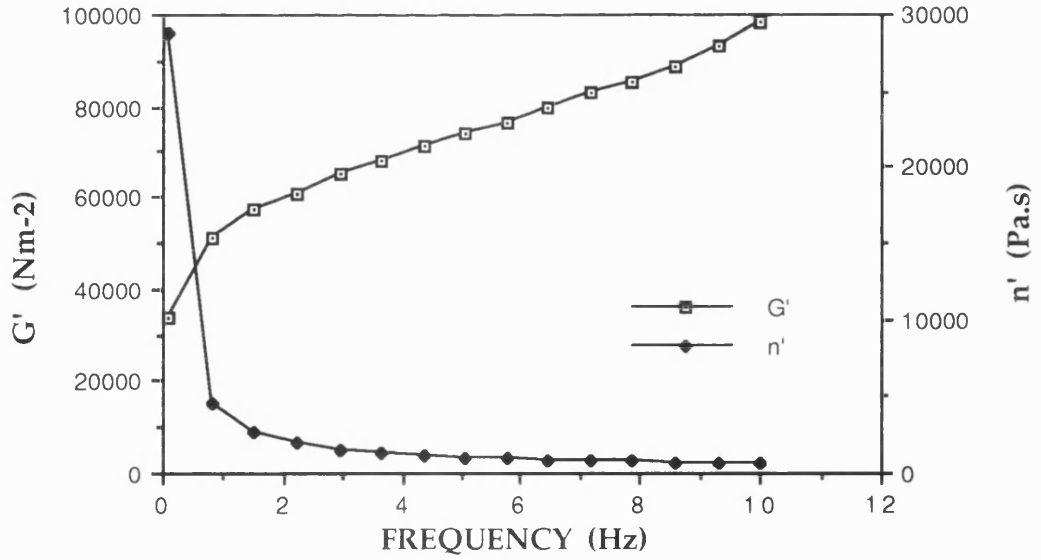


Figure 6.4 Effect of various values of Frequency on the Storage Modulus (G') and the Dynamic Viscosity (n') of the plug containing Chitosan and **Lactic acid** at a Torque of 4000 microN.m.

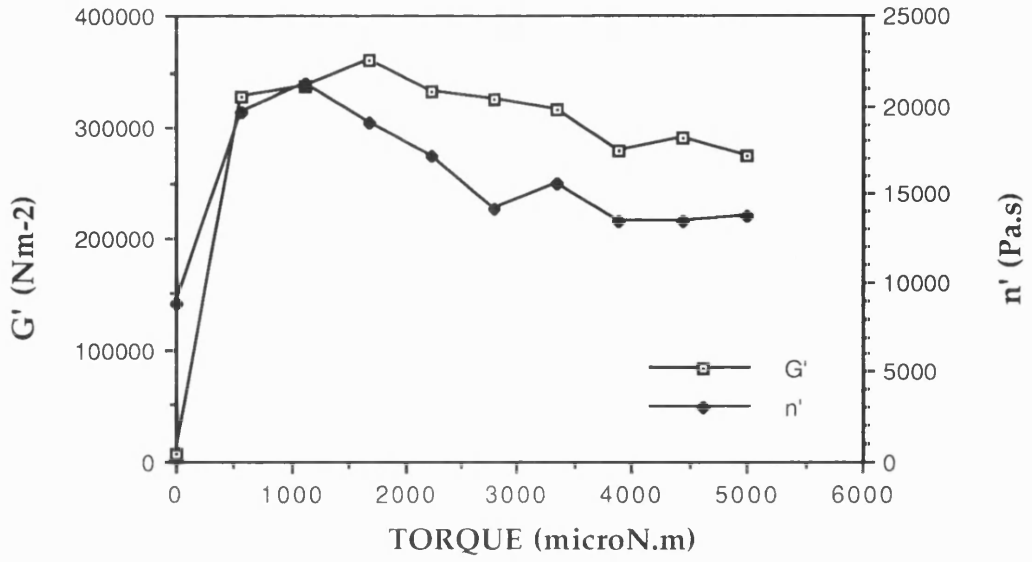


Figure 6.5 The Effect of various values of Torque on the Storage Modulus (G') and the Dynamic Viscosity (n') of the plug containing Chitosan and **Citric acid** at an Oscillatory Frequency of 1Hz.

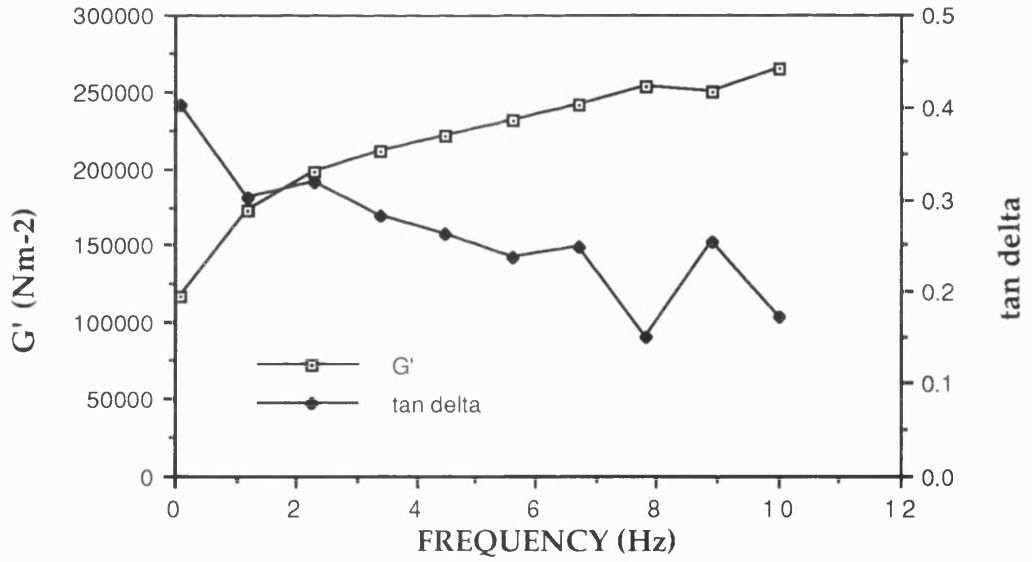


Figure 6.6 Effect of various values of Frequency on the Storage Modulus (G') and the Tan Delta value of the plug containing Chitosan and **Citric acid** at a Torque of 4000 microN.m.

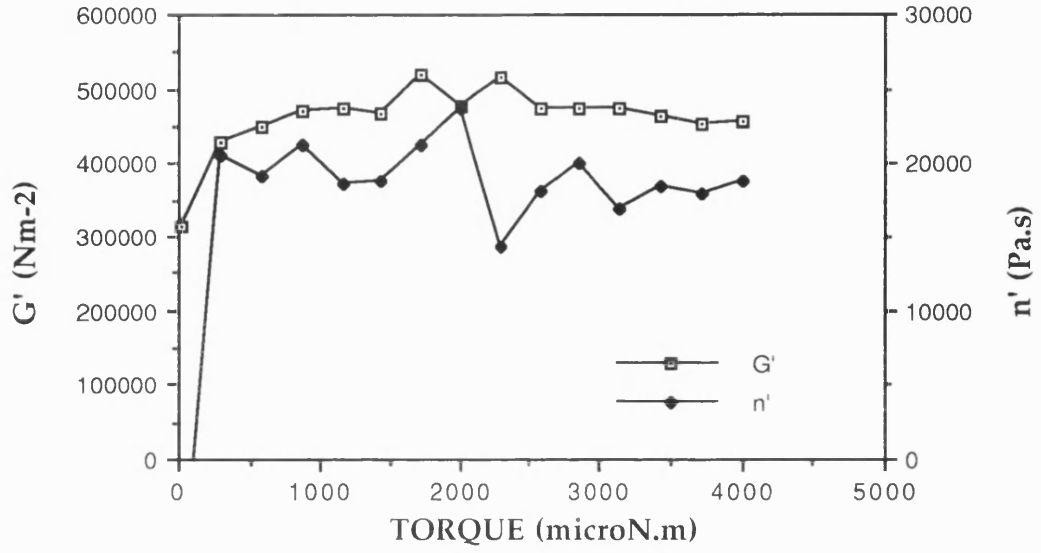


Figure 6.7 The Effect of various values of Torque on the Storage Modulus (G') and the Dynamic Viscosity (n') of the plug containing Chitosan and **Tartaric acid** at an Oscillatory Frequency of 1Hz.

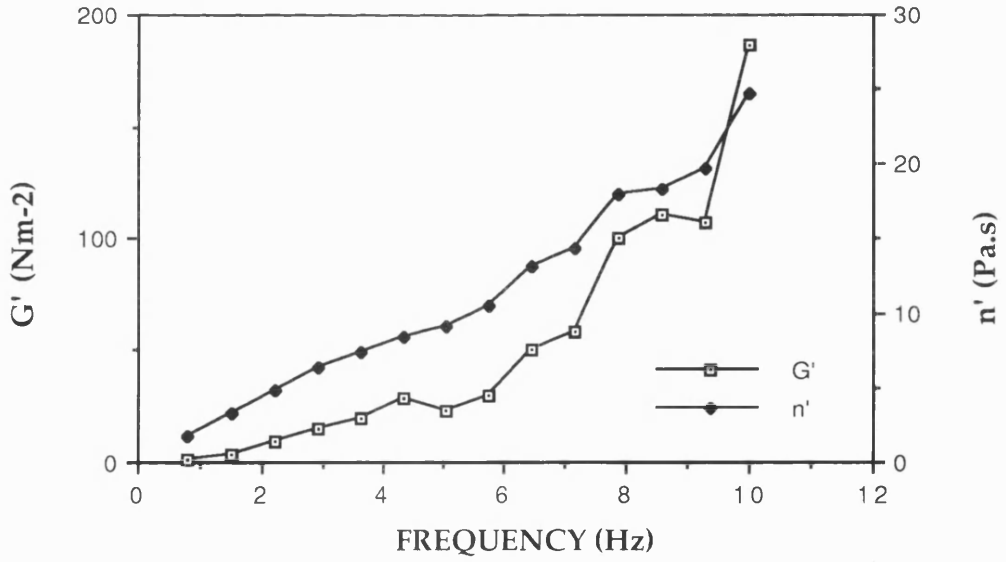


Figure 6.8 Effect of various values of Frequency on the Storage Modulus (G') and the Dynamic Viscosity (n') of the plug containing Chitosan and **Tartaric acid** at a Torque of 3000 microN.m.

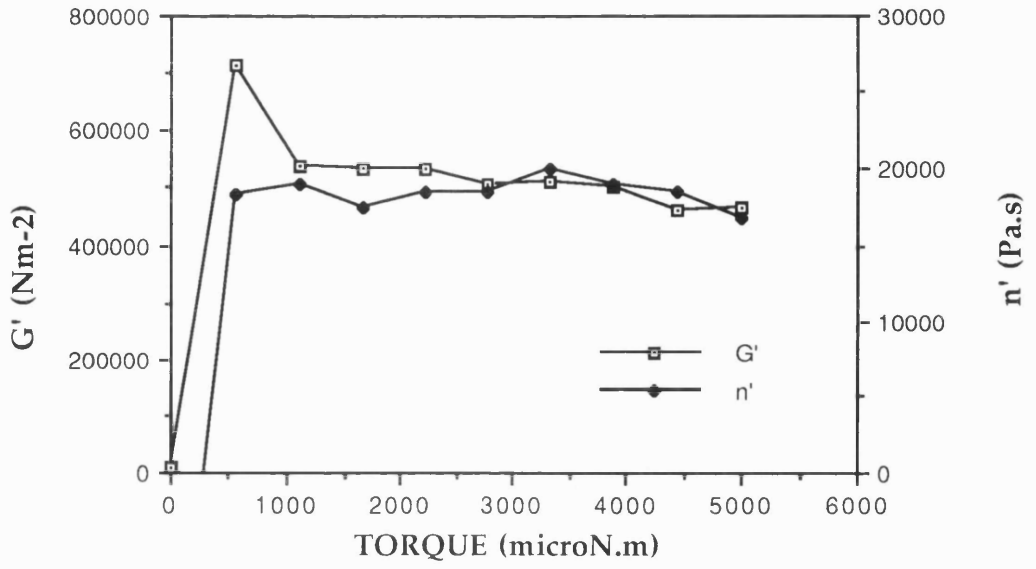


Figure 6.9 The Effect of various values of Torque on the Storage Modulus (G') and the Dynamic Viscosity (n') of the plug containing Chitosan and **Ascorbic acid** at an Oscillatory Frequency of 1Hz.

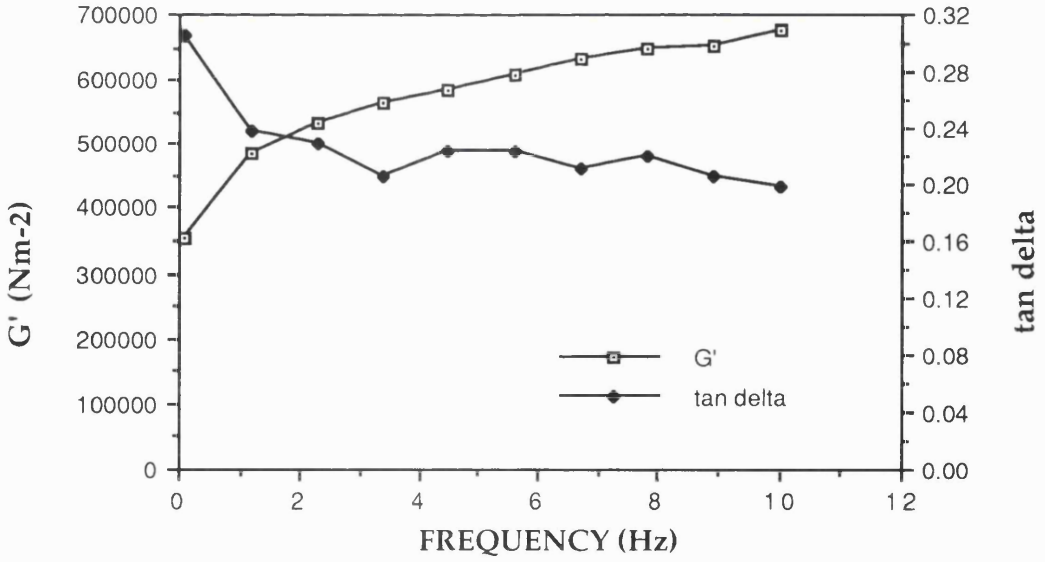


Figure 6.10 Effect of various values of Frequency on the Storage Modulus (G') and the Tan Delta value of the plug containing Chitosan and **Ascorbic acid** at a Torque of 3000 microN.m.

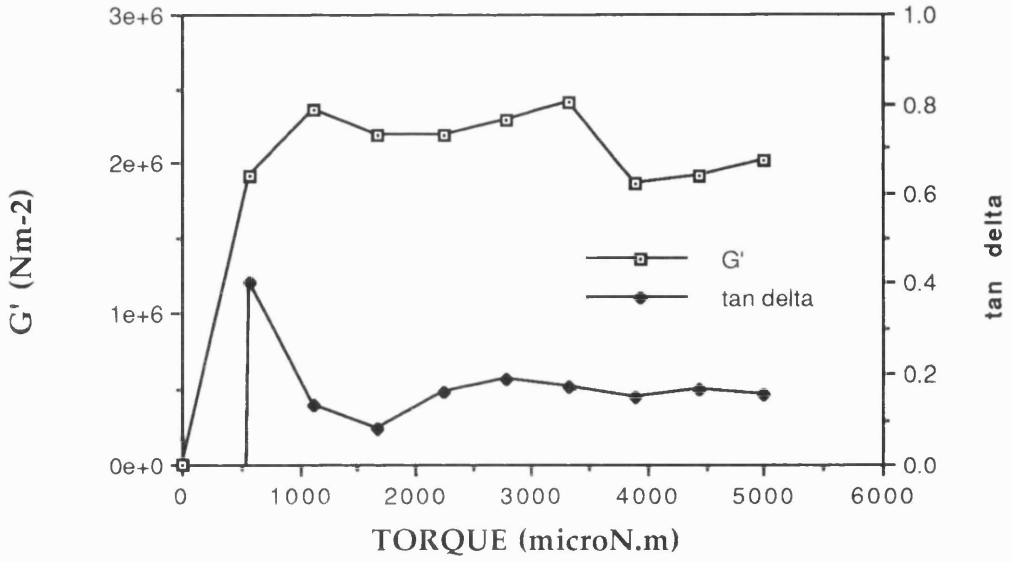


Figure 6.11 The Effect of various values of Torque on the Storage Modulus (G') and the Tan Delta value of the plug containing Chitosan and **Maleic acid** at an Oscillatory Frequency of 1Hz.

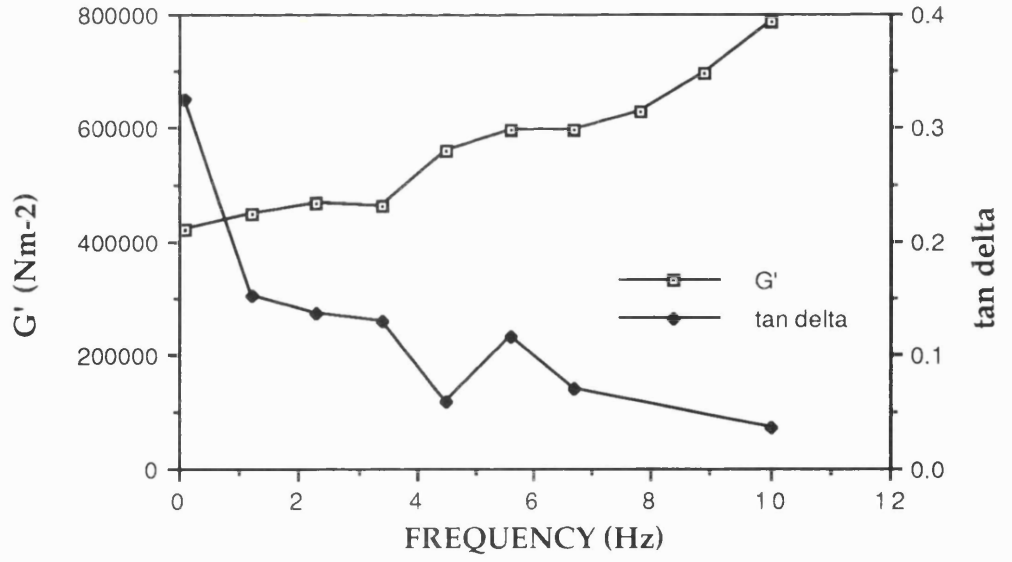


Figure 6.12 Effect of various values of Frequency on the Storage Modulus (G') and the Tan Delta value of the plug containing Chitosan and **Maleic acid** at a Torque of 3000 microN.m.

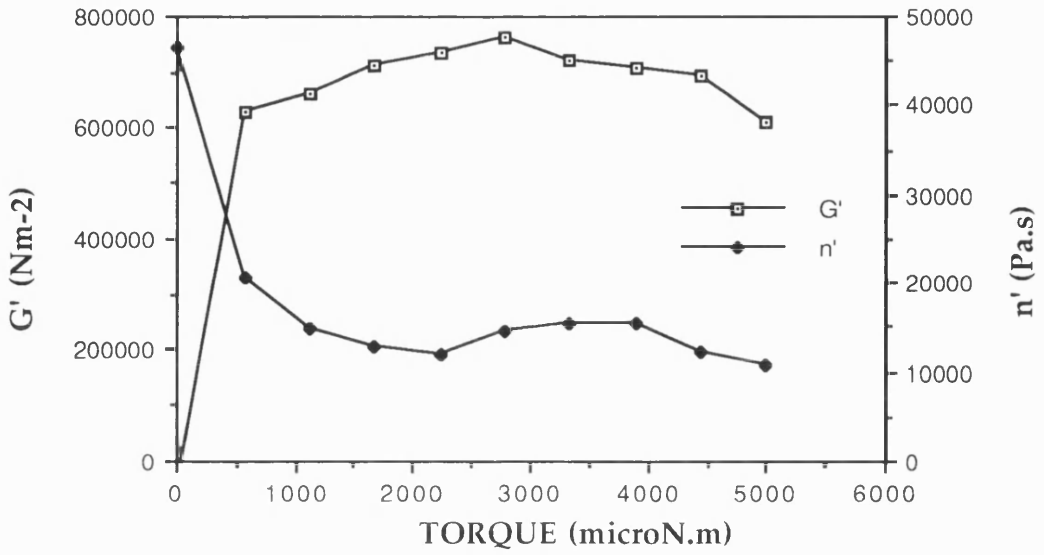


Figure 6.13 The Effect of various values of Torque on the Storage Modulus (G') and the Dynamic Viscosity (n') of the plug containing Chitosan and **Hydrochloric acid** at an Oscillatory Frequency of 1Hz.

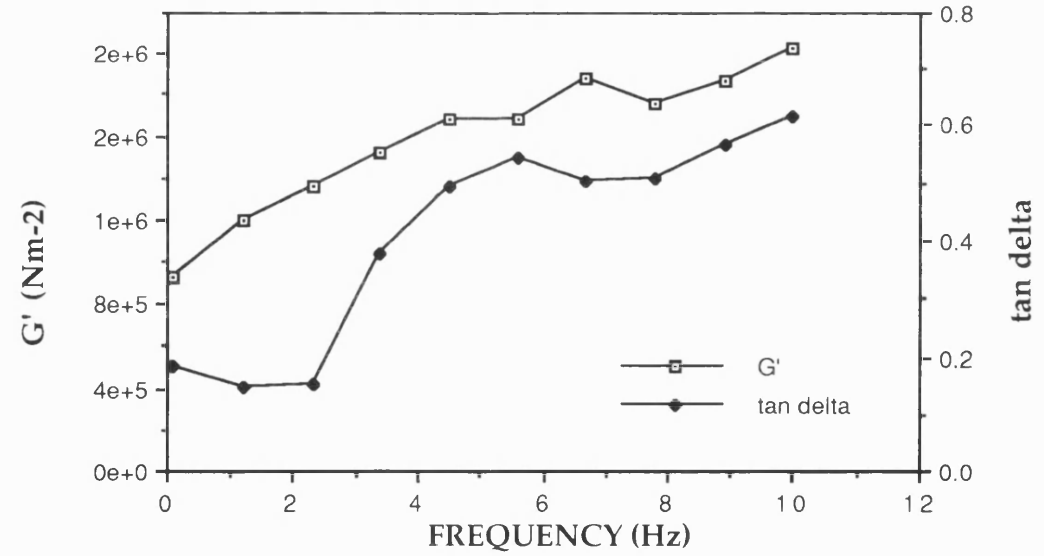


Figure 6.14 Effect of various values of Frequency on the Storage Modulus (G') and the Tan Delta value of the plug containing Chitosan and **Hydrochloric acid** at a Torque of 3000 microN.m.

frequency values decrease towards zero. This implies that the acetic acid plug appears to be slightly more viscous at the lower frequency values. The effect of the different stresses applied do not appear to affect the viscous/elastic balance of the material. Thus the acetic acid plugs appear to be able to withstand the torque values without unduly affecting its elasticity or viscosity at a frequency of 1Hz.

The lactic acid plugs are subjected to the same torque and frequency sweeps and the effect on the storage modulus and dynamic viscosity plotted in Figures 6.3 and 6.4. The storage modulus and dynamic viscosity both increase until the torque reaches a value of 500 microN.m and then begin to decrease in a linear fashion. Therefore as the torque increases the material becomes less elastic and less viscous. The frequency sweep carried out at a torque value of 4000 microN.m shows the same crossover at a frequency of less than 1Hz as the acetic acid plug. The storage modulus values gradually increases as the frequency values increase, while the viscosity falls and then levels off to a plateau. The tan delta values show a slight decrease initially and then a gradual increase as the torque values increase from 10 -5000 microN.m. The tan delta values remain constant for the frequency sweep. This implies that the lactic acid plug behaves as an elastic body initially and then the viscous behaviour becomes predominant.

Figures 6.5 and 6.6 show the results of the torque and frequency sweeps respectively on the plugs containing citric acid. The torque sweep shows that as the torque increases the storage modulus and the dynamic viscosity increase until the torque reaches a value of 500 microN.m and then the storage modulus levels off slightly decreasing while the viscosity drops off and then also levels out. Between torque values of 4000 - 5000 microN.m the storage modulus and the dynamic viscosity are linear. The frequency sweep shows that as the frequency increases the storage modulus increases and the tan delta value plotted here decreases. This implies that the citric acid plug behaves as an elastic body at higher frequencies and as a more viscous one at lower frequencies. The tan delta values for the torque sweep at a frequency of 1Hz are low indicating a predominantly

elastic body and the values remain relatively constant and then decrease slightly.

The plugs containing tartaric acid are subjected to the same torque and frequency sweeps and the results are shown in Figures 6.7 and 6.8. The torque sweep at a frequency of 1 Hz shows the same initial increase in both the storage modulus and the dynamic viscosity and then the levelling off. Both plots are similar apart from a few increases or decreases in the general plateau level. The plot of the frequency sweep (Figure 6.8) shows that both the storage modulus and the dynamic viscosity increase at a steady rate as the frequency increases from 1 -10 Hz. The tan delta values for the frequency curve are lower initially and then increase for frequency values between 5-7.5 Hz. The tartaric acid plug appears to behave more as a viscous body at higher frequencies. The tan delta values for the torque sweep decrease slightly as the torque values increase showing elastic behaviour at a frequency of 1Hz.

The effect of the torque sweep on the plug containing chitosan and ascorbic acid is shown in Figure 6.9. The storage modulus (G') and the dynamic viscosity (n') show an initial increase until the torque reaches a value of 500 microN.m and from 1000 - 5000 microN.m both are constant. The storage modulus begins to drop off at higher torque values. Thus this is the linear viscoelastic region for the ascorbic acid plug. The frequency sweep (Figure 6.10) at a torque of 3000 microN.m shows that as the frequency increases, the storage modulus value also increases, while the tan delta value gradually decreases. The values of tan delta are low (0.3 - 0.2) indicating a predominantly elastic body. The tan delta values for the torque sweep increase gradually as the torque increases. This indicates that the material becomes more affected by its viscous component as the stresses on the plug of material increases.

Figure 6.11 shows the effect of a range of torque values on the storage modulus and tan delta values for the plugs containing chitosan and maleic acid. High values for G' were recorded at a frequency of 1Hz. The tan delta values are approximately zero indicating mainly elastic behaviour in the rheological test. The

frequency sweep at a torque of 3000 microN.m in Figure 6.12 shows that as the frequency increases the storage modulus value increases to a maximum. The tan delta values decrease as the frequency increases but remains close to zero. The maleic acid plug exhibits slightly more viscous behaviour at lower frequencies but the elastic behaviour still predominates.

The torque and frequency sweeps for the plugs containing chitosan and hydrochloric acid are shown in Figures 6.13 and 6.14. The torque sweep at a frequency of 1Hz shows an initial increase in the storage modulus which then levels off to a plateau in the linear region from 1000 - 4000 Nm-2 and then drops off and the material appears to be less elastic. The dynamic viscosity drops initially and from torque values between 2500 - 4000 microN.m the curve levels out. Both plots are fairly constant from 1000 - 4000 microN.m. The tan delta values for the torque sweep decrease gradually and this reduction is characteristic of an elastic material. The frequency sweep (Figure 6.14) shows that as the frequency increases the value for the Storage Modulus and tan delta increases also. The values of G' are high reflecting the response of the material to the applied stress. The material behaves as a more elastic body at lower frequencies.

6.1.3 SINGLE POINT TORQUE AND FREQUENCY MEASUREMENT.

Further oscillatory measurements are only carried out in the region where the range of stresses and/or strains over which the Storage Modulus and viscosity are constant. In this linear region, the strain is proportional to stress and G' is constant over a range of strains. A frequency of 1Hz was selected as the most appropriate for all the test samples and this was used for the subsequent tests. The single torque value is selected from the linear region for each sample. In order to compare the Storage and Loss Modulus values for all the acids, a value of torque of 3000 microNm was selected as the most suitable for all the samples. The Storage Modulus was constant at this value and a frequency of 1 Hz. Plugs of the material containing each of the seven acids were subjected to repeated measurements at these values until the resulting Storage and Loss

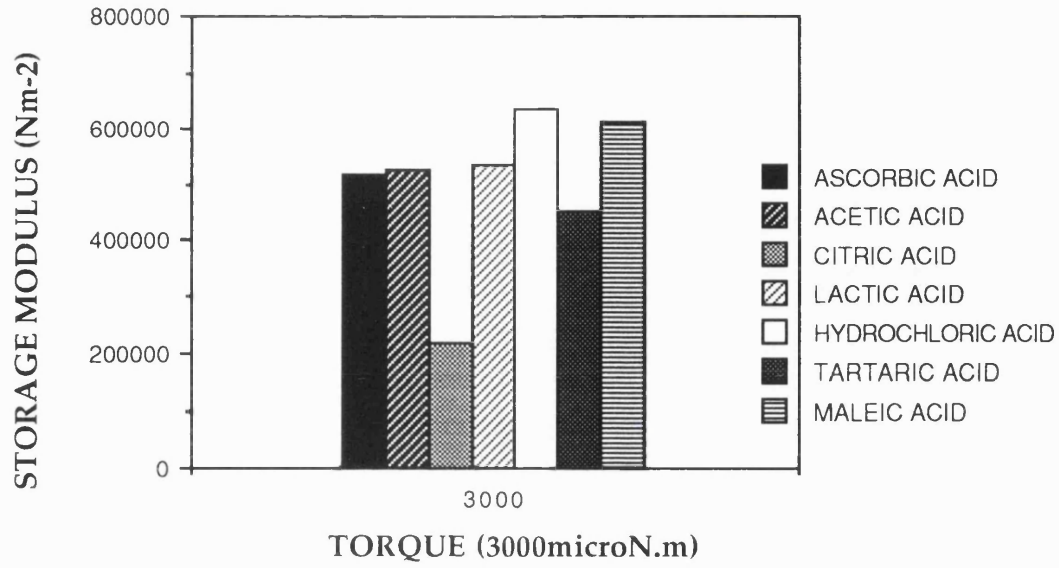


Figure 6.15 Comparison of the Storage Modulus values for the various acids at a set Torque (3000 microNm) and frequency (1Hz).

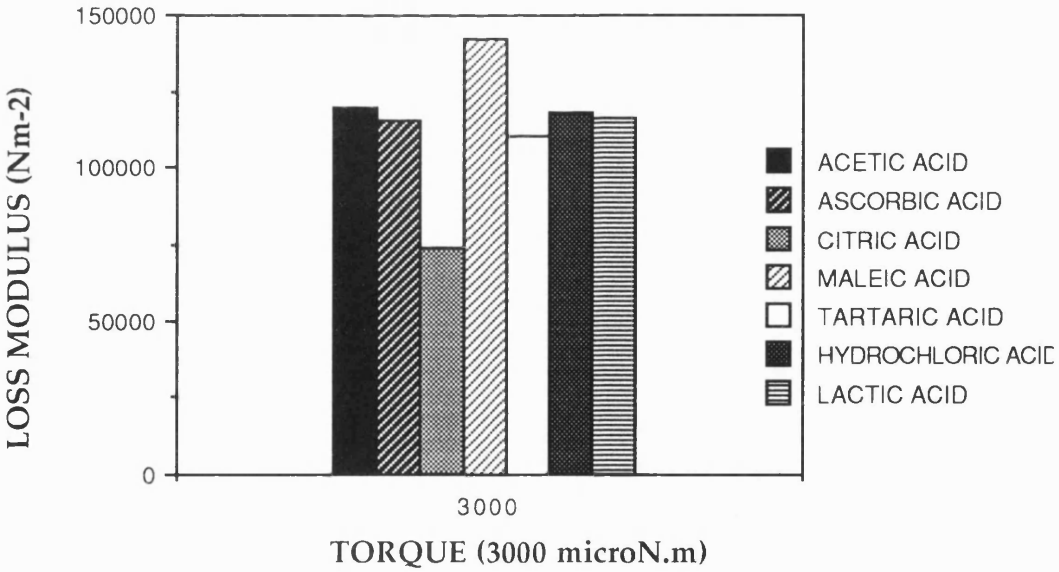


Figure 6.16 Comparison of the Loss Modulus values for the various acids at a set Torque (3000 microNm) and frequency (1Hz).

Modulus values were reproducible giving well resolved displacement amplitudes. The results are shown in Figures 6.15 and 6.16. The Storage Modulus - a measure of the energy stored and recovered per cycle is shown to be highest for hydrochloric acid and maleic acid plugs and lowest for citric and tartaric acid plugs. The Loss Modulus or Viscous Modulus - a measure of the energy dissipated per cycle of deformation is highest for maleic and acetic acid and lowest for citric and tartaric acid. MacRitchie, (1993) shows that as the moisture content of the plug of material increases the values for both the Storage and Loss Modulus tend to decrease. The Loss Modulus values for the plugs containing acetic, ascorbic, tartaric, hydrochloric and lactic acid are similar. Citric acid would therefore appear to act as though it has a higher moisture content than the other plugs with hydrochloric and maleic acid having a lower moisture content. The Storage Modulus values are relatively constant for lactic, tartaric, acetic and ascorbic acid plugs and the high values suggest quite elastic materials.

6.1.4 DISCUSSION

The Storage Modulus values for the plugs of material investigated have a higher value than those for the Loss Modulus values and this suggests that the materials are mainly elastic in nature. The effect on the Complex Modulus recorded for each plug of material follows the pattern of the Storage Modulus values, showing that the behaviour of the materials is predominantly elastic. The results from the torque sweeps show that as the applied stress increases the values for the Storage Modulus decrease and the dynamic viscosity also decreases once the stress exceeds that of the linear region. The tan delta values were low overall indicating an elastic body. The frequency sweeps show that the Storage Modulus values tend to increase as the frequency is increased from 0.1 - 10Hz. The linear region, where the Storage Modulus and dynamic viscosity were constant and parallel, tended to occur at the lower frequency values and values of torque of ~ 3000 - 4000 microNm. The plugs appear to withstand a range of torque values without losing their elasticity. The frequency sweeps showed that at as the frequency increases, the tan delta values decreased indicating that viscous

behaviour becomes more important at low frequencies except for the hydrochloric acid plugs. These plugs showed higher tan delta values as the frequency increases implying that the elastic behaviour predominates at low frequencies. Thus the response of the plugs of material varies with the oscillatory frequency, the applied stress and the nature of the material and hence the linear viscoelastic region is subject to change.

6.2 CREEP

Creep testing involves the application of a controlled stress to a material for a set length of time and then measuring its recovery. These experiments yield information on the instantaneous compliance, J_0 and the Newtonian viscosity value calculated at a particular shear rate (Section 2.2.10.4). Creep measurement can be used to characterize the viscoelastic behaviour of the sample without changing its structure. Creep behaviour is a result of viscous flow and retarded elastic deformation and can be represented by a viscoelastic model, consisting of several dashpots and springs in series and in parallel. The gels consisting of chitosan dissolved in the series of seven different acids are subjected to a creep test at three values of applied stress to investigate their response. The sample is treated as an elastic solid, a stress is applied to the material and the deformation which results is measured and recorded. The strain response represents the amount of deformation expressed as a change in a certain dimension of the sample and strain is expressed as values of compliance by dividing the strain by the applied stress.

6.2.1 ANALYSIS OF RESULTS

The analysis program used by the Carrimed CSL 500 models the data to a theoretical curve made up of three components; an instantaneous compliance (J_0) from the initial movement the sample makes once the stress is applied, a Newtonian viscosity (η_0) from the constant shear component at large values of time and a spectrum of

decayed exponential movement for the relaxation of the sample. Voigt units will continue to be fitted to the data until less than ten points remain or four Voigt units have been fitted. A creep curve should tend to a straight line of constant slope at large time values. This straight line represents Newtonian shear and the strain rate is calculated and the stress divided by this to yield the viscosity at that particular shear rate. In the analysis process, the instantaneous compliance is set from the curve intercept on the compliance axis of the graph, and the Newtonian viscosity is derived from the slope (the strain rate) at large values of time. When these terms are removed from the data only the exponential tems remain. Voigt units are presented in terms of springs and dashpots; springs denoting elastic behaviour and dashpots denote viscous flowing behaviour. This is the general model used to describe materials by adapting the number of viscoelastic Voigt units fitted. When the stress is applied initially there is an immediate deformation of the sample due to the undamped elastic behaviour described by the spring. The Voigt units may start to move under the effect of the constant stress applied. The rate and extent to which an individual Voigt unit moves depends on the damping effect of the dashpot and the strength of the spring. The ratio of these is the retardation time. The spring and dashpot with the shortest retardation time reaches equilibrium first and then ceases to move. This implies that any remaining movement is due to further Voigt units with longer retardation times. These reach equilibrium in ascending order of retardation time until there is no further movement due to Voigt units.

The manner in which Voigt units reach equilibrium in ascending order of retardation time means that unlike oscillation, creep can give measurements over the desired time scale in one determination. However as the analysis proceeds the movement of one Voigt unit is overlaid with that of another with shorter retardation times. The structural information obtained by this technique can be directly related to the bonds and mechanisms that operate in the sample under stress.

6.2.1.1 CITRIC ACID

The gel containing chitosan dissolved in citric acid is subjected to an applied stress of 1.1936, .59680 or .29840 Nm^{-2} corresponding to a torque of 20, 10, and 5 microNm respectively. A creep test is performed in the linear viscoelastic region and the torque values corresponding to this are measured for the gels and the three points are included for comparison purposes between the various gels where appropriate. A stress is applied for two minutes and the gel then allowed to relax for two minutes. The Tables 6.1 (a) and 6.1(b) show the instantaneous compliance, Newtonian viscosity and shear rate for the retardation and relaxation portions of the graph at the different torque values. At an applied stress of 10 microNm the gel is characterized by a single Voigt unit. The retardation time is 19.6 seconds with a compliance of 9.091×10^{-3} m^2N^{-1}. Voigt unit analysis is useful in determining compliance values and the retardation times for each unit. The retardation time is the time taken for the strain in a material that obeys the Kelvin model to reduce to 1/e of its original equilibrium value after the removal of the stress, (Barnes et al, 1989). Thus the retardation time is an indication of the time required for the elastic component of the gel to relax under the given stress. The relaxation time is the time taken for the shear stress of a fluid that obeys the Maxwell model to reduce to 1/e of its original equilibrium value after the cessation of steady shear flow, (Barnes et al, 1989). At a torque of 20 microNm the retardation time for the Voigt unit fitted is 11.86 seconds and the retardation time at a torque of 5 microNm is 25.40 seconds. Thus it appears that the sample takes longer to recover from the strain at lower torque values. The retardation times are quite short which implies that the sample readily recovers its elasticity once the stress is removed.

TORQUE (microNm)	INSTANTANEOUS COMPLIANCE(m^2/N)	NEWTONIAN VISCOSITY (Pa.s)	SHEAR RATE (s^{-1})
20	2.448×10^{-3}	108600	1.099×10^{-5}
10	1.854×10^{-3}	5333	1.119×10^{-4}
5	3.356×10^{-4}	9745	3.062×10^{-5}

Table 6.1 (a) Retardation Curve Measurements for Citric Acid.

TORQUE (microNm)	INSTANTANEOUS COMPLIANCE(m^2/N)	NEWTONIAN VISCOSITY (Pa.s)	SHEAR RATE (s^{-1})
10	1.700×10^{-3}	5486	1.088×10^{-4}
5	3.269×10^{-3}	6689	4.461×10^{-5}

Table 6.1 (b) Relaxation Curve Measurements for Citric Acid.

The relaxation curve of citric acid/chitosan gel at a torque of 10 microNm is unsuitable for Voigt unit analysis. The higher torque value of 20 microNm is also unsuitable and is outside the linear region. An applied stress of 5 microNm has a relaxation time of 21.09 seconds and a compliance of 4.246 x 10-3 m^2N^{-1} at a single Voigt unit. The Newtonian viscosity of the gel is 6689 Pa.s and this is measured once the response to the applied stress is linear at the time point during relaxation.

6.2.1.2 MALEIC ACID

The gel containing chitosan and maleic acid at the same molar strength as the other acids underwent the same applied stresses and the results can be seen in Table 6.2 (a) for the retardation curve and in Table 6.2 (b) for the relaxation curve. The Newtonian viscosity varies with the shear rate showing an increase in viscosity as the applied stress decreases and the shear rate decreases also. The retardation curve at a torque of 5 microNm is unsuitable for Voigt unit analysis. At a torque of 10 microNm the retardation time is 17.55 seconds and has a compliance value of .01263 m^2/N and a viscosity of 1390 Pa.s. Only one Voigt unit is fitted to the curve. At a torque of 20 microNm, the retardation time is 28.79 seconds and the compliance is .01114 m^2/N with a viscosity of 2584 Pa.s. Thus as the applied stress increases so does the retardation time. The retardation values are low showing the material regains its elasticity once the stress is removed.

TORQUE (microNm)	INSTANTANEOUS COMPLIANCE(m^2/N)	NEWTONIAN VISCOSITY (Pa.s)	SHEAR RATE (s^{-1})
20	2.182×10^{-3}	6454	1.849×10^{-4}
10	1.083×10^{-3}	7361	8.107×10^{-5}
5	1.651×10^{-3}	7646	3.903×10^{-5}

Table 6.2 (a) Retardation Curve Measurements for Maleic Acid.

TORQUE (microNm)	INSTANTANEOUS COMPLIANCE(m^2/N)	NEWTONIAN VISCOSITY (Pa.s)	SHEAR RATE (s^{-1})
20	6.789×10^{-4}	5042	2.367×10^{-4}
10	3.727×10^{-4}	5264	1.134×10^{-4}
5	1.778×10^{-3}	11600	2.572×10^{-5}

Table 6.2 (b) Relaxation Curve Measurements for Maleic Acid.

The relaxation curve at a torque of 20 microNm is unsuitable for Voigt analysis and at a torque of 10 microNm the relaxation time is 35.61 seconds with a compliance of 0.00658 m^2/N and a viscosity of 5411 Pa.s. At a torque of 5 microNm the relaxation time is much shorter 13.93 seconds with a compliance of 0.00484 m^2/N and a viscosity of 2875 Pa.s. Therefore as the applied stress increases, the time for relaxation increases.

6.2.1.3 HYDROCHLORIC ACID

Table 6.3 (a) shows the retardation curve values for instantaneous compliance and Newtonian viscosity at a torque of 5 microNm and Table 6.3 (b) the same values for the relaxation curve. The higher applied stresses were outside the linear region for this sample and could not be measured.

TORQUE (microNm)	INSTANTANEOUS COMPLIANCE(m^2/N)	NEWTONIAN VISCOSITY (Pa.s)	SHEAR RATE (s^{-1})
5	3.087×10^{-4}	4572	6.527×10^{-5}

Table 6.3 (a) Retardation Curve Measurement for Hydrochloric Acid.

TORQUE (microNm)	INSTANTANEOUS COMPLIANCE(m^2/N)	NEWTONIAN VISCOSITY (Pa.s)	SHEAR RATE (s^{-1})
5	2.593×10^{-3}	62430	4.780×10^{-6}

Table 6.3 (b) Relaxation Curve Measurement for Hydrochloric Acid.

The retardation curve has one Voigt unit fitted with the longest retardation time of any of the gels, 80.04 seconds. The compliance is 0.02865 m^2/N with a viscosity of 2794 Pa.s. The data is unsuitable for analysis at Voigt unit 2. The relaxation curve was also unsuitable for analysis at any Voigt unit. The elastic component of the gel takes a longer time to reach equilibrium as it does not recover its elasticity as quickly as the other gels.

6.2.1.4 LACTIC ACID

The chitosan - lactic acid gel is subjected to creep measurements at three values of torque. The instantaneous compliance values for the retardation curve increase as the applied stress increases. At a torque value of 20 microNm the Voigt unit fitted has a retardation time of 22.49 seconds and a compliance of 6.975×10^{-3} m^2/N with a viscosity of 3224 Pa.s. The retardation time at 10 microNm is 77.94 seconds for the first Voigt unit and 13.27 seconds for the second Voigt unit fitted. The compliance at the first Voigt unit is 3.202×10^{-3} m^2/N and 2.824×10^{-3} m^2/N at the second Voigt unit. The first Voigt unit reaches equilibrium at 13 seconds and ceases to move while the second Voigt unit takes longer to reach equilibrium. The retardation curve at the applied stress of 5 microNm is unsuitable for Voigt analysis.

TORQUE (microNm)	INSTANTANEOUS COMPLIANCE(m^2/N)	NEWTONIAN VISCOSITY (Pa.s)	SHEAR RATE (s^{-1})
20	8.619×10^{-4}	8244	1.448×10^{-4}
10	1.497×10^{-3}	6042	9.878×10^{-5}
5	3.051×10^{-5}	9275	3.217×10^{-5}

Table 6.4 (a) Retardation Curve Measurement for Lactic Acid.

TORQUE (microNm)	INSTANTANEOUS COMPLIANCE(m^2/N)	NEWTONIAN VISCOSITY (Pa.s)	SHEAR RATE (s^{-1})
20	1.480×10^{-3}	12110	9.856×10^{-5}
10	3.185×10^{-3}	4985	1.197×10^{-4}
5	5.675×10^{-3}	21150	1.411×10^{-5}

Table 6.4 (b) Relaxation Curve Measurement for Lactic Acid.

The relaxation curve parameters are shown in Table 6.4 (b) and the instantaneous compliance values increase as the applied stress decreases. The analysis of the relaxation curve at 20 microNm shows one Voigt unit fitted with a relaxation time of 93.46 seconds and a compliance of 0.01144 m^2/N with a viscosity of 8166 Pa.s. The sample takes longer to regain its elasticity at this applied stress. At a torque of 10 microNm and 5 microNm both curves were unsuitable for Voigt unit analysis. The gel containing lactic acid is affected by the shear stress applied and takes longer to recover.

6.2.1.5 ACETIC ACID

Table 6.5 (a) shows the retardation curve values for instantaneous compliance and Newtonian viscosity at an applied stress of 20, 10 and 5 microNm. The Voigt unit fitted to the curve of compliance against time at a torque of 5 microNm shows a retardation time of 25.34 seconds at a compliance of 0.02365 m^2/N and a viscosity of 1071 Pa.s. This structure regains its elasticity in a

TORQUE (microNm)	INSTANTANEOUS COMPLIANCE(m^2/N)	NEWTONIAN VISCOSITY (Pa.s)	SHEAR RATE (s^{-1})
20	1.076×10^{-3}	8774	1.360×10^{-4}
10	1.045×10^{-3}	13380	4.459×10^{-5}
5	3.675×10^{-3}	6921	4.311×10^{-5}

Table 6.5 (a) Retardation Curve Measurement for Acetic Acid.

short time interval. At an applied stress of 10 microNm the curve is unsuitable for Voigt analysis. A retardation time of 22.62 seconds is seen at a torque of 20 microNm with a compliance of 5.080×10^{-3} m^2/N and a viscosity of 4452 Pa.s. Thus the retardation time decreases slightly as the applied stress increases.

TORQUE (microNm)	INSTANTANEOUS COMPLIANCE(m^2/N)	NEWTONIAN VISCOSITY (Pa.s)	SHEAR RATE (s^{-1})
20	1.304×10^{-3}	8638	1.382×10^{-4}
10	1.167×10^{-3}	15570	3.050×10^{-5}
5	1.617×10^{-3}	3099	9.628×10^{-5}

Table 6.5 (b) Relaxation Curve Measurement for Acetic Acid.

The relaxation curve at a torque of 20 microNm has two Voigt units fitted. Voigt unit one shows a relaxation time of 43.15 seconds and a compliance of 2.715 x 10-3 m^2/N and a viscosity of 15890 Pa.s. The second Voigt unit has a relaxation time of 7.53 seconds at a compliance of 1.635 x 10-3 m^2/N and a viscosity of 4609 Pa.s. Two Voigt units are also fitted to the curve at a torque of 10 microNm. The relaxation times are 91.44 seconds and 18.82 seconds for the first and second units respectively. The compliance values are 6.926×10^{-3} and 8.99×10^{-4} m^2/N and the viscosity values are 13200 and 20930 Pa.s. The curve was unsuitable for analysis at a torque of 5 microNm at insufficient points were available.

6.2.1.6 ASCORBIC ACID

Table 6.6 (a) shows the instantaneous compliance, the Newtonian viscosity and the shear rate for the three applied stress values. The instantaneous compliance and the viscosity decrease as the applied stress decreases. At a torque of 5 microNm one Voigt unit is fitted to the curve which shows a retardation time of 21.8 seconds at a compliance of 0.02135 m^2/N and a viscosity of 1021 Pa.s. The retardation time at a torque of 10 microNm is 14.95 seconds at a compliance of 8.432 x 10-3 m^2/N and a viscosity of 1773 Pa.s. At a torque of 20 microNm the retardation time is 19.31 seconds at a compliance of 5.891 x 10-3 m^2/N and a viscosity of 3278 Pa.s. Th retardation times are all short showing the sample recovers its elasticity once the stress is released.

TORQUE (microNm)	INSTANTANEOUS COMPLIANCE(m^2/N)	NEWTONIAN VISCOSITY (Pa.s)	SHEAR RATE (s^{-1})
20	1.449×10^{-4}	21110	5.655×10^{-5}
10	9.153×10^{-3}	9293	6.422×10^{-5}
5	3.661×10^{-4}	2920	1.022×10^{-4}

Table 6.6 (a) Retardation Curve Measurement for Ascorbic Acid.

The relaxation curve at a torque of 5 microNm is unsuitable for analysis at one Voigt unit. The relaxation curve at a torque of 10 microNm has one Voigt unit fitted with a relaxation time of 47.71 seconds for the sample with a compliance value of 7.043×10^{-3} m^2/N and a viscosity of 6773 Pa.s. At a torque of 20 microNm two Voigt units are fitted to the curve with relaxation times of 5.25 and 38.49 seconds. The compliance values are 2.134×10^{-3} m^2/N and 3.445×10^{-3} m^2/N and the viscosity values are 2464 and 11170 Pa.s. The ascorbic acid curve has a shorter relaxation time at a higher value of applied stress.

TORQUE (microNm)	INSTANTANEOUS COMPLIANCE(m^2/N)	NEWTONIAN VISCOSITY (Pa.s)	SHEAR RATE (s^{-1})
20	1.274×10^{-3}	23010	5.187×10^{-5}
10	6.863×10^{-3}	8151	7.322×10^{-5}
5	2.328×10^{-8}	1746	1.709×10^{-4}

Table 6.6 (b) Relaxation Curve Measurement for Ascorbic Acid.

6.2.1.7 TARTARIC ACID

Table 6.7 (a) shows the parameters measured at the three torque values for the tartaric acid gel retardation curve. At a torque value of 5 microNm there are insufficient data points to fit a Voigt model to the curve. At a torque of 10 microNm, the retardation time is 23.29 seconds and the compliance is .02189 m^2/N and the viscosity 1064 Pa.s. There are only enough data points to fit one Voigt unit. The retardation time at a torque of 20 microNm is 11.48 seconds; the compliance 2.546 x 10-3 m^2/N and the viscosity 4509 Pa.s. Thus as the applied stress increases the sample regains its elasticity more rapidly and all the retardation times are short suggesting the material is quite elastic.

Table 6.7 (b) shows the relaxation curve data at the different torque values. At a torque of 5 microNm the relaxation time is 66.45 seconds, the compliance 0.02933 m^2/N and the viscosity 2266 Pa.s at Voigt unit one. There are insufficient points to fit a second Voigt unit.

TORQUE (microNm)	INSTANTANEOUS COMPLIANCE(m^2/N)	NEWTONIAN VISCOSITY (Pa.s)	SHEAR RATE (s^{-1})
20	1.464×10^{-3}	55390	2.155×10^{-5}
10	1.526×10^{-3}	6456	9.244×10^{-5}
5	1.556×10^{-3}	7491	3.983×10^{-5}

Table 6.7 (a) Retardation Curve Measurement for Tartaric Acid.

TORQUE (microNm)	INSTANTANEOUS COMPLIANCE(m^2/N)	NEWTONIAN VISCOSITY (Pa.s)	SHEAR RATE (s^{-1})
20	1.746×10^{-3}	50910	2.344×10^{-5}
10	1.830×10^{-3}	4214	1.416×10^{-4}
5	2.441×10^{-3}	18210	1.639×10^{-5}

Table 6.7 (b) Relaxation Curve Measurement for Tartaric Acid.

The relaxation curve at a torque value of 10 microNm has two Voigt units fitted. The relaxation times are 9.8 seconds and 18.44 seconds, the compliance values 6.891×10^{-3} m^2/N and 8.353×10^{-3} m^2/N, and the viscosity values 4220 Pa.s and 2207 Pa.s. The relaxation time at a torque of 20 microNm is 27.62 seconds, the compliance 2.934×10^{-3} m^2/N and the viscosity 9415 Pa.s. The compliance values are higher at lower applied stresses.

6.2.2 INSTANTANEOUS COMPLIANCE COMPARISON

Figure 6.17 shows the instantaneous compliance values for the retardation curves of the various gels at a torque of 10 microNm. No plot is shown for the hydrochloric acid gel as this gel did not produce a reproducible response to this applied stress. The response of the gels to the initial stress varies with ascorbic acid showing the most response with maleic and acetic acid gels showing the lowest instantaneous compliance values. The gels are all within $\pm 2 \times 10^{-3}$ m^2/N except for the ascorbic acid gel.

Figure 6.18 shows the relaxation curves for the same gels at the same torque value. The instantaneous compliance values are of higher magnitude for the relaxation curves of maleic and lactic acid. Ascorbic acid has the largest compliance value (which is lower than its retardation compliance value) followed by the maleic acid gel. The sample size can affect the instantaneous compliance values; as the sample size increases the relaxation instantaneous compliance values will show a reduction in magnitude. The sample size was small to avoid edge effects also. The difference in instantaneous compliance

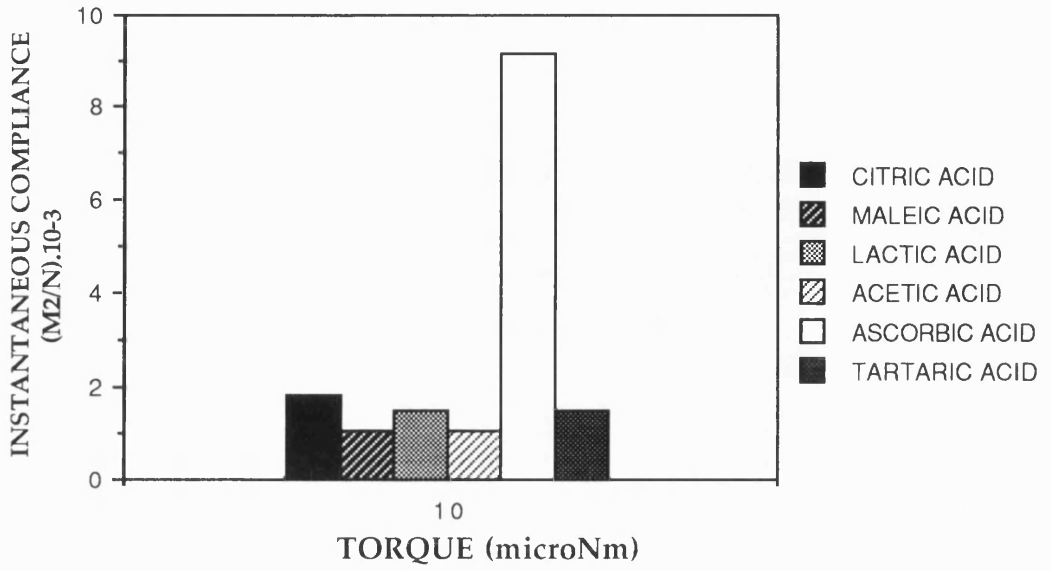


Figure 6.17 Comparison of the Instantaneous Compliance values for the Retardation curves for the gels indicated at an applied stress of 10 microNm.

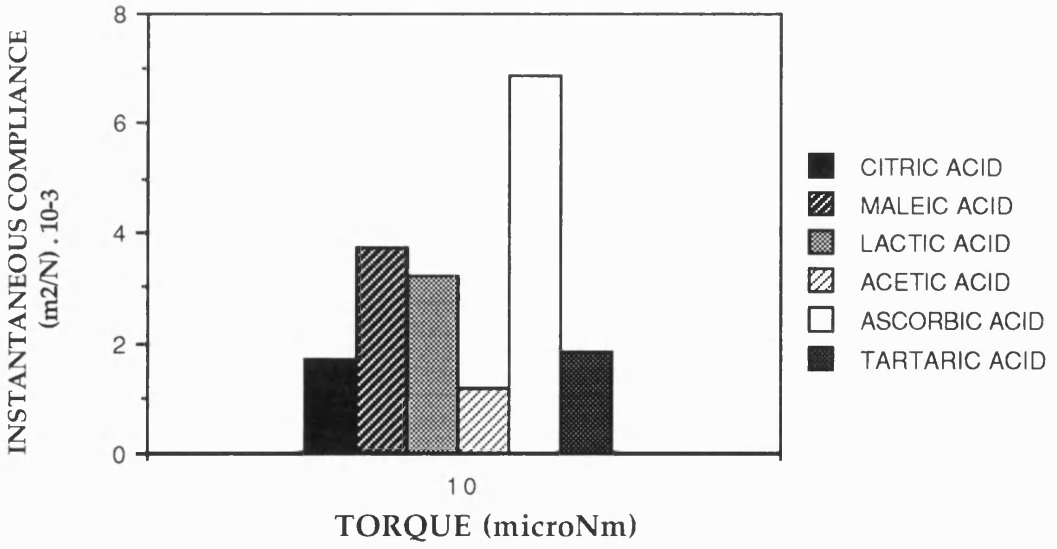


Figure 6.18 Comparison of the Instantaneous Compliance values for the Relaxation curves for the gels indicated at an applied stress of 10 microNm.

values for the ascorbic acid sample suggests a partial loss of the elastic component when the stress is applied to the sample. The other gels all appear to retain their elastic component.

6.2.3 DISCUSSION

The measurement of the various parameters from the creep test gives information on the viscoelastic behaviour of the different chitosan gels. The instantaneous compliance is a measure of the elasticity of the samples, ascorbic acid gel shows the largest compliance value for the retardation curves and also appears to lose some of its elasticity as shown by the relaxation curve. The Newtonian viscosity is measured for each sample at the applied stress. The viscosity tends to be higher at lower values of torque for citric, maleic, lactic acid gels, while higher at the intermediate value for acetic acid, and higher at increasing torque values for ascorbic acid and tartaric acid. The retardation times are short overall indicating the gels are elastic materials and recover rapidly from the applied stress. Citric acid gels have longer retardation times as the applied stress is reduced. This is also seen with the lactic, acetic, ascorbic, and tartaric acid gels. The maleic acid gel shows the opposite; as the applied stress is increased the retardation time is increased. Hydrochloric acid gel shows one retardation value which is considerably longer than the other gels implying the sample takes longer to recover from the applied stress and hence is not as elastic a material as the other samples.

The shear rate at a torque of 10 microNm (5 microNm for hydrochloric acid) decreases slightly for the citric and acetic acid gels, and increases for maleic, hydrochloric, lactic, ascorbic and tartaric acid gels. The increase in shear rate for some of the relaxation curves suggests that the applied stress may have caused some structure breakdown during retardation. Both acetic and citric acid appear not to undergo this structure breakdown, neither showing any increase in shear rate. The viscosity of both citric and acetic acid gels measured shows a slight increase between the retardation and relaxation curves while the other gels all show a decrease in viscosity at 10 microNm.

The oscillation results showed that the plugs of material were predominantly elastic in nature with low tan delta values and the viscous behaviour became more evident at low frequencies apart from the plugs containing hydrochloric acid. Thus the gels appear to show the same elastic behaviour when subjected to a creep test and the differences between them may lie in the way they interact with the chitosan polymer in the acidic solution. The acids are equimolar in concentration used to dissolve chitosan. The solubility of chitosan is known to decrease as the concentration of ions in a solution increases. The type of ions can affect polymer hydration to varying degrees and this would in turn affect the viscosity of the gels formed. Chitosan has a high charge density and in solution the positive ion from the acid interacts with the amino group in chitosan and the polymer will extend itself. Thus the differences seen between the various acids must be due to the interaction with the chitosan polymer.

CHAPTER 7

DISCUSSION

7.1 DISCUSSION

An attempt has been made to formulate controlled drug release pellets containing chitosan, Avicel and lactose as the main excipients. The overall effect of manipulating the variables in the formulation will be discussed with respect to the effect on the drug release. The addition or removal of some ingredients has been shown to affect the release and the ability of the formulation to form pellets. Pellets were formed with low extrusion forces, typically of the order of 3 kN and did not disintegrate in the dissolution medium. The inclusion of chitosan in a gel resulted in a slower controlled release pellet compared to its inclusion in the powder form, and the viscosity and elasticity of these gels were investigated to see if this had an effect on the drug release from the matrix pellet.

The traditional formulation ingredients for extrusion - spheronization consist of Avicel, lactose and a drug, with water added as the granulating fluid to give the mixture sufficient plasticity to extrude and deform into spheres. The rheological properties of this type of formulation have been documented by a number of workers, including Harrison et al. (1987). He describes the rheological characteristics required of the wet powder mass to produce satisfactory extrudate. These properties must also be compatible with the rheological characteristics of the extrudate if it is to undergo successful spheronization. The formulation requires a rigidity to retain its structure when wet and yet must be brittle enough to break into shorter lengths in the spheronizer. Raines et al. (1989) observed the different rheological properties of the mixtures of various types of microcrystalline celluloses with lactose and water. Newton et al. (1992) used three brands of powdered microcrystalline cellulose to prepare spheres with different quantities of water and found that the brands cannot be interchanged, without also changing the water content of the formulation. The inclusion of sodium carboxymethylcellulose (NaCMC) renders the formulation more plastic, Raines et al. (1989). The flow curves of the traditional mixes show non - Newtonian behaviour, with less resistance to flow at higher shear rates. The flow curves with the inclusion of NaCMC in the

formulation appear to be plastic rather than shear thinning. The presence of NaCMC increases the viscosity and is more resistant to flow as shown by the higher values for the die wall shear stress reported by Raines et al. (1989). The formulations presented in this work include the presence of a polymer, chitosan, which will result in a further increase in the viscosity of this complex system. The polymer is incorporated as a gel which shows non-Newtonian characteristics, into the traditional type of system. The rheological properties of the chitosan gels were shown in Chapter 6 and these gels are mixed into a non - Newtonian system and a quantity of water added. The manner in which the MCC reacts to water has been shown to be critical and different sources have been shown to give different rheological characteristics. Thus this complex formulation is not covered by conventional rheological models and the final product is as a result of a number of different materials interacting with each other to produce an extrudate with the required rheological properties to produce spheres.

7.2 PELLET FORMATION & ABILITY TO CONTROL RELEASE

The results suggest that chitosan has potential in a sustained release formulation via extrusion - spheronisation, without the need for pellet coating. Chitosan shows a slower release profile when the polymer is incorporated in a gel (Section 3.3.4) rather than as a dry powder (Section 3.2.3). The polymer dissolves in dilute acids to form a gel and dilute solutions of 10% w/w were shown to be equally suitable as a more concentrated acidic solution (40% w/w). The size of the pellet shows a marked effect on the drug release in each of the formulations. The 1mm and 2mm pellet containing the same formulation appear to show different mechanisms of drug release as seen from the analysis of the dissolution profiles. The 2mm pellet has a slower release profile in each formulation studied and the release appears to follow the first order model, being a diffusion controlled release system. Drug release from the 1mm pellets did not follow the diffusion model and showed a curvilinear shape for the plots of percentage drug released vs time implying the release is not zero order but may be first order.

The presence of barium sulphate also appears to have an effect in delaying the drug release from the sphere (Section 3.4). Barium sulphate is water insoluble and Anderson and Newton, (1990) found that with mixtures of microcrystalline cellulose and barium sulphate, particle size and water content influenced the rheology of the wet powder mass and the subsequent ability of the extrudate to spheronize. The concentration of microcrystalline cellulose also influences the rheological properties and the ability of the mix to extrude and spheronize. The pellets formed appear to be uniformly mixed, when observed in the scanning electron micrographs (Figure 3.24), and present a smooth outer surface with some pores visible. The sphericity of the various pellets can be seen from the micrographs.

Drug release was shown to be pH dependent, as diclofenac sodium is an acidic drug (pKa 3.8), its solubility is limited in an acidic medium. The salt form has however, been shown to be more soluble (Fini et al. 1985). Faster dissolution is obtained in media without acid present, at the higher pH values, when sink conditions are observed. There is also a strong formulation effect on the dissolution profiles seen (Section 3.3.5). No major swelling of the pellets was evident in the acidic media after twelve hours dissolution. The results of the sieve analysis shows that the pellets have quite narrow size distributions.

The 2mm pellets gave slower release profiles from the release rate graphs and appear to approach zero order. The 1mm pellets showed faster initial release and then reached a constant plateau (Figures 3.5 - 3.9). The 1.5mm pellet showed an intermediate release between the 1mm and 2mm pellet. The release per unit surface area (Figure 3.21) for each of the pellets shows that there is not a linear relationship between the amount of drug released per unit surface area. The 2mm pellet shows the slowest release and the 1mm pellet the fastest. Therefore the depth of the pellet matrix and the surface area available to the dissolution medium appears to influence the release. As the pellets are manufactured by extrusion-spheronization a complex diffusional process may be involved, where the drug has to

diffuse through the core prior to dissolving in the dissolution medium. The pellet matrix system has been shown (Scott and Hollenbeck, 1991) to be insensitive to changes in the stirring conditions as diffusion from the non-eroding matrix is the rate controlling factor. Tapia et al. 1993 also found that dissolution testing of pellets containing chitosan at different stirring speeds did not alter the rate of drug release.

7.3 THE INFLUENCE OF ELECTROLYTES ON DRUG RELEASE

Four different electrolytes were incorporated into the pellet system and the release from these pellets investigated in Chapter 4. It has been shown that in general (Li et al. 1992), as the concentration of ions in a polymer solution increases, the polymer solubility decreases. The amount of water available to hydrate the polymer is reduced as more water is required to keep the ions in solution. The changes in the viscosity of the gels is as a result of the inclusion of the electrolytes and the affect they have on the hydration of the chitosan polymer. Sodium chloride and potassium chloride are monovalent electrolytes and therefore, their ionic strength is equal to to their molality (unit = mol kg^{-1}) (Florence and Attwood, 1981). The divalent salts are more complex in their ionisation behaviour and have higher ionic strengths. For the salt solutions, the ionic strength may be calculated from their molarity providing the density of the solution is known. The sodium and magnesium salts at equimolar concentrations, were shown to produce the more viscous gels, as assessed by the shear rates from the flow curves (Section 4.7.1), compared to the potassium salts especially at the higher chitosan concentration at both levels of the electrolyte, and at the higher electrolyte and lower chitosan concentration. The lower chitosan and lower electrolyte concentration showed that the potassium salts produced the more viscous gels.

The dissolution profiles for the pellets containing 3.5 or 7% chitosan with one of the electrolyte concentrations (0.8 or 1.6M) are shown below. Figure 7.1 shows that at the higher chitosan and higher electrolyte concentration, potassium hydrogen carbonate pellets

are the slowest releasing followed by the sodium salt while the potassium chloride salt is the fastest releasing. Figure 7.2 shows the dissolution profiles of the pellets containing the lower chitosan concentration and the higher electrolyte concentration. Once again the potassium hydrogen carbonate pellet is the slowest releasing followed by the sodium salt while the magnesium salt is marginally the fastest releasing. Thus at the higher electrolyte concentration the drug release from the pellets does not appear to reflect the viscosity of the gels. The sodium salt which was the second most viscous gel also was the second slowest releasing pellet. The magnesium salt which was the other viscous gel (Figure 4.12) was one of the faster releasing pellets in both samples. Potassium hydrogen carbonate appears to be one of the less viscous gels (Figure 4.13) and produced the slowest releasing pellet. Thus drug release does not appear to follow the gel viscosity for these pellets at the higher electrolyte concentration. The sodium salt produces a more viscous gel and is slower releasing while the two potassium salts produce less viscous gels and are both slow and fast releasing.

The dissolution profiles of the pellets containing the lower electrolyte concentration are shown in Figures 7.3 and 7.4. The higher chitosan and lower electrolyte concentration shown in Figure 7.3 for the pellets results in potassium hydrogen carbonate showing the slowest release followed by the magnesium salt and potassium chloride having the fastest release. The gel viscosities for these formulations showed sodium and magnesium being the more viscous gels. The release is slower from the formulations containing the more viscous gels apart from the potassium hydrogen carbonate gel which produces the slowest releasing pellet.

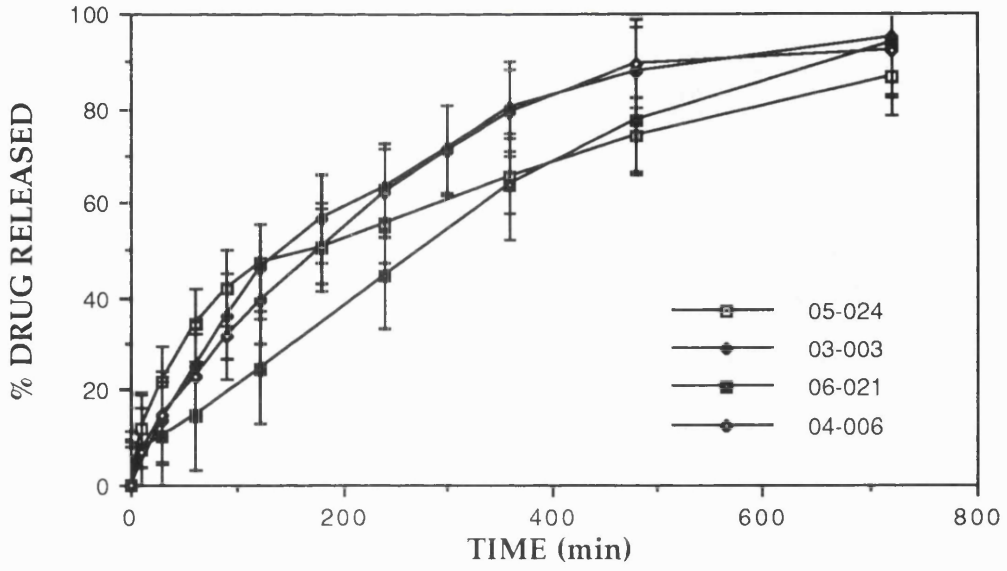


Figure 7.1 Dissolution profile of pellets (05-Sodium chloride, 03-Potassium chloride, 06-Potassium hydrogen carbonate, 04-Magnesium) containing 7% chitosan and the higher level of electrolyte.

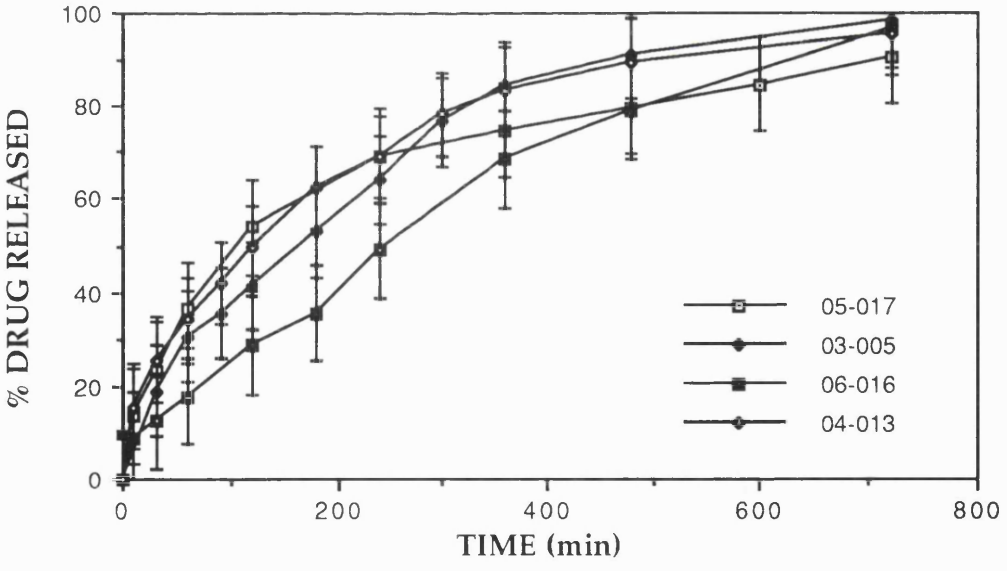


Figure 7.2 Dissolution profile of pellets (05-Sodium chloride, 03-Potassium chloride, 06-Potassium hydrogen carbonate, 04-Magnesium) containing 3.5% chitosan and the higher level of electrolyte.

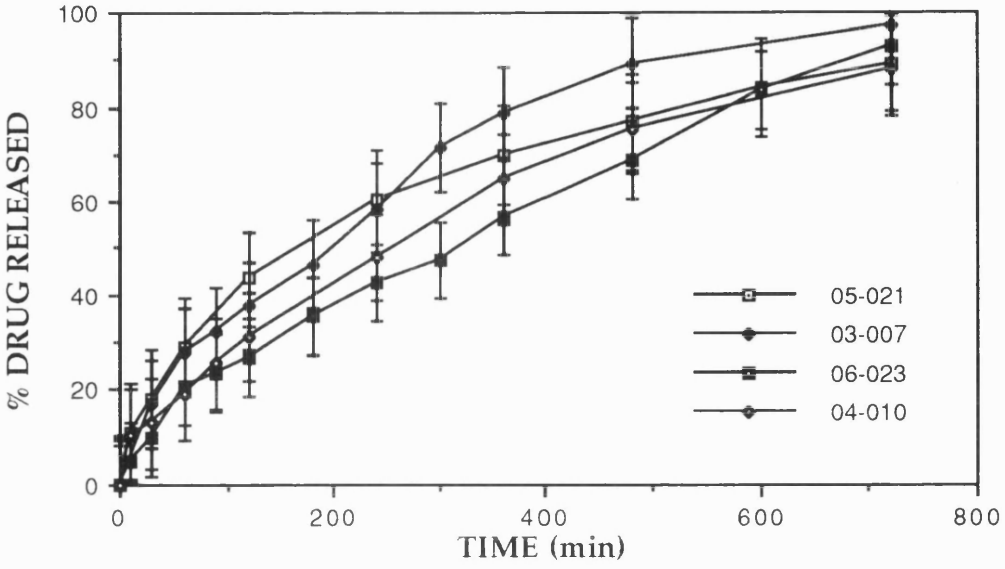


Figure 7.3 Dissolution profile of pellets (05-Sodium chloride, 03-Potassium chloride, 06-Potassium hydrogen carbonate, 04-Magnesium) containing 7% chitosan and the lower level of electrolyte.

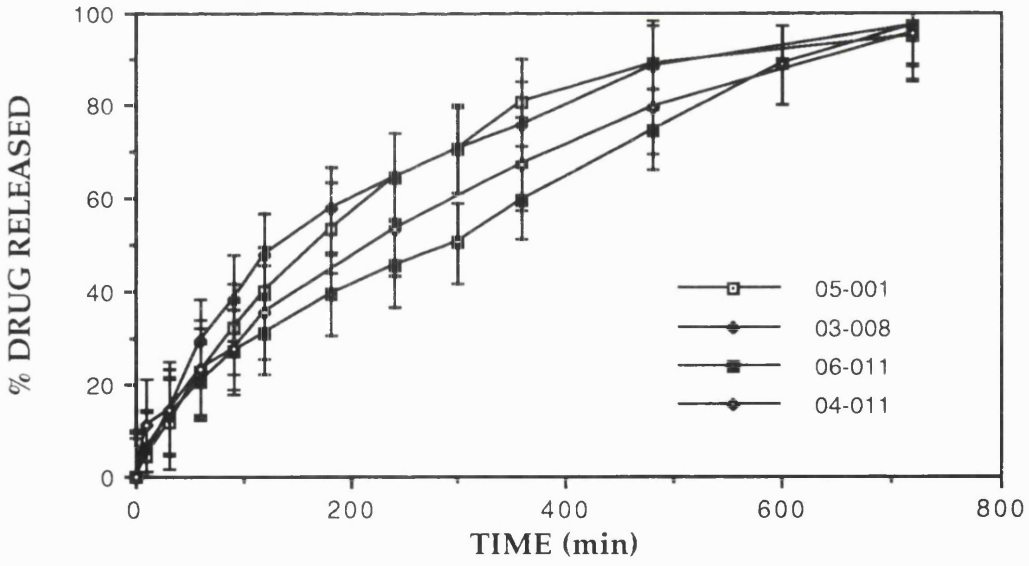


Figure 7.4 Dissolution profile of pellets (05-Sodium chloride, 03-Potassium chloride, 06-Potassium hydrogen carbonate, 04-Magnesium) containing 3.5% chitosan and the lower level of electrolyte.

Ming-Thau Sheu et al. (1992) investigated the release from matrix tablets containing diclofenac sodium as the drug with the addition of sodium or potassium chloride to the dissolution medium. They found that the addition of these salts decreased the solubility of the drug and slowed the dissolution rate with the effect of sodium chloride being greater. The effect of sodium chloride on slowing the dissolution rate was also greater in this study, than that of potassium chloride. The solubility of the drug, diclofenac sodium will have a major impact on the dissolution rate. Thus the salt potassium hydrogen carbonate may inhibit the solubility of the acidic drug resulting in a slower dissolution rate.

The dissolution profiles for the pellets containing the lower chitosan and electrolyte concentration is shown in Figure 7.4. The potassium hydrogen carbonate pellet is once again the slowest releasing, followed by the magnesium containing pellet. The potassium chloride pellet is the fastest releasing. The viscosity for these gels is; potassium hydrogen carbonate > potassium chloride > sodium chloride > magnesium chloride. The release from these pellets does not appear to follow the viscosity of the gels incorporated in the formulation.

Therefore, in conclusion, there does not appear to be a direct relationship between the gel viscosity and the drug release from the pellet. The slowest releasing formulations are the ones that contain the highest chitosan and lowest electrolyte concentration for magnesium chloride, potassium hydrogen carbonate and potassium chloride. This formulation is also slow releasing for sodium chloride along with the formulation containing the highest chitosan and highest electrolyte concentration. The solubility of the drug will be significantly affected by the both the type of salt present and the hydrogen ion concentration in the dissolution medium at pH 7.4.

7.4 CHOICE OF A SOLVENT SYSTEM FOR CHITOSAN

Chitosan is soluble in a wide number of dilute inorganic acids and forms gels (Kienzle-Sterzer et al. 1982). Acetic acid is the most

widely used solvent system but, it is corrosive and has an unpleasant odour, and alternative acids were investigated (see Chapter 5). Seven different acids were used to formulate pellets and their dissolution profiles investigated. Citric acid when used as a solvent did not form pellets and hence could not be tested by dissolution. Adusumilli and Bolton (1991) prepared chitosan citrate complexes and investigated the drug release from matrix tablets containing the complex. They concluded that these complexes formed excellent hydrophilic matrix systems for controlling drug release. Nigalaye et al. (1990) also investigated the sustained release characteristics of chitosan in the presence of citric acid in a tablet formulation. The rate of drug release was shown to be slower in tablets containing citric acid compared to those containing chitosan alone.

The drug release at 120 minutes is shown in Figure 5.16 for the pellets containing the various acids and this graph shows that pellets, prepared by dissolving chitosan in hydrochloric acid are the fastest releasing. These dissolution experiments all took place in phosphate buffer pH 7.4. The pellets containing ascorbic acid are the next fastest releasing closely followed by the pellets containing tartaric acid. Pellets containing maleic acid were the slowest releasing followed by acetic and lactic acid. Therefore, either of these acids, maleic or lactic would be a suitable alternative to acetic acid in terms of drug release and ease of pellet formation. Both produced an extrudate that readily deforms in the spheronizer to produce spheres. The hydrochloric acid extrudate readily produced spheres of various sizes depending on the moisture content of the extrudate, all of which were fast releasing. Hydrochloric acid is the strongest acid in the series (pKa -6.1) and as only a low drug concentration is present in the dissolution medium, it does not appear to affect the solubility of the acidic drug, diclofenac sodium.

The flow curves (Section 5.7.1) for the various chitosan gels dissolved in the equivalent strength acids showed that ascorbic acid produced the most viscous gel followed by lactic, citric, tartaric, acetic and maleic. The hydrochloric acid gel appears visually to be the most fluid but gave high viscosity readings probably as the layer between

the cone and plate was very thin. Another measuring system would be more suitable for a gel of this consistency. The pellets containing hydrochloric acid are the fastest releasing and contain the most viscous gel measured. Maleic acid containing pellets were the slowest releasing and contained the least viscous gel measured by the cone and plate rheometer. Pellets containing acetic and lactic acid were shown to be the next slowest releasing, acetic acid gel being not very viscous and the lactic acid gel being one of the most viscous gels. Ascorbic acid produced the most viscous gel and the pellets made with the gel were the fastest releasing ones after the pellets containing hydrochloric acid. The trend appears to be that the less viscous gels (maleic acid) produce pellets that are slow releasing while the more viscous gels (ascorbic acid) produce pellets that are faster releasing.

The choice of a solvent system for chitosan depends on the release required from the final pellet. All of the acids tested here apart from citric acid, produced spheres by extrusion-spheronization. The slowest releasing pellets were those containing maleic acid while the fastest releasing were the pellets containing hydrochloric acid. The less viscous gels appear to produce pellets with slower drug release rates in the phosphate buffer dissolution medium. Acetic acid is a good solvent for chitosan but the practical difficulties associated (corrosion of the barrel and dies) render it not the solvent of choice.

7.5 CREEP AND OSCILLATION RHEOMETRY RESULTS

The application of an oscillating stress to the plugs of material containing Avicel, lactose, drug, chitosan dissolved in a gel in one of the seven acids (at equimolar concentrations) and water gives information on the viscoelastic properties of the material, as measured by tan δ. The release from the formulations containing the different gels has already been discussed and maleic acid was found to be the slowest releasing. The single point measurement at a torque of 3000 microN.m and a frequency of 1Hz showed that the plugs containing hydrochloric and maleic acid were the most elastic. Citric acid containing plugs were the least elastic as shown by their

low values for the Storage Modulus. Citric acid formulations did not deform sufficiently in the spheronizer to form spheres. They produced dumbells or on addition of excess water agglomeration resulted. Thus the oscillation of the plug of material is useful in determining the elasticity and this will provide information on whether the extrudate will be sufficiently elastic to deform into spheres. Figure 7.5 shows the tan delta values for the plugs of material at two values of torque.

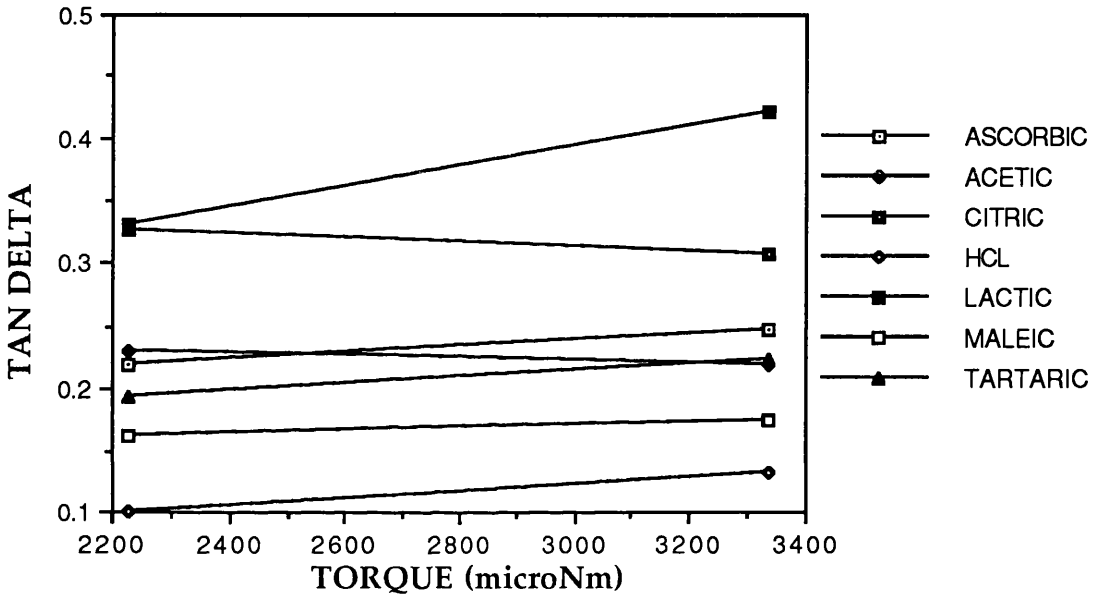


Figure 7.5 Plot of Tan Delta values for the plugs of material at two different values of Torque.

Tan delta values are an indicator of viscoelasticity for the formulations and the lower the value, the more elastic and less viscous is a material. Hydrochloric acid shows the lowest tan delta value followed by maleic acid and the values remain steady over a range of applied stresses. The plug containing lactic acid has the highest tan delta value and this increases slightly as the value of torque increases. Citric acid also shows one of the higher tan delta values. However the values overall are low indicating that the plugs are elastic in nature.

Plugs containing hydrochloric acid were shown to be the most elastic in nature and these readily deformed to produce spheres on adding different amounts of additional water. The extrusion force for these pellets was lower than for any of the others typically 2kN. Maleic acid plugs (extrusion force ~ 3.4kN) were the second most elastic ones measured and produced spheres readily in the spheronizer also. Citric acid formulations extruded at a value of 2.8 - 3 kN but did not form pellets, therefore the elastic nature appears to influence the ability to form spheres.

The Storage (Elastic) Modulus values for the plugs of material examined are of a higher value than those recorded for the Loss (Viscous) Modulus (Section 6.1.4). This suggests from these results that the materials are predominantly elastic and yet readily extrude and deform in the spheronizer to form spheres. The frequency sweeps on the individual plugs showed that, at the lower frequency values the viscous behaviour becomes more important, apart from the hydrochloric acid plugs. The plugs containing hydrochloric acid are elastic in nature, extrude at low forces and readily form spheres. However they do not produce a controlled release pellet. Plugs containing maleic acid, the second most elastic plug of material readily produces pellets, extrudes at a slightly higher force and produces a good controlled release pellet. The other plugs of material all produce an elastic response to the applied stress and also produce a controlled release pellet to different degrees. The rheological properties of a material required to produce spheres have been documented by other workers, including, Newton (1990) and Fielden (1987). The initial formulation must have sufficient viscosity to flow through the die and enough rigidity to withstand the processing conditions and sufficient plasticity to deform into spheres. The formulations tested all showed this ability apart from citric acid formulations which do not appear to have sufficient plasticity to mould into spheres under the influence of the spheronizing force.

The creep tests were performed on the chitosan gels dissolved in the various acids unlike the oscillation tests, which were performed on the plugs of material containing the chitosan gels

consisting of the same formulations as those that were extruded. The instantaneous compliance shows that the ascorbic acid gel (Figure 6.17) is the most elastic but does not recover its elasticity on the removal of the stress as rapidly as some of the other gels. Hydrochloric acid gel also took a long time to recover from the applied stress.

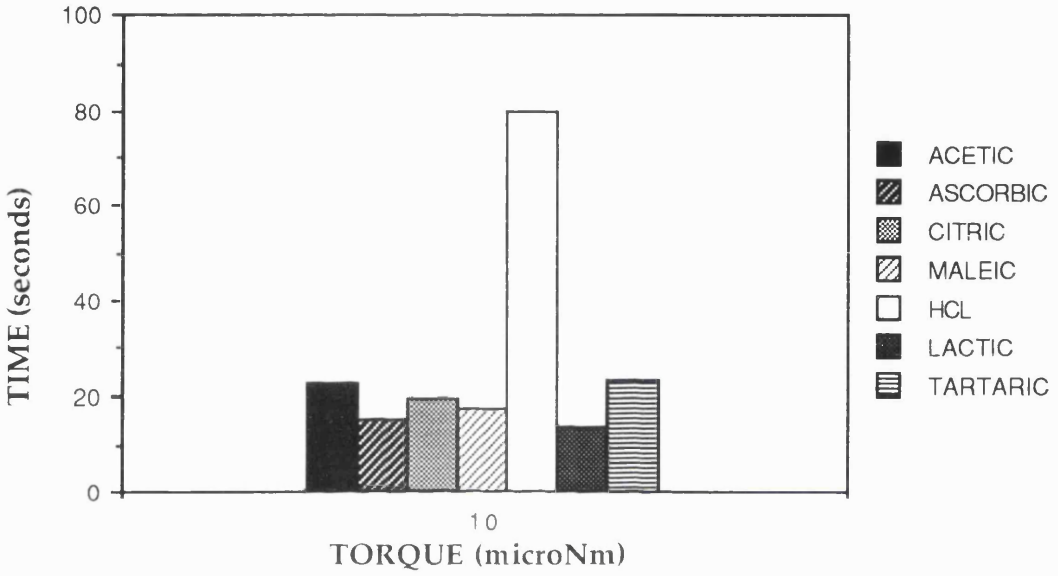


Figure 7.6 Retardation times for the gels at an applied stress of 10 microNm.

The retardation times for the gels are shown in Figure 7.6, which is a measure of the time taken to recover after the removal of the stress. The hydrochloric acid gel takes longest to recover and lactic acid gel recovers fastest. The other gels all show a recovery of ~ 20 seconds which is quite rapid, once again indicative of an elastic material. Maleic acid recovers in 17.5s.

The shear rate was shown to increase for some of the gels during the relaxation plots and this implies some sort of structure breakdown (Sherman, 1970). Chitosan gels containing citric and acetic acid did not show this effect. The comparison of oscillation and creep results shows that the materials are elastic in nature and some

recover more rapidly than others from applied stresses. Citric acid is shown to have less elasticity in the plug of material while the hydrochloric acid plug was shown to be more elastic. This reflects the ease of pellet formation with the materials.

CHAPTER 8

CONCLUSION

8.1 GENERAL CONCLUSIONS

The term chitosan covers a whole range of related polymers depending on the source and the degree of deacetylation, which will alter its physical and chemical properties. Thus the exact properties of the polymer must be specified initially in any work. Formulations containing different concentrations of chitosan have the ability to sucessfully produce pellets from an extrusion - spheronization process. A controlled release pellet can be produced from a matrix type system eliminating the need for a coating process. The concentration of chitosan in the pellet affects the release, an increasing chitosan concentration delaying drug release. The inclusion of chitosan dissolved in a dilute inorganic solvent results in an improved controlled release pellet compared to the dry powder form. The manipulation of the excipients (lactose, Avicel and barium sulphate) in the pellet has been shown to alter the drug release. Increasing barium sulphate concentration has been shown to help in slowing drug release. The quantity of water added as a granulating fluid has been shown to be of primary importance in determining the ability of the formulation to form spheres. Too little, results in dumbell shapes and too much in agglomeration of the pellets. The exact quantity of water is determined by trial and error. Pellet size was shown to modify the drug release and the larger pellet shows diffusion controlled release (Section 3.4). Dissolution profiles were found to be pH dependent, as the drug diclofenac sodium is not very soluble in acidic media.

The addition of an electrolyte reduces the viscosity of the chitosan gel and the slowest release profiles were seen with the pellets containing the higher chitosan concentrations and the lower electrolyte concentration while the other variables in the formulation were kept constant. Pellets containing potassium hydrogen carbonate showed the slowest release profiles.

Chitosan has the ability to form pellets with a number of different acids as a solvent for the polymer. Acetic acid is the most widely used but maleic acid is shown to be a good alternative, readily producing spheres which show a controlled release effect.

The controlled stress rheology performed on the plugs of Avicel, lactose, drug, water and chitosan dissolved in the different acids, and the creep experiments performed on the gels, showed the elastic nature of the material. The low values found for the storage modulus reflected the difficulties in producing spheres in the formulations, especially with citric acid. The higher the value for the storage (elastic) modulus the easier it was to produce spheres. The tan delta values were low overall indicating primarily elastic materials. Thus the creep and oscillation tests help to identify formulations that will readily deform in the spheronizer to produce spheres.

8.2 FUTURE WORK

Formulations containing chitosan as a gel have been shown to produce spheres by the process of extrusion-spheronization. The grades of chitosan tested could be extended to evaluate the results with polymers with different degrees of deacetylation. Further work is required with increasing concentrations of the polymer in the pellet, to determine its effect on the rate of drug release. At present the concentration of chitosan has been shown to affect the drug release, increasing concentrations delaying the release but the maximum concentration that may be included has not been determined. The concentration that can be included is limited by the rheological properties of the mixture.

The inclusion of diclofenac sodium as the model drug in this study could be extended to include a wider range including some basic drugs. The amount of water added to produce the final product has not been quantified and is determined by trial and error at present. Further investigations into this extra water required to produce a pellet are needed. The controlled stress rheology is useful in assessing the viscous and elastic properties of the plugs of material and in identifying the formulations that will sucessfully spheronize. Further manipulations of the pellet formulations and addition of electrolytes may give rise to the optimum controlled release pellet.

REFERENCES

Acarturk,F. Preparation of a prolonged - release tablet formulation of diclofenac sodium. Pharmazie 1989, 44 (8), 547-549.

Adusumilli,P.S. and Bolton,S.M. Evaluation of Chitosan Citrate Complexes as Matrices for Controlled Release Formulations using a 3^2 Full Factorial Design. Drug Dev. Ind. Pharm. 1991, 17 (14), 1931-1945.

Aiba,S. Studies on Chitosan: 1. Determination of the degree of N-acetylation of chitosan by ultraviolet spectrophotometry and gel permeation chromatography. Int. J. Biol. Macromol. 1986, 8, 173-176.

Aiba,S. Studies on Chitosan: 2. Solution stability and reactivity of partially N-acetylated chitosan derivitives in aqueous media. Int. J. Biol. Macromol. 1989, 11 (4), 249-252.

Aiba,S., Minoura,N., Taguchi,K. and Fujiwara,Y. Covalent immobilisation of Chitosan derivitives onto polymeric film surfaces with the use of a photosensitive hetero-bifunctional crosslinking reagent. Biomaterials 1987, 8 (6), 481-488.

Aiguelles-Monal,W. and Peniche-Covas,C. Study of the interpolyelectrolyte reaction between chitosan and carboxymethylcellulose. Makromol. Chem. (Rapid Commun.) 1988, 9 (10), 693-697.

Akbuga,J. Use of chitosonium malate as a matrix in sustained-release tablets. Int. J. Pharm. 1993, 89, 19-24.

Anderson,A.H. and Newton,J.M. The influence of Moisture Content and Particle Size of Barium Sulphate on the Extrusion Properties of Mixtures with Microcrystalline Cellulose. p258-267 'Rheology of Food, Pharmaceutical and Biological Materials.' 1990, Elsevier, Amsterdam.

Arai,K., Kinumaki,T. and Fujita,T. Toxicity of Chitosan. Bull. Tokai Reg. Fish. Res. Lab. 1968, 56, 89-95.

Ashford,N.A., Hattis,D. and Murray,A.E. Industrial prospects for chitin and protein from shellfish wastes. MIT Sea Grant Report MITSG 77-3, 1977, MIT Cambridge Mass.

Austin,P.R., Brine,C.J., Castle,J.E. and Zikakis,J.P. Chitin: New Facets of Research. Science 1981, 212, 749.

Baert,L. and Remon,J.P. Influence of amount of granulation liquid on the drug release rate from pellets made by extrusion-spheronization. Int. J. Pharm. 1993, 95, 135-141.

Baert,L., Vermeersch,H., Remon,J.P., Smeyers-Verbeke,J. and Massart,D.L. Study of parameters important in the spheronization process. Int. J. Pharm. 1993, 96, 225-229.

Bagley,E.B. End corrections in the capillary flow of polyethylene. J. Appl. Physics 1957, 28, 624-627.

Barrau,J.P., Bataille,B. and Jacob,M. The Influence of Spheronizer Load in Extrusion/Spheronization. Pharm. Tech. Int. September 1993, 66-70.

Barry,B.W. Advances in Pharmaceutical Sciences. Academic Press, London 1974, 4, 1-72.

Bechgaard,H. and Nielsen, G.H. Controlled Release Multiple-Unit and Single-Unit Doses. Drug Dev. Ind. Pharm. 1978, 4, 53-67.

Bodmeier,R. and Paeratakul,O. Spherical agglomerates of water insoluble drugs. J. Pharm.Sci. 1989, 78 (11), 964-967.

Bodmeier,R., Oh,K.H. and Pramar,Y. Preparation and Evaluation of drug containing Chitosan beads. Drug Dev. Ind. Pharm. 1989, 15 (9), 1475-1494.

Braconnot,H. Sur la nature des champignons. Ann. Chi. Phys., 1811, 79, 265-304.

British Pharmacopeia 1993 Her Majesty's Stationery Office, London.

Chandy,T. and Sharma,C.P. Biodegradable Chitosan Matrix for the Controlled Release of Steroids. Biomat., Art. Cells & Immob. Biotech., 1991, 19 (4), 745-760.

Chandy,T. and Sharma,C.P. Chitosan - as a biomaterial. Biomat.,Art. Cells, Art. Org. 1990, 18, 1-24.

Chapman,S. The influence of process variables on the preparation of spherical granules. PhD Thesis, University of London, 1985.

Cho,I. and Chul,Y. A potential source of clean chitin and its film properties. Int. J. Biol. Macromol., 1980, 2, 52.

Conine,J.W. and Hadley,H.R. Preparation of small solid pharmaceutical spheres. Drug and Cosmet. Ind. 1970, 106 (4), 38-41.

Davis,S.S., Shotton,E. and Warburton,B. Some limitations of continuous shear methods for the study of pharmaceutical semisolids. J. Pharm. Pharmacol. Suppl. 1968, 20, 157-167.

Deasy,P.B. Microencapsulation and Related Drug Processes. 1984, Marcel Dekker, New York.

Domard,A. and Rinaudo,M. Preparation and characterization of fully deacetylated chitosan. Int. J. Biol. Macromol. 1983, 5(1), 49-52.

Domard,A. pH and cd measurements on a fully deacetylated chitosan: application to Cu^{II} - polymer interactions. Int. J. Biol. Macromol., 1987, 9, 98-104.

Domard,A., Gey,C., Rinaudo,M. and Terrassin,C. ^{13}C and proton NMR spectroscopy of Chitosan and N-trimethyl chloride derivitives. Int. J. Biol. Macromol. 1987, 9 (4), 233-237.

Domard,A., Rinaudo,M. and Terrassin,C. Adsorption of Chitosan and a quaternized derivitive on kaolin. J. Appl. Poly. Sci. 1989, 38 (10), 1799-1806.

Domard,A., Rinaudo,M. and Terrassin,C. New method for the quarternization of chitosan. Int. J. Biol. Macromol., 1986, 8, 105-107.

Domszy,J.G. and Roberts,G.A.F. Reactions of chitosan: 5. The reaction of chitosan with 2,4-dinitrofluorobenzene and determination of the extent of the reaction. Int. J. Biol. Macromol., 1985, 7, 45.

Elbers,J.A.C., Bakkenes,H.W. and Fokkens,J.G. Effect of Amount and Composition of Granulation Liquid on Mixing, Extrusion and Spheronization. Drug Dev. Ind. Pharm. 1992, 18 (5), 501-517.

Eskilson,C. Controlled Release by Microencapsulation. Manuf. Chem. March 1985, 35-39.

Fairbrother,J.E. Thermal Methods of Analysis. The Pharmaceutical Journal, June 1983, 730-732.

Fenner,R.T. Extruder Screw Design. 1970, Iliffe Press, London.

Ferry,J.D. Viscoelastic properties of polymers. 3rd Edition, 1980, Wiley, New York.

Fielden,K.E., Newton,J.M., O'Brien,P. and Rowe,R.C. Thermal studies on the interaction of water and microcrystalline cellulose. J. Pharm. Pharmacol. 1988, 40, 674-678.

Fielden,K.E. and Newton, J.M. 'Extrusion and Extruders', in "Encyclopedia of Pharmaceutical Technology" Ed. Swarbrick,J. and Boylan,J.C. Volume 5, Marcel Dekker, New York, 1992, 395-442.

Fielden,K.E. Extrusion and spheronization of microcrystalline cellulose and lactose mixtures. PhD Thesis, University of London, 1987.

Filar,L.J. and Wirick,M.G. Bulk and solution properties of chitosan. 'Proceedings of the 1st Int. Conf. Chitin/Chitosan', 1978, 169-181. Ed. R.A.A. Muzzarelli and E.R. Pariser MIT Sea Grant Program, Cambridge, Mass.

Fini,A., Zecchi,V. and Tartarini,A. Dissolution Profiles of NSAID Carboxylic acids and their salts with different Counter Ions. Pharm. Acta. Helv. 1985, 60 (2), 58-62.

Florence,A.T. and Attwood,D. Physicochemical Principles of Pharmacy. MacMillan 1981, 88-90.

Ghebre-Sellassie,I., Gordon,R., Fawzi,M.B. and Nesbitt,R.U. Evaluation of a high speed pelletization process and equipment. Drug Dev. Ind. Pharm. 1985, 11, 1523-1541.

Gooday,G.W. The microbial synthesis of cellulose, chitin and chitosan. Prog. Ind. Microbiol. 1983, 18, 85-127.

Gordon,D.T. and Williford,C.B. Influence of chitin and chitosan on element absorption in the rat. ACS Symposium Series 1983, Amer. Chem. Soc., Washington DC.

Goskonda,S.R. and Upadrashta,S.M. Avicel RC-591/Chitosan Beads by Extrusion-Spheronization Technology. Drug Dev. Ind. Pharm. 1993, 19 (8), 915-927.

Gurny,R., Doelker,E. and Peppas,N.A.Modelling of sustained release of water - soluble drugs from porous, hydrophobic polymers. Biomaterials 1982, 3 (1), 27-32.

Harrison,P.J. Extrusion of Wet Powder Masses. PhD Thesis, University of London, 1982.

Harrison,P.J., Newton,J.M. and Rowe,R.C. The application of capillary rheometry to the extrusion of wet powder masses. Int. J. Pharm. 1987, 35, 235-242.

Hasznos,L., Langer,I. and Gyarmathy,M. Some Factors Influencing Pellet Characteristics made by an Extrusion/Spheronization Process. Drug Dev. Ind. Pharm. 1992, 18 (4), 409-437.

Henriksen,I., Skaugrod,O. and Karlsen,J. Use of chitosan and chitosan malate as an excipient in wet granulation of three water soluble drugs. Int. J. Pharm. 1993, 98, 181-188.

Higuchi,T. Mechanism of sustained action medication. J. Pharm. Sci. 1963, 52, 1145-1149.

Higuchi,T. Rate of release of medicaments from ointment bases containing drugs in suspension. J. Pharm. Sci. 1961, 50, 874-875.

Hillyard,I.W., Doczi,J. and Kiernan,P.B. Pharmacological uses of chitin and chitosan. Proc. Soc. Exp. Biol. Med. 1964, 115, 1108-1112.

Hirano,S. in "Chitin and Chitosan", ed. Skjak-Braek,G., Anthonsen,T., and Sandford,P., Elsevier Applied Science, New York. 1989, 37-43.

Hirano,S. and Yano,H. Some nitrated derivitives of N-acylchitosan. Int. J. Biol. Macromol., 1986, 8, 153-156.

Hirano,S., Ilakura,C., Seino,H., Akiyama,Y., Nanaka,I., Kanbura,N. and Kawakami,T. Chitosan as an Ingredient for Domestic Animal Feeds. J. Agric. Food Chem. 1990, 38 (5), 1214-1217.

Hopfenberg,H.B. "Controlled release from erodible slabs, cylinders and spheres", in Controlled Release Polymeric Formulations. Eds Paul,D.R. and Harris,F.W. ACS Washington DC, 1976, 26 -32.

Hou,W.M., Miyazaki,S., Takada,M. and Kamai,T. Sustained release of indomethacin from chitosan granules. Chem. Pharm. Bull. 1985, 33 (9), 3986-3992.

Ikeda,I., Tamari,Y. and Sugano,M. Interrelated effects of Dietary Fibre and Fat on Lymphatic Cholesterol triglyceride absorption in Rats. J. Nutr. 1989, 119 (10), 1383-1389.

Imai,T., Shiraishi,S., Saito,H. and Otagiri,H. Interaction of indomethacin with low molecular weight chitosan, and improvements of some pharmaceutical properties of indomethacin by low molecular weight chitosans. Int. J. Pharm. 1991, 67, 11-20.

Inouye,K., Machida,Y., Sannan,T. and Nagai,T. Buoyant sustained release granules based on chitosan. Drug Design and Delivery 1989, 4 (1), 55-67.

Inouye,K., Machida,Y., Sannan,T. and Nagai,T. Buoyant sustained release tablets based on chitosan. Drug Design and Delivery 1988, 2, 165-175.

Jasberg,B.K., Mustakas,G.C. and Bagley,E.G. J. Rheol. 1979, 23, 437-449.

Kanke,M., Katayama,H., Tsuzuki,S. and Kuramoto,H. Application of Chitin and Chitosan to Pharmaceutical Preparations.1. Film Preparation and in Vitro Evaluation. Chem. Pharm. Bull. 1989, 37 (2), 523-525.

Karlsen,J. and Skaugrud,O. Excipient properties of chitosan. Manuf. Chem. June 1991, 18-19.

Kawashima,Y., Lin,S.Y., Kasai,A., Handa,T. and Takenaka,H. Preparation of a Prolonged Release Tablet of Aspirin with Chitosan. Chem. Pharm. Bull. 1985, 33 (5), 2107-2113.

Kay,R. Communication Bioshell Inc. 1982 Albany Ore.

Kienzle-Sterzer,C., Rodriguez-Sanchez,D. and Rha,C.K. Dilute solution behaviour of a cationic polyelectrolyte. J. Appl. Polym. Sci. 1982, 27, 4467.

Kienzle-Sterzer,C., Rodriguez-Sanchez,D. and Rha,C.K. p383-393 in Chitin, Chitosan and related Enzymes, 1984, Academic Press.

Kienzle-Sterzer,C.A., Rodriguez-Sanchez,D. and Rha,C.K. p338 in Chitin in Nature and Technology. Eds. Muzzarelli, R.A.A.,Jeuniaux,C. and Gooday,G.W. Plenum Press, New York, 1986.

Knapczyk,J. Chitosan Hydrogel as a base for semisolid drug forms. Int. J. Pharm. 1993, 93, 233-237.

Knapczyk,J. Excipient ability of chitosan for direct tabletting. Int. J. Pharm. 1993, 89, 1-7.

Knorr,D. Dye binding properties of chitin and chitosan. J. Food. Sci. 1983, 48, 36.

Knorr,D. Use of Chitinous Polymers in Food. Food Technol. (Chicago) 1984, 38 (1), 85-97.

Kurita,K., Koyama,Y., Nishimura,S. and Kamiya,M. Facile Preparation of water-soluble Chitin from Chitosan. Chem. Lett. 1989, (9), 1597-1598.

Kurita,K., Yoshida,A. and Koyama,Y. Studies on Chitin. 13. New Polysaccharide/Polypeptide hybrid Materials based on Chitin and Poly(γ - methyl L - glutamate). Macromolecules 1988, 21, 1579-1583.

Kushino,S. and Asano,H. Water Soluble Chitosan. Jpn. Kokai Tokkyo Koho. 1988 JP 63,225,602.

Landes,D.R. and Bough,W.A. Effects of Chitosan - a coagulating agent for food processing wastes - in the diet of rats on growth and liver

and blood consumption. Bull. Environmental Contam. Toxicol. 1976, 15, 555.

Lee, V.H.L. and Robinson,J.R. Methods to achieve sustained drug delivery, Chapter 3 in "Sustained and Controlled Release Drug Delivery Systems" ed. Robinson,J.R. Marcel Dekker, New York, 1978, 123-173.

Lee,P. "Controlled drug release from polymeric matrices". in Recent Advances in Drug Delivery Systems. Eds Anderson,J.M. and Kim,S.W. 1984, Plenum Press, New York.

Lee,V. Solution and Shear Properties of Chitin and Chitosan, University of Washington, PhD Thesis, 1974.

Lehr,C-M., Bouwstra,J.A., Schacht,E.H. and Juninger,H.E. In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. Int. J. Pharm. 1992, 78, 43-48.

Li,Q., Dunn,E.T., Grandmaison,E.W. and Goosen,M.F. Applications and Properties of Chitosan. J. Bioactive and Compatible Polymers 1992, 7, 370-397.

Machida,Y. and Nagai,T. Chitin/Chitosan as pharmaceutical excipients. p211-221 In 'Topics in Pharmaceutical Science' Ed. Breimer,D.D., Crommelin,D.J.A. and Midha,K.K. 1989, SDU The Hague.

Machida,Y., Nagai,T., Abe,M. and Sannan,K. Use of chitosan and hydroxypropylchitosan in drug formulations to effect sustained release. Drug Design and Del. 1986, 1, 119-130.

Machida,Y., Nagai,T., Inouye,K. and Sannan,K. Preparation and Evaluation of Buoyant Sustained Release Dosage Forms based on Chitosan. in "Chitin and Chitosan", ed. Skjak-Braek,G., Anthonsen,T. and Sandford,P. Elsevier Applied Science, New York, 1989, 693-702.

Malette,W.G. and Quigley,H.J. Method of achieving hemostasis, inhibiting fibroplasis and promoting tissue regeneration in a tissue wound. US Patent 4,532,134. July 1985.

Malette,W.G., Quigley,H.J., Gaines,R.D., Johnston,N.D. and Rainer,W.G. Chitosan: a new hemostatic. Am. Thoracic Surgery, 1983, 36, 55-58.

Malinowski, H.J. and Smith,W.E. Use of factorial design to evaluate granulations prepared by spheronization. J. Pharm. Sci. 1975, 64, 1688 - 1692.

McKay,G., Blair,H.S. and Findon,A. Equilibrium studies for the sorption of metal ions onto Chitosan. Indian J. Chem., Sect. A, 1989, 28A (5), 356 -360.

McRitchie,K. Rheological Evaluation of Mixtures of Lactose, Microcrystalline cellulose, and Water suitable for the Preparation of spherical granules. PhD Thesis, University of London, 1993.

Mehta,A.M. Factors in the development of oral controlled release dosage forms, Pharm. Manuf. 1986, 3 (1), 23-29.

Meshali,M.M. and Gabr,K.E. Effect of interpolymer complex formation of chitosan with pectin or acacia on the release behaviour of chlorpromazine HCL. Int. J. Pharm. 1993, 89, 177-181.

Meshali,M.M., El-Dien,E.Z., Omar,S.A. and Luzzi,L.A. A new approach to encapsulating non-steroidal anti-inflammatory drugs. III. Coating acidic as well as basic non-steroidal anti-inflammatory drugs with cellulose derivitives having different functional groups. J. Microencapsulation 1989, 6 (3), 339-353.

Miya,M., Iwamoto,R., Yoshikawa,S. and Mima,S. IR Spectroscopic determination of CONH content in highly deacetylated chitosan. Int. J. Biol. Macromol., 1980, 2, 323-324.

Miyazaki,S., Ishii,K. and Nadai,T. The use of Chitin and Chitosan as Drug Carriers. Chem. Pharm. Bull. 1981, 29 (10), 3067-3069.

Miyazaki,S., Yamaguchi,H., Takada,M., Hou,W., Takeichi,Y. and Yasubuchi,H. Pharmaceutical Application of Biomedical Polymers. XXIX. Preliminary study on film dosage form prepared from chitosan for oral drug delivery. Acta Pharm. Nord. 1990, 2 (6), 401-406.

Miyazaki,S., Yamaguchi,H., Yokouchi,C., Takada,M. and Hou,W. Sustained -Release and Intragastric-Floating Granules of Indomethacin using Chitosan in Rabbits. Chem. Pharm. Bull. 1988, 36 (10), 4033-4038.

Moore,G.K. and Roberts,G.A.F. Chitosan gels: 1. Study of reaction variables. Int. J. Biol. Macromol., 1980, 2, 73-77.

Moore,G.K. and Roberts,G.A.F. Chitosan gels: 2. Mechanism of gelation. Int. J. Biol. Macromol., 1980, 2, 78-80.

Moore,G.K. and Roberts,G.A.F. Determination of the degree of N-acetylation of chitosan. Int. J. Biol. Macromol., 1980, 2, 115-116.

Moore,G.K. and Roberts,G.A.F. Reactions of Chitosan: 4 Preparation of organosoluble derivites of Chitosan. Int. J. Biol. Macromol., 1982, 4, 246-249.

Murata,Y., Maeda,T., Miyamoto,E. and Kawashima,S. Preparation of chitosan-reinforced alginate gel beads - effects of chitosan on gel matrix erosion. Int. J. Pharm. 1993, 96, 139-145.

Muzzarelli, R.A.A., Lough,C. and Emanuelli,M. The Molecular Weight of Chitosans studied by laser light - scattering. Carbo. Res. 1987, 164, 433-442.

Muzzarelli, R.A.A., Tanfani,F., Emanuelli,M., Muzzarelli,M.G. and Celia,G. The Production of Chitosans of superior quality. J. Appl. Biochem. 1981, 3 (4), 316-321.

Muzzarelli,R.A.A. 'Chitin', Pergamon Press, Oxford, 1977.

Muzzarelli,R.A.A. 'Natural Chelating Polymers', Pergamon Press, New York, 1973.

Muzzarelli,R.A.A. Water - Soluble Chitosan Derivitives. Carbohydrate Polymers 1988, 8, 1-21.

Muzzarelli,R.A.A., Baldassarre,V., Conti,F., Ferrara,P., Biagini,G., Gazzanelli,G. and Vasi,V. Biological Activity of Chitosan. Biomaterials, 1988, 9, 247-252.

Muzzarelli,R.A.A., Jeuniaux,C. and Gooday,G.W. 'Chitin in Nature and Technology', Plenum Press, New York, 1986.

Nagasawa,K., Tohira,Y., Inoue,Y. and Tanoura,N. Reaction between carbohydrates and sulfuric acid. 1 Depolymerization and sulphonation of polysaccharides by sulfuric acid. Carbo. Res. 1971, 18, 95-102.

Nernst,W. and Brunner,E. Z. Phys. Chem. 1904, 47, 52.

Newton,J.M. The preparation of spherical granules by extrusion/spheronisation. S.T.P. Pharma 1990, 6 (6), 396-398.

Newton,J.M., Chow,A.K. and Jeewa,K.B. The Effect of Excipient Source on Spherical Granules made by Extrusion/Spheronization. Pharm. Technol. Oct. 1992, 52-58.

Nigalaye,A.G., Adusumilli,P. and Bolton,S. Investigation of Prolonged Drug Release from Matrix Formulations of Chitosan. Drug Dev. Ind. Pharm. 1990, 16 (3), 449-467.

Nishi,N., Ebina,A., Nishimura,S., Tsutsumi,A., Hasegawa,O. and Tokura,S. Highly phosphorylated derivitives of Chitin, partially deacetylated Chitin and Chitosan as new functional polymers: preparation and characterization. Int. J. Biol. Macromol. 1986, 8, 311-317.

Nishi,N., Nishimura,S., Ebina,A., Tsutsumi,A. and Tokura,S. Preparation and characterization of water - soluble chitin phosphate. Int. J. Biol. Macromol., 1984, 6, 53-54.

Nishioka,Y., Kyotani,S., Masui,H., Okamura,M., Miyazaki,M., Okazaki,K., Ohnishi,S., Yamamoto,Y. and Ito,K. Preparation and Release Characteristics of Cisplatin Albumin Microspheres containing Chitin and treated with Chitosan. Chem. Pharm. Bull. 1989, 37 (11), 3074-3077.

Niskanen,M., Niskanen,T., Yliruusi,J.K. and Kristoffersson,E. Pelletization in a centrifugal granulator. Pharm. Technol. Int. Oct. 1990, pp 22-28.

O'Connor,R.E. and Schwartz,J.B. Extrusion and Spheronization Technology, Chapter 9 of "Pharmaceutical Pelletization Technology" Ed. Ghebre-Sellassie,I. Marcel Dekker, New York, 1989.

Odier,A. Memorie sur la composition Chimique des parties cornees des insects. Mem. Soc. Hist. Nat. Paris. 1823, 29-42.

Ogawa,K. and Inukai,S. X - Ray Diffraction study of Sulfuric, Nitric and Halogen acid salts of Chitosan. Carbohydrate Res. 1987, 160, 425.

Ogawa,K., Hirano,S., Miyanishi,T., Yui,T. and Watanabe,T. A new polymorph of Chitosan. Macromolecules 1984, 17 (4), 973-975.

Ogura,K., Kanamoto,T., Sannan,T., Tanaka,K. and Iwakura,Y. Proceedings of the Second International Conference of Chitin and Chitosan, Sapporo Japan. 1982, 39.

Ovenston,A. and Benbow,J.J. Effects of die geometry on extrusion of clay like materials. Trans. Br. Ceram. Soc. 1968, 67, 543-567.

Park,J.W., Park,M.O. and Park,K.K. Mechanism of Metal Ion Binding to Chitosan in Solution. Cooperative Inter- and Intramolecular Chelations. Bull. Korean Chem. Soc. 1984, 5 (3), 108-112.

Peppas,N.A. and Korsmeyer,R.W. "Dynamically swelling hydrogels in Controlled Release Applications" in Hydrogels in Medicine and Pharmacy. Ed Peppas,N.A., CRC Press Florida, 1987, Volume 3, 109-135.

Raines,C.L., Newton,J.M. and Rowe,R.C. 'Extrusion of Microcrystalline Cellulose Formulations' in Proceedings of the Annual Meeting of the British Society of Rheology, Ed. Carter,R.E. Elsevier Applied Science, London, 1989, 248-257.

Ranga,K.V. and Devi,K.P. Swelling Controlled Release Systems: recent developments and applications. Int. J. Pharm. 1988, 48, 1-13.

Reynolds,A.D. A new technique for the production of spherical particles. Manuf. Chem. Aerosol News June 1970, 40-43.

Rittidej,G.C., Chomto,P., Pummangura,S. and Menasveta,P. Chitin and Chitosan as Disintegrants in Paracetamol Tablets. Drug Dev. Ind. Pharm. 1994, 20 (13), 2109-2134.

Roberts,G.A.F. and Domszy,J.G. Determination of the viscometric constants for chitosan. Int. J. Biol. Macromol., 1982, 4, 374-377.

Roberts,G.A.F. and Taylor,K.E. The preparation and characterisation if chitin beads for use in chromatography. p577 In 'Chitin and Chitosan' Ed. Skjak-Braek,G., Anthonsen,T. and Sandford,P. 1989, Elsevier Applied Science, London and New York.

Roberts,G.A.F. Chitosan Gels: Part 4. Chitosan - based Thermally Reversible Gels. p479-485 In 'Chitin and Chitosan' Ed. Skjak-Braek,G., Anthonsen,T. and Sandford,P. 1989, Elsevier Applied Science, London and New York.

Rodriguez-Sanchez,D. and Rha,C. Chitosan globules. J. Food. Technol. 1981, 16, 469.

Rodriguez-Sanchez,D., Kienzle-Sterzer,C.A. and Rha,C. Intrinsic viscosity of chitosan solutions as affected by ionic strength. The Second International Conference on Chitin and Chitosan, Sapporo, Japan, 1982.

Rouget,C. Comp. Rend. 1859, 48, 792-795.

Rowe,R.C. Spheronization: A novel pill-making process?. Pharm. Int. 1985, 6, 119-123.

Ruiz-Herrara,J. The distribution and quantative importance of chitin in fungi. In 'Proceedings of the 1st International Conference on Chitin and Chitosan' Ed Muzzarelli,R.A.A. and Pariser,E.R. 1978, MIT Sea Grant program, Cambridge, Mass.

Saito,H., Tabeta,R. and Ogawa,K. High-Resolution Solid-State ^{13}C NMR Study of Chitosan and its salts with Acids: Conformational Characterization of Polymorphs and Helical Structures as Viewed from the Conformation-Dependent ^{13}C Chemical Shifts. Macromolecules 1987, 20 (10), 2424-2430.

Sandford,P. Chitosan:Commercial Uses and Potential Applications. in "Chitin and Chitosan", ed. Skjak-Braek,G., Anthonsen,T., and Sandford,P., Elsevier Applied Science, New York. 1989, 51-69

.

Sapelli,P.L., Baldassarre,V., Emanuelli,M. and Muzzarelli, R.A.A. Chitosan in Dentistry. p507 In 'Chitin in Nature and Technology', Eds. Muzzarelli, R.A.A., Jeuniaux,C. and Gooday,G.W. Plenum Press, New York, 1986.

Sawayanagi,Y., Nambu,N. and Nagai,T. Directly compressed tablets containing chitin or chitosan in addition to mannitol. Chem. Pharm. Bull. 1982 (c), 30 (11), 4216-4218.

Sawayanagi,Y., Nambu,N. and Nagai,T. Permeation of Drugs through Chitosan Membranes. Chem. Pharm. Bull. 1982(a), 30 (9), 3297-3301.

Sawayanagi,Y., Nambu,N. and Nagai,T. Use of chitosan for sustained release preparations of water-soluble drugs. Chem. Pharm. Bull. 1982 (b), 30 (11), 4213-4215.

Scott,D.C. and Hollenbeck,R.G. Design and manufacture of a zero-order sustained release pellet dosage from non-uniform drug distribution in a diffusional matrix. Pharm. Res. 1991, 8 (2), 156-161.

Sherman,S. Industrial Rheology. 1970, Academic Press.

Sheu,Ming-Thau, Chou,Huei-Lan, Kao,Ching-Cheng, Liu,Cheng-Hsiung and Sokoloski,T.D. Dissolution of diclofenac sodium from matrix tablets. Int. J. Pharm. 1992, 85, 57-63.

Shin,S.C., Oh,I.J., Lee,K.C., Lee,Y.B. and Koh,I.B. Enhancement of dissolution properties of poorly soluble drugs. Yakche Hakhoechi 1987, 4 (17), 175-181.

Shiraishi,S., Arahira,M., Imai,T. and Otagiri,M. Enhancement of Dissolution Rates of Several Drugs by Low-Molecular Chitosan and Alginate. Chem. Pharm. Bull. 1990, 38 (1), 185-187.

Shiraishi,S., Imai,T. and Otagiri,M. Controlled release of indomethacin by chitosan-polyelectrolyte complex: optimization and in vivo/in vitro evaluation. J. Cont. Release. 1993 25, 217-225.

Skaugrud,O. Chitosan makes the grade. Manuf. Chem. Oct. 1989, 31-33.

Skjak-Braek,G., Anthonsen,T. and Sandford,P. 'Chitin and Chitosan, Sources, Chemistry, Biochemistry, Physical Properties and Applications', Elsevier Applied Science, 1989, London and New York.

Sugano,M., Watanabe,S., Kishi,A., Izume,M. and Otakara,A. Hypocholesterolemic Action of Chitosans with Different Viscosities in Rats. Lipids 1988, 23 (3), 187-191.

Takahashi,T., Takayama,K., Machida,Y. and Nagai,T. Characteristics of polyion complexes of chitosan with sodium alginate and sodium polyacrylate. Int. J. Pharm. 1990, 61, 35-41.

Takayama,K., Hirata,M., Machida,Y., Masada,T., Sannan,T. and Nagai,T. Effect of Interpolymer Complex Formation on Bioadhesive Property and Drug Release Phenomenon of Compressed Tablet Consisting of Chitosan and Sodium Hyaluronate. Chem. Pharm. Bull. 1990, 38 (7), 1993-1997.

Tapia,C., Buckton,G. and Newton,J.M. Factors influencing the mechanism of release from sustained release matrix pellets, produced by extrusion/spheronisation. Int. J. Pharm. 1993, 92.211-218.

Terbojevich,M., Cosani,A., Conio,G., Marsano,E. and Bianchi,E. Chitosan: chain rigidity and mesophase formation. Carbo. Res. 1991, 209, 251-260.

Terbojevich,M., Cosani,A., Focher,B., Naggi,A. and Torri,G. Chitosans from Euphausia superba. 1: Solution properties. Carbo. Polym. 1992, 18, 35-42.

Thanoo,B.C., Sunny,M.C. and Jayakrishnan,A. Cross-Linked Chitosan Microspheres: Preparation and Evaluation as a Matrix for the Controlled Release of Pharmaceuticals. J. Pharm. Pharmacol. 1992, 44, 283-286.

Upadrashta,S.M., Katikaneni,P.R. and Nuessle,N.O. Chitosan as a Tablet Binder. Drug Dev. Ind. Pharm. 1992, 18 (15), 1701-1708.

Van Wazer,J.B., Lyons,J.W., Kim,K.Y. and Colwell,R.E. Viscosity and flow measurements: A laboratory handbook of rheology. 1963, Interscience, New York.

Vorlop,K.D. and Klein,J. Formation of spherical chitosan biocatalysts by ionotropic gelation. Biotechnol. Letters 1981, 3 (1), 9.

Wang,W., Bo,S., Li,S. and Qin,W. Determination of the Mark-Houwink equation for chitosans with different degrees of acetylation. Int. J. Biol. Macromol. 1991, 13, 281-285.

Warburton,B. and Barry,B.W. Concentric cylinder creep investigations of pharmaceutical semi-solids. J. Pharm. Pharmacol. 1968, 20, 255-268.

Watanabe,K., Saiki,I., Uraki,Y., Tokura,S. and Azuma,I. 6-O-Carboxymethyl-chitin (CM-chitin) as a Drug Carrier. Chem. Pharm. Bull. 1990, 38 (2), 506-509.

Yalpani,M., Hall,L.D., Tung,M.A. and Brooks,D.E. Unusual rheology of a branched, water-soluble chitosan derivitive. Nature (London) 1983, 302 (5911), 812-814.

Yamaguchi,H., Yokouchi,C., Takada,M. and Hou,W. Sustained release of indomethacin from chitosan granules in beagle dogs. J. Pharm. Pharmacol. 1988, 40 (9), 642-643.

Zikakis,J.P. 'Chitin, Chitosan and Related Enzymes', Academic Press, 1984.