THE INTERACTION OF CHITOSAN WITH CELLULOSE AND ITS USE IN PAPER

by

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Thesis submitted in partial
fulfilment of the requirements
for the degree of Doctor of Philosophy (C.N.A.A.)

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Submitted December, 1983.

ACKNOWLEDGEMENTS

The work described in this thesis was carried out in the laboratory of both the Department of Physical Sciences, Trent Polytechnic, Nottingham and P.I.R.A., Leatherhead, between September 1980 and August 1983.

I wish to thank Dr. G.A.F. Roberts for his excellent supervision and guidance throughout the course of this work.

Thanks are also due to: the SERC and P.I.R.A. for the award of a CASE research studentship, and to the latter for their generosity in extending the use of their facilities to me over the duration of this research project; Dr. G.K. Moore of P.I.R.A. for his expertise and guidance in the field of paper; Mrs F.A. Wood for typing the manuscript and preparing the Figures and, together with Mrs A.J. Wild, for technical assistance.

Part of this thesis has been published in the International Journal of Biological Macromolecules, and a copy of this is included as Appendix V.

ABSTRACT

Title: Interaction of chitosan with cellulose and its use in paper.

By: Julian G. Domszy.

The aims of this study were to investigate the adsorption of chitosan onto cellulose and to apply the knowledge obtained to the application of chitosan to paper.

The molecular weight and structure of chitosan are important in its adsorption. The viscometric constants of the modified Staudinger equation were therefore determined in 0.1M acetic acid/0.2M sodium chloride solution and found to be $a = 0.93$ and $K = 1.81 \times 10^{-5}$ cm^3 g^{-1}.

Novel methods for determining the extent of residual N-acetylation have been developed based on the reaction between salicylaldehyde and chitosan, together with spectroscopic determination of the N-salicylidene chitosan produced or of the residual salicylaldehyde for homogeneous and heterogeneous reaction respectively. The results obtained agree well with those obtained by infrared spectroscopy and by alkalimetric titrations of chitosan hydrobromide. Measurement of the N-salicylidene chitosan chromophore has been extended to determining the concentration of chitosan in solution and, through use of reflectance spectroscopy, to measurement of chitosan adsorbed on paper handsheets. The adsorption of chitosan on cellulose was found to be an equilibrium process dependant on polymer-adsorbent, polymer-solvent and solvent-adsorbent interactions.

Factors that decrease the effective volume of the chitosan molecule in solution, such as increasing degree of N-acetylation and addition of electrolyte, tend to increase the equilibrium uptake. Neutral electrolyte has a second effect, namely suppression of the surface charge, that predominates at higher concentrations and reduces the uptake. Although increasing the surface charge of the cellulose increases the extent of adsorption, the experimental evidence suggests that very few ionic links are formed between chitosan and cellulose. The proposed mechanism involves initial electrostatic attraction of the chitosan to the vicinity of the cellulose surface where it is adsorbed predominately through van der Waals forces and hydrophobic interaction. The influence of molecular weight of the chitosan indicates that the cellulose is acting as a porous substrate.

Dedication

- to my parents -

"Ὑπότε ποιήσει τὸν

καρκινον ὀρθὰ βαδίζειν"

- Aristophanes -

The Peace, 1. 1083. 421 B.C.

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CHAPTER 1

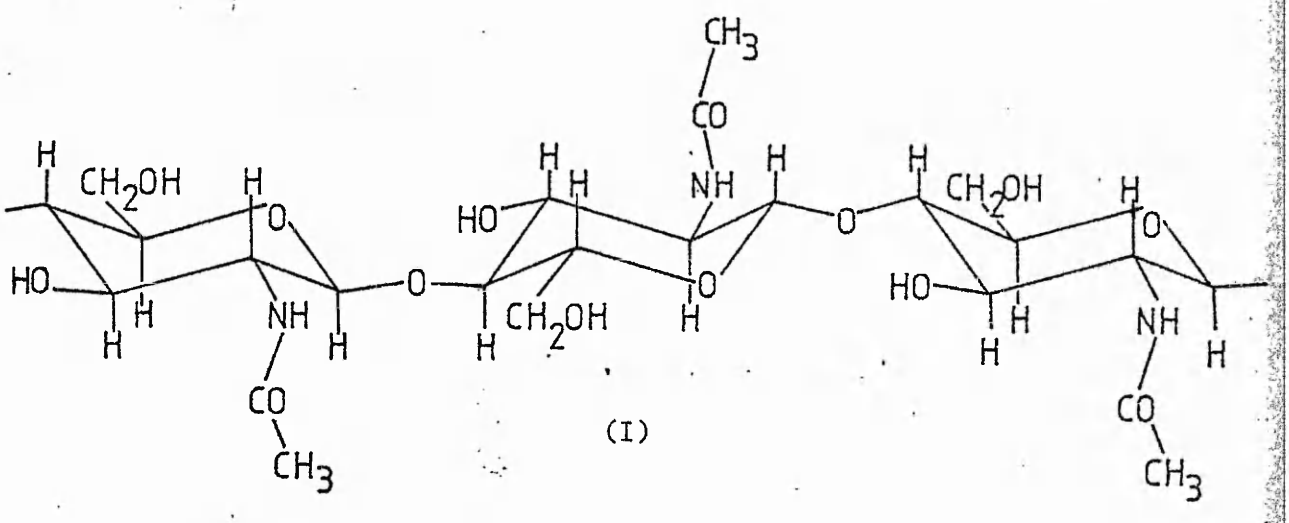
INTRODUCTION

The existence of chitin as a constituent of living matter has been well established since its initial isolation by Bracannot[1] in 1811. It was during work on chitin that Rouget[2] in 1859 discovered chitosan, the modified product of chitin, and thus initiated the diverse research programmes that the polymer now attracts.

Both chitin and chitosan are polysaccharides. Chitin in its ideal form is poly[(1→4)-2-acetamido-2-deoxy- β-D-glucose] and chitosan is poly[(1→4)-2-amino-2-deoxy- β-D-glucose]. Figure 1 shows the idealised structures of chitin (I) and chitosan (II). However in practice it is unlikely that chitin or chitosan will exist entirely in their idealised forms. In chitosan there is usually a certain proportion of the units that exist as the acetamido and thus the term chitosan usually denotes a co-polymeric system consisting of both units. In general it is assumed that the amine-containing units are dominant in chitosan. Similarly most chitin samples contain a small proportion of de-N-acetylated sugar residues, the concentration of these depending on the source and isolation procedures used, and hence are also copolymers. A rough indication to whether a particular sample should be labelled as chitin or chitosan is its solubility in dilute acid. Chitosan is assumed to be soluble whereas chitin is not. Thus for accurate work it is necessary to determine the degree of N-acetylation for any specific chitosan sample.

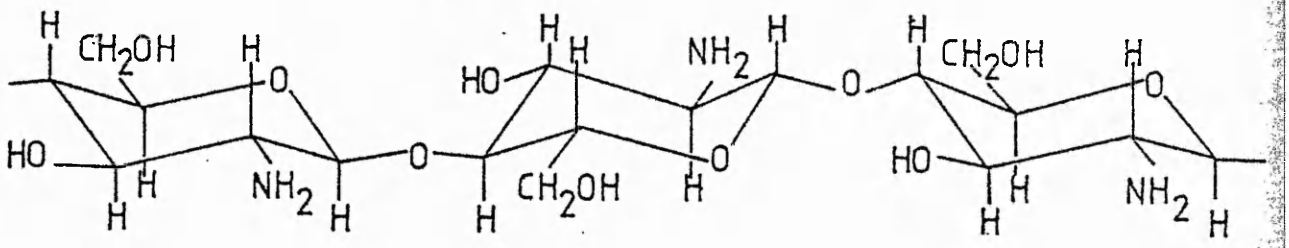
Chitin is a crystalline polysaccharide that has a highly ordered structure. It occurs in three polymorphic forms, namely α-, β- and γ- chitins, which differ in the arrangement of the molecular chains

Figure 1



(I)

Idealised structure of chitin.



(II)

Idealised structure of chitosan.

within the crystal cell, as shown by X-ray crystallographic studies[3,4]. α-Chitin is the most crystalline polymorphic form and has the chains arranged in an antiparallel fashion. In β-chitin the chains are parallel whilst in γ- chitin two chains are "up" to every one "down"[5]. α-Chitin is by far the most abundant form.

The occurence of chitin is widespread[6] throughout nature in both animal and plant kingdoms. Its mode of isolation and the quality of the final product with respect to extent of residual N-acetylation and molecular weight is dependant upon the nature of the source of the chitinous material. In nature chitin is normally found in close association with other materials, and in order to separate them the use of harsh conditions are often required, which have a detrimental effect on the final product. It has been found[7] that of all the sources of chitin the crustaceans form the best potential source for commercial manufacture. Chitin production from such sources involves the removal of the calcium carbonate and proteins associated with the chitin, and the majority of methods employed[8-10] involve an acid treatment for decalcification and an alkali treatment for deproteination. However there are also milder methods of treatment using ethylenediamine tetraacetic acid for decalcification[11] and proteolytic enzymes for deproteination[12].

Preparation of chitosan from chitin involves the cleavage of the amide linkage to form the free amine. The N-acetyl group cannot be removed under acidic conditions because the acid will readily hydrolyse the glycosidic bonds in the polysaccharide, so alkaline techniques are used for de-N-acetylation. Under basic conditions acetamido groups next to cis related hydroxyls may undergo de-N-acetylation but trans related analogues are more resistant[13]. Chitin has a

C2 - C3 trans arrangement of the acetamido and hydroxyl groups and thus is stable to most reagents. Hence drastic conditions are required for de-N-acetylation, e.g. Winterstein[14] showed that fusion of chitin with potassium hydroxide at 180°C led to chitosan. Often deacetylation is incomplete, but with hot concentrated sodium hydroxide an almost completely de-N-acetylated product can be obtained although it is highly degraded and of low molecular weight. Hence in most cases in order to preserve the high molecular weight the final product is often not completely de-N-acetylated. The variation in the degree of N-acetylation and in the molecular weight, due to processing variations, is one reason why a substantial amount of this thesis has been concerned with the accurate characterisation of chitosan.

Variations in the degree of N-acetylation and in molecular weight of chitosan samples are just two of the factors considered to be important in the interaction between chitosan and cellulose, from the view point of chitosan as a strength improvement additive for paper. It is assumed that in paper the fibres are held together by hydrogen bonds, thus when in the presence of water they are more likely to be hydrogen bonded to water molecules than to themselves. Thus with water present the fibres are joined together by a fluid bridge of hydrogen bonded water molecules, the weakness of which is shown by the wet strength of the paper. An additive that improves the wet strength operates by allowing the interfibre bonding areas to remain linked in the presence of water. The dry strength is also improved by materials that aid the interfibre bonding. Chitosan, due to its cationic nature in solution, has been suggested as a strength additive because of its potential bonding ability with cellulose. In this thesis a detailed study has been carried out into the adsorption of chitosan

onto cellulose from solution. Solution adsorption as a technique for the uptake of chitosan by cellulose was chosen because of its relevence to the wet end process of practical paper making, with respect to ease of addition.

1.1 The paper making system

A schematic diagram of a typical paper making system is shown in Figure 2.

The input is composed of virgin fibre and broke or scrap from the paper making operation. It also includes other additives such as fillers, chemicals, dyes, etc. These are all run through the stock proportionater and then stored in the machine chest until needed. The thick stock from the machine chest is added to the white water which comes from the initial part of the fourdrinier wire. This goes through a fan pump and various cleaning and screening devices into a head box. The stock is now roughly at a concentration of 0.5% and is allowed to flow onto the fourdrinier wire where most of the water is drained in the tray section and recirculated. The vacuum drainage devices on the wire allow water to be collected in the seal pit. This water is usually of a lower solids content than that of the tray water and is used to feed the saveall, although some of it may be used for reslurrying the broke. The saveall is essentially a solids-liquid separater used to remove additional solids from the process water before it leaves the system. For high cost papers the recovered solids are returned to the process. The trim from the edges of the sheets coming off the wire are dropped into the couch pit where they are removed to the saveall for solids recovery.

Once the sheet leaves the wire it goes through a press section,

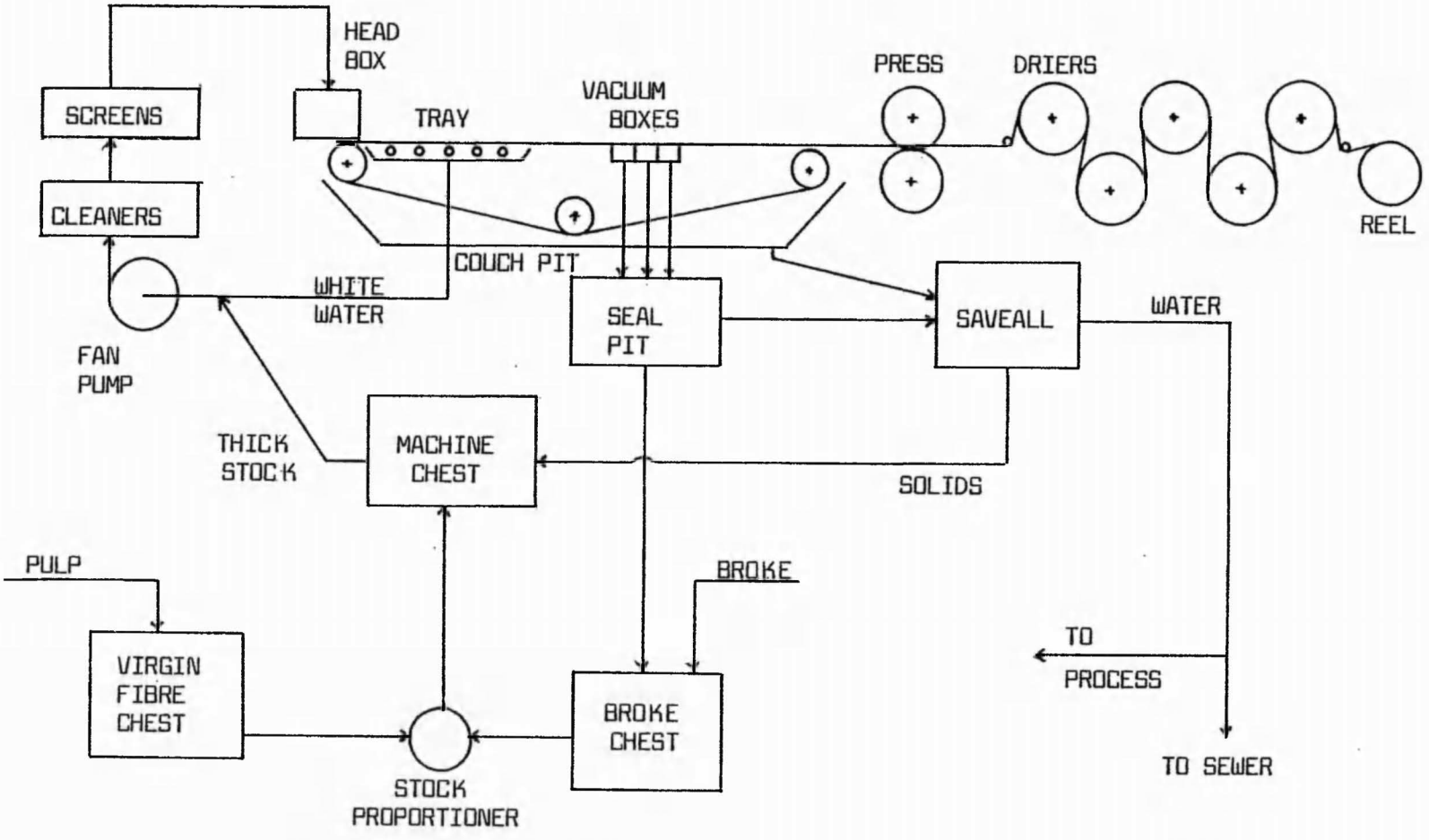


Figure 2

Paper machine wet end system.

where it is wedged between two felts before being run through mechanical presses. More water is pressed out at this stage and is picked up by the felts, which run through washers to remove any fibres or chemicals picked up from the sheet. The sheets then run over a number of steam heated drier drums to evaporate the remaining water, before being wound onto the reel. Paper making can be carried out at a pH of between 4.5 - 5, which is ideal for chitosan since it is soluble in dilute acid solutions.

Within the system there are various points where additions of chemicals can be made. These are usually at the initial input or after the thick stock has been added to the recirculating tray water, or after points of high shear. In the latter case the addition must be made far enough ahead of the headbox to give good mixing e.g. at the outlet of the screens.

CHAPTER 2

HISTORICAL REVIEW

2.1 Molecular weight measurements on chitin and chitosan

2.1.1 Average molecular weights

High polymers, in contrast to low molecular weight compounds, contain molecules with many different molecular weights. Therefore a particular sample is likely to have a spread of molecular weights (Figure 3), and the experimental determination of molecular weight will only give an average value. Several different averages are of importance e.g. some methods of molecular weight measurement count the number of molecules in a known mass of material. This leads to the number average molecular weight $\bar{M}n$. This is defined as:

$$\bar{M}n = \frac{\Sigma N_i M_i}{\Sigma N_i}$$

where N_i is the number of molecules of molecular weight M_i.

In other experiments such as light scattering the contribution of a molecule to the observed effect is a function of its mass. Heavy molecules are favoured in the averaging process, giving a weight average molecular weight $\bar{M}w$ defined as:

$$\bar{M}w = \frac{\Sigma N_i M_i^2}{\Sigma N_i M_i}$$

Since $\bar{M}w$ is more sensitive to the heavier molecules it is larger than $\bar{M}n$. The ratio $\bar{M}w/\bar{M}n$ for a polymer is used as a measure of the breadth of the molecular weight distribution.

It is possible to define any number of molecular weight averages,

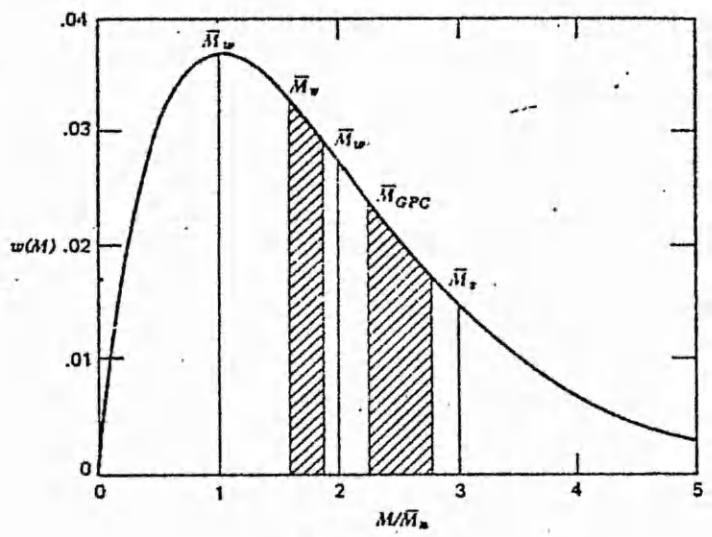


Figure 3

Distribution of molecular weights in a typical polymer showing the positions of important averages.

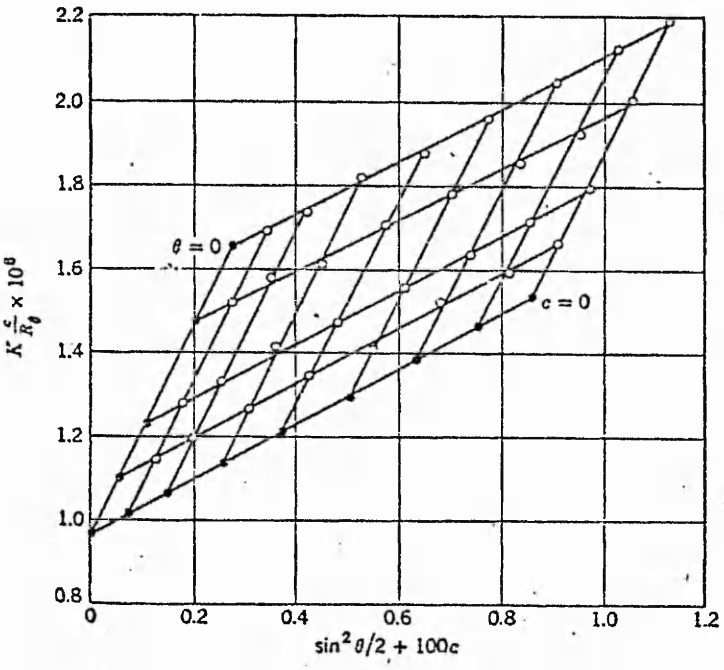


Figure 4

An example of a rectilinear Zimm plot.

but there is little point unless they have a specific use

e.g. Z - average molecular weight $\bar{M}z$

$$\bar{M}z = \frac{\Sigma N_i M_i^3}{\Sigma N_i M_i^2}$$

The technique of ultracentrifugation lends itself to the measurement of this average.

Determination of the intrinsic viscosity of a polymer leads to the viscosity average molecular weight $\bar{M}v$ defined as:

$$\bar{M}v = \left[\frac{\Sigma N_i M_i^{1+a}}{\Sigma N_i M_i} \right]^{1/a}$$

where a is the constant in the modified Staudinger equation for the empirical relationship between molecular weight and viscosity.

2.1.2 End group analysis techniques

2.1.2.1 Introduction

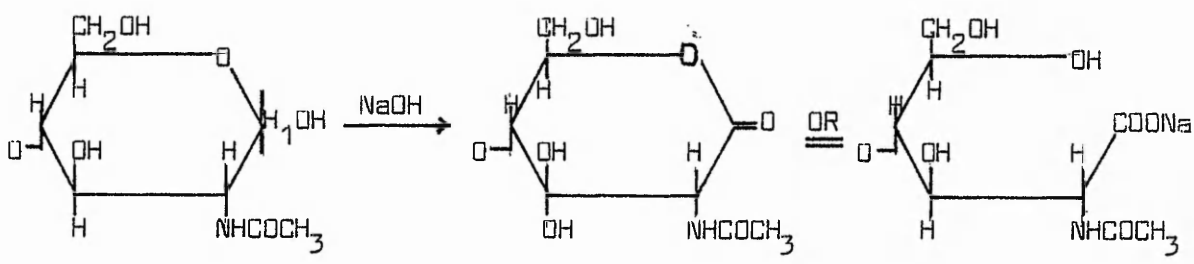
Molecular weight determination via end group analysis requires that the polymer contain a known number of determinable groups per molecule. The long chain nature of polymers limits such groups to the ends of the molecules and hence the method is usually referred to as end group analysis. Such methods measure the number of molecules in a given weight of sample and yield the number average molecular weight. The technique tends to become insensitive at high molecular weights as the fraction of end groups becomes too small to be measured with precision. The procedure in general requires some chemical or physical determination of the end groups on the polymer chain.

2.1.2.2 Copper Number techniques

The determination of the molecular weight of chitin samples was first carried out by Meyer and Wehrli[15]. They determined the copper number of chitin and related this to the chain length which was found to be 103 glucosamine units for the particular sample examined. Although the authors only considered this value to be a minimum, due to polymer degradation, the values are still extremely low. The copper number technique measures the number of reducing groups in a system and can only be applied to molecular weights if the reducing groups are the end groups. Copper in the form of the Cu^{2+} salt is reduced to Cu^{+} by the aldehyde of a chitin end group:

$$R - CHO + Cu^{2+} \xrightarrow[H_2O]{} RCOOH + Cu^{+}$$

This method has been applied in cellulose chemistry and found to be too insensitive for quantitative molecular weight study, especially with high molecular weight polymers. Its main use is to determine the extent of acid hydrolysis of hydrocelluloses. Other errors that will effect the copper number method may be due to the presence of non-reducing end groups on the chitin as a direct result of the alkaline processing conditions which may oxidise the terminal carbonyl group to a carboxyl (III) or a lactone group (IV)



(IV) (III)

Also the amine groups along the polymer chain will form a complex with copper[16], hence the extremely low value of between 20 - 30 for the degree of polymerisation of chitosan found by Meyer and Wehrli. They also carried out a comparative viscometric study of chitin with cellulose in 50% nitric acid and found that the K values in the original Staudinger viscosity equation for both chitin and wood cellulose were similar thus indicating a similarity in molecular weights.

2.1.2.3 Hypoiodite method

For low molecular weight chitosans, resulting from severe polymer degradation by sulphuric acid, the number average molecular weight $\bar{M}n$ was determined by the hypoiodite method[17]. This is a technique that was developed for measuring cellulose molecular weights and involves the determination of the aldehyde end group by oxidation. The hypoiodite solution consists of an alkaline solution of iodine with the oxidising species being undissociated hypoiodous acid. The reactions involved may be represented by equations (1) and (2)

$$I_2 + OH^- \longrightarrow HIO + I^- \quad \text{.....(1)}$$

$$R\text{-}CHO + HIO \longrightarrow RCO_2H + HI \quad \text{.....(2)}$$

The amount of iodine converted to iodide is determined by thiosulphate titration and is used to calculate the number of aldehyde groups oxidised. In its application to cellulose the method has been the subject of considerable criticism, owing to the fact that when using scoured cotton, which has no aldehyde groups, iodine is still consumed, which makes the technique too insensitive for low aldehyde contents.

A second flaw, of importance with oxidised samples, is the ability of the alkaline reagent to produce new reducing groups, by chain scission, while the measurement is being made. When considering chitosan rather than cellulose, due to its alkaline production techniques, end groups are likely to be in non-reducing forms and this, allied to the fact that iodine has been shown to have an excellent affinity for chitosan[18], casts some doubt on the accuracy of the results using this method.

2.1.3 Light scattering techniques

2.1.3.1 Introduction

The scattering of light occurs whenever a beam of light encounters matter. The nuclei and electrons undergo induced vibrations in phase with the incident light and act as sources of light which is propagated in all directions, with the same wavelength as the exciting beam. The theory of light scattering when applied to the molecular weight of large molecules is complex but is expressed in the modified Debye equation (3).

$$K.c/R_{90} = H.c/\tau = 1/\bar{M}w\ P(\theta) + 2A_2c \quad \ldots\ldots\ldots\ldots(3)$$

where $K = \dfrac{2\pi^2 n^2}{N_o \lambda^2} \left(\dfrac{dn}{dc}\right)^2$ and $H = \dfrac{32\pi^3 n^2}{3 N_o \lambda^4} \left(\dfrac{dn}{dc}\right)^2$

τ = turbidity - the total scattering intergrated over all angles

n = refractive index

λ = wavelength of the light

N_o = Avogadro's number

c = concentration

$\bar{M}w$ = weight average molecular weight

R_{90} = Rayleigh ratio at 90^0

A_2 = second virial coefficient

$\frac{dn}{dc}$ = specific refractive increment

$P(\theta)$ = $\bar{z}$ - average dimension

In practice the left hand side of the equation is plotted against $Sin^2 \theta /2 + kc$ resulting in a rectilinear grid known as a Zimm plot (Figure 4). Extrapolation of this plot to both c = o and θ = o gives conditions where $P(\theta) = 1$ and thus

$$K.c/R_{90} = \frac{1}{\bar{M}_w} + 2A_2c \quad \text{.....(4)}$$

Thus beyond the measurement of τ or $R\theta$ only the refractive index and the specific refractive increment require experimental determination. The latter quantity is a constant for a given polymer, solvent and temperature and is measured with an interferometer or a differential refractometer. τ is related to the intensity of the primary beam before and after passing through the scattering medium.

$$I/I_o = e^{-\tau l} \quad \text{.....(5)}$$

where I_o = initial intensity

I = reduced intensity

l = path length

The light scattering instrument measures the scattered light intensity at different angles, using photoelectronic instrumentation.

Samples prepared for light scattering must be free from extraneous materials that may themselves cause scattering, and require to be

clarified by filtration or ultracentrigufation. The solvent choice is also important, with the difference in refractive index between the polymer and solvent being as large as possible. In the Zimm plot $\bar{M}w$ is the inverse of the intercept at $c = 0$ and $\theta = 0$. The second virial coefficient is calculated from the slopes of the $\theta = 0$ line by equation (4). The radius of gyration is derived from the slope of the zero concentration line as a function of angle. The radius of gyration gives a rough indication of the size of the random polymer coil.

2.1.3.2 Light scattering studies on chitin and chitosan

Van Duin and Hermans[19] in experiments investigating the effect of ionic strength on the molceular weight of chitosan, used light scattering as a technique for molecular weight determinations. Using the water soluble chitosan hydrochloride in aqueous sodium chloride they reported that the molecular weight increased with decreasing ionic strength and that the radius of gyration also increased with decrease in ionic strength. The latter trend is one that would be expected because of the osmotic effect which increases with decreasing salt concentration. The specific molecular weights were found to be of the order of 2×10^5 g with the increase in molecular weight with decrease in ionic strength being attributed to association of the polymer.

The use of aqueous sodium chloride solutions as a light scattering solvent was reported by Nagasawa et al.[20] in their investigation of the sulphation of polysaccharides by sulphuric acid. The molecular weight $\bar{M}w$ was reported to be 1.2×10^5 g for chitosan. The assumption that chitin also had the same molecular weight was made although it will almost certainly be higher as a result of the inevitable degradation in producing chitosan from chitin.

Muzzarelli, Ferrero and Pizzoli[21] determined the molecular weight of chitosan by light scattering in 8.5% formic acid plus 0.5M sodium formate and found good agreement with the results obtained by Nagasawa[20] for commercially prepared chitosans. However since the methods of isolation and de-N-acetylation will affect the molecular weight, exact agreement between two groups of workers must be fortuitous unless they both used the same samples. The sodium formate was added to the solvent to reduce the polyelectrolyte effect. This is caused by the ability of the amino groups on the polymer to become protonated. At high concentrations the chitosan molecules are close together and the formate counter ions remain in the molecular domain. At lower concentrations the counter ions tend to diffuse away from the polymer molecules, to a limited extent. This causes an increase in the effective charge resulting in a more extended polymeric structure. The presence of sodium formate helps to reduce this.

2.1.3.3 Light scattering studies on chitin and chitosan derivatives

The reluctance of chitin to dissolve in conventional solvent systems appeared to be the major drawback for the determination of molecular weight by light scattering. However Hackman and Goldberg[22] used water soluble chitin derivatives for light scattering studies. They used two derivatives, O-carboxymethylchitin and O-(2-hydroxyethyl)chitin.

Carboxymethyl chitin can be prepared by the action of monochloroacetic acid on alkali chitin[23]. The chitin has to be pretreated with alkali prior to reaction, and this may cause degradation. For good water solubility the sodium content of the carboxymethyl chitin must

be in excess of 6%. It is also possible to produce carboxymethyl chitosan from carboxymethyl chitin via de-N-acetylation. The chitosan analogue has been produced by Okimasu[24] and used for light scattering experiments.

Hydroxyethyl chitins can be prepared by the etherification of alkali chitin with ethylene oxide[25]. For light scattering work the two water-soluble chitin derivatives were dissolved in aqueous sodium chloride. The salt concentration range investigated was between 0.01 and 2.5M. In addition to the two soluble derivatives, chitin itself was dissolved in aqueous lithium thiocyanate and from the Zimm plots obtained, the weight-average molecular weights, the Z- average radii of gyration and the second virial coefficients were calculated. For carboxymethyl chitin in 2.5M NaCl and chitin in 5.5M LiSCN the average degree of polymerisation was found to be approximately 5200. At these high salt concentrations there is little likelyhood of association of the molecular chains and thus no or very little degradation has occured in the manufacture of the carboxymethyl chitin. For comparative studies the degree of polymerisation is a more relevent value than molecular weight since the repeat unit molecular weights for the chitin derivatives are higher than that for chitin alone. When the salt concentration was reduced the measured degree of polymerisation increased due to molecular association. Similar trends were observed for the hydroxyethyl chitin. These trends along with the decrease in the radius of gyration with increasing salt concentration were similar to those observed by VanDuin[19]. The values obtained for the second virial coefficient were low indicating that little interaction between polymer and solvent had occured. From their results Hackman and Goldberg concluded that in solution chitin and its two derivatives existed as random coils and

were polydisperse in molecular weight.

The contribution of light scattering studies to the molecular weight of chitosan and chitin confirmed that the molecular weights of chitin and chitosan were very different. These differences are attributed to the severe degradation of the chain that takes place during the production process caused by the strongly alkaline conditions required for deacetylation. By the use of less severe reagents[11,12] and conditions it is possible to reduce this degradation to some extent.

2.1.4 Ultracentrifugation techniques

2.1.4.1 Description

Ultracentrifugation techniques are the most intricate of the existing methods for determining the molecular weight of high polymers, although they are far more successful in application to compact protein molecules than to random coil polymers. The ultracentrifuge method operates on the principle that heavy particles sediment faster in a gravitational field than light ones. The polymer solution is spun in a cell which is held in a rotor. Both cell and rotor are provided with windows that permit observation of the course of sedimentation by optical methods. Experimental data is obtained in the form of a curve of dn/dr versus r where n is the refractive index and r is the distance from the centre of rotation to the point of observation in the cell. It is possible to relate this data to a molecular weight value, usually $\bar{M}w$. There are two modes of operation of the ultracentrifuge.

a) Sedimentation equilibrium;

In the sedimentation equilibrium method the ultracentrifuge is operated at low speeds of rotation for up to two weeks under constant conditions. A thermodynamic equilibrium is reached in which the polymer is distributed in the cell solely according to molecular weight and molecular weight distribution, the force of sedimentation on each species being just balanced by their tendency to diffuse back against the concentration gradient. Under these conditions it is possible to derive an equation based on thermodynamic principles that correlates the molecular weight with the refractive index data from the ultracentrifuge. It is also possible to reduce the time of the experiment by taking readings at the meniscus and at the bottom of the cell, where it is assumed that conditions approach those of equilibrium. A series of measurements of dn/dr are made at these two points at varying time intervals and extrapolated to zero time. For this modified technique higher speeds are used.

b) Sedimentation velocity;

In this experiment the ultracentrifuge is operated at high speeds. At the start of sedimentation the concentration of polymer in solution is uniform throughout the cell. As the solution is spun the molecules move downwards. After a time, t, there is a band δx centimetres long at the top of the cell where there are no molecules. Also during the time interval t all the molecules initially in a band δx centimetres above the bottom of the cell become packed at the bottom. The polymer-free volume of the cell is separated from the bulk of the solution by a boundary known as the sedimentation boundary. The boundary moves towards the bottom of the cell as sedimentation

proceeds. It is the velocity and position of the boundary that is measured in an analytical ultracentrifuge. The theory assumes that diffusion is negligible although this is not usually the case. At the start of sedimentation the molecules are accelerated but later when centrifugal force is balanced by frictional resistance the velocity of sedimentation becomes constant. Under these conditions it is possible to obtain an expression for the molecular weight in terms of measurable quantities.

2.1.4.2 Ultracentrifugation studies on chitin and chitosan

Lee[26] reported both the weight average and the number average molecular weights via ultracentrifugation for three samples of chitosan. The weight average molecular weight was used for the determination of K and a, the constants from the modified Staudinger equation (see Section 2.1.6.2).

2.1.5. High pressure liquid chromatography techniques

2.1.5.1 Description

High pressure liquid chromatography (HPLC) applied to molecular weight determinations, is basically a high pressure modification of gel permeation techniques (GPC). High pressures are used to obtain shorter analysis times and faster flow rates. Columns are usually packed with either cross-linked polystyrenes or silicas.

In general chromatographic processes may be defined as those in which the solute is transferred between two phases, one of which is stationary and the other mobile. In GPC both phases are liquid but

in contrast to most liquid-liquid chromatography, where the two liquids are immiscible, the two phases are the same liquid (solvent). The column is packed with porous gel particles with the two liquid phases being differentiated only in that the stationary phase is that part of the solvent which is inside the porous gel, while the mobile phase is outside. The pores in the gel should be the same size as the dimensions of the polymer molecule.

A sample of dilute polymer solution is introduced into the solvent stream, as it flows past the porous gel the polymer molecules can diffuse into the internal pore structure of the gel to an extent depending on their size and on the pore-size distribution of the gel. Larger molecules can only enter a small fraction of the internal portion of the gel, or are completely excluded, whereas smaller molecules penetrate a greater fraction of the interior of the gel. Hence the larger the molecule the less time it spends inside the gel and the quicker it flows through the column. The different molecular species are eluted from the column in order of their molecular size, the largest emerging first.

It is usual to measure the retention of a series of polymers of narrow molecular weight distributions to provide a calibration curve of retention volume *versus* molecular weight.

2.1.5.2 HPLC studies with chitosan

In order to study the variables in the chitosan manufacturing process in relation to molecular weight distribution[27], Wu *et al.*[28] developed a method using HPLC. A calibration curve was obtained using a series of dextran samples whose molecular weights ranged from 10,000 to 2×10^6. The number average and weight average

molecular weights of these samples had previously been determined by gel filtration and light scattering techniques respectively. The calibration curve for the dextran standards was plotted, based on the peak elution volumes against the logarithm of the molecular weight. From these plots the elution volumes of chitosan samples under similar conditoins could be related to molecular weight. The technique makes the assumption that dextran and chitosan have similar relationships between their molecular volumes and molecular weights. The criteria for separation in the column is based on size and not weight. Chitosan in solution behaves as a polyelectrolyte and in the absence of added electrolyte will be more expanded than for the non-ionic dextran. Thus the assumption may not be entirely valid.

2.1.6 Viscometric techniques

2.1.6.1 Description

One of the most useful methods for the evaluation of polymer molecular weights, with respect to accuracy, speed and expense, is dilute solution viscometry. This is basically a measure of the size or extension in space of polymer molecules and is empirically related to molecular weight for linear polymers.

Measurements of solution viscosity are usually made by comparing the efflux time, t, required for a specific volume of polymer solution to flow through a capillary tube with the corresponding efflux time, t_o, for the solvent. Using t, t_o and c, the solute concentration, the following definitions can be made:

η_o = viscosity of solvent

η = viscosity of solution

η_r = viscosity ratio = $\eta / \eta_o = t/t_o$

η_{sp} = specific viscosity = $\eta_r - 1 = (t - t_o)/t_o$

$\eta_{red} = \eta_{sp}/c$ = viscosity number

$[\eta]$ = limiting viscosity number = $(\eta_{sp}/c)_{c=0}$

Viscosity is related to molecular weight using the modified Staudinger equation (equation 6)

$$[\eta] = K\bar{M}v^a \quad \text{.....(6)}$$

where $\bar{M}v$ = viscosity average molecular weight, and K and a are constants. They are functions of both the polymer and solvent. The constant a is a measure of the extension of the polymer chains, and varies between 0.5 for completely coiled polymers to 2 for totally extended chains. If the value of a is unity then the weight average molecular weight can be substituted into the equation. If however a is not unity and the polymer is polydisperse then only the value of $\bar{M}v$ is applicable to the equation.

For the determination of K and a it is necessary to measure the viscosities of a series of fractionated samples of known molecular weights and having narrow molecular weight distributions, where $\bar{M}v = \bar{M}w$ and $a = 1$.

Prior to this thesis and the authors publication[29] there has been only one report in the literature concerning viscometric constants for chitosan[26]. The K and a values were reported as $8.93 \times 10^{-2} cm^3 g^{-1}$ and 0.71 respectively in a solvent system of 0.2M acetic acid - 0.1M sodium chloride - 4M urea. The constants were determined using three samples of chitosan of varying molecular weights produced by mechanical shear degradation. The limiting viscosity number of each sample was determined and related to the weight average molecular weight of the sample which was found by ultracentrifugation methods

in the same solvent system. However the results from the ultracentrifuge technique show that the chitosan samples used did not have narrow molecular weight distributions and thus $\bar{M}w$ cannot be assumed to be the same as $\bar{M}v$. Thus at best the constants can only be applied to the determination of molecular weight for samples with a similar molecular weight distribution to those used in the determination of K and a.

2.2 Structural characterisation of chitosan

2.2.1 Introduction

There are two approachs to the determination of the extent of N-acetylation of chitosan - either direct estimation of the amide group or determination of the residual free amine content. The latter approach is more often used because in general the amine groups are more abundant in chitosan, and are more versatile with respect to their ability to react with a range of reagents. Various methods have been developed to determine the degree of N-acetylation, although few of them have been directly compared. The various techniques may be conveniently divided into chemical and physical methods.

2.2.2 Chemical techniques

2.2.2.1 Titrimetric methods

a) Potentiometric methods;

This method was developed by Broussignac[12] and follows the titration of a solution of chitosan in the presence of excess acid, with alkali, by potentiometric means. Broussignac reported that the titration curve has two inflection points. The first point was interpreted as being equivalent to the amount of alkali required

to neutralise the excess acid present. The volume difference between the two inflection points was assumed to correspond to the amount of alkali required to neutralise the protonated amine groups and from this the free amine groups and thus the degree of N-acetylation could be assessed.

The extent of de-N-acetylation[30] of an alkali chitin, prepared[31] by steeping chitin in aqueous sodium hydroxide and dissolving by the addition of crushed ice, was found by following the uptake of hydrochloric acid by the free amine groups using a pH meter. This technique is a modification of the method[32] for the determination of the degree of de-N-acetylation of glycol chitin.

b) Indicator titrations

Moore[33] made use of the insolubility of chitosan in relatively concentrated acid solutions to evaluate the free amine content of the sample. Heterogeneous treatment of chitosan was carried out using 0.5M hydrochloric acid with treatment times of 72 hours. The excess acid was then titrated against sodium hydroxide using phenolphthalein as indicator, thereby enabling the volume of acid required to neutralise the primary amine groups to be determined. The results for the degree of N-acetylation when compared to other methods were consistantly high and were explained on the basis of the Donnan membrane effect. This theory is applicable to semi-permeable membranes, and in the case of heterogeneous acidimetry the chitosan can be considered to act in a similar way to a semi-permeable membrane. The theory takes into account the relationship of the ionic concentrations on either side of the membrane as follows:

In the external phase

$$[Cl^-]_{ext} = [H^+]_{ext}$$

whilst in the internal phase

$$[Cl^-]_{int} = [H^+]_{int} + [-NH_3^+]_{int}$$

since

$$[Cl^-]_{int} \times [H^+]_{int} = [Cl^-]_{ext} \times [H^+]_{ext}$$

it follows that

$$[H^+]_{ext} > [H^+]_{int}$$

Hence the internal aqueous phase will have a lower concentration of hydrogen ions than will the external phase and hence the results will indicate a lower free amine content.

The problem of the Donnan effect can be readily negated by the use of water-soluble derivatives. Hayes and Davies[34] used the water-soluble chitosan hydrochloride salt in titrations against sodium hydroxide. These were carried out both potentiometrically and by the use of phenolphthalein as indicator to yield identical results. The method has the advantage that it is directly measuring the protonated free amine groups, without having to initially neutralise any excess acid. Furthermore there is no problem of ensuring that the amine groups are accessible during the protonation step. The hydrochloride salts were prepared from chitosan solutions in aqueous acetic acid by the addition of concentrated hydrochloric acid which causes the chitosan to precipitate as the hydrochloride salt.

c) Colloid titration;

The term colloid titration[35] is the name given to the titration between positively and negatively charged high molecular weight ionic compounds. The ionic combination between the ions generally occurs stoichiometrically and rapidly, even in dilute solutions, and hence can be used for volumetric titrations. The end points of such reactions can be followed by the metachromic effect of the polymer ions on certain dyes which exhibit large shifts in λ max on addition of minute excesses of a particular polymer ion.

There are two methods used for the titrations. The first involves the direct titration of a cationic polyelectrolyte with an anionic polyelectrolyte using a metachromic cationic dye as an indicator. The molarity of the titre solution must be known and is defined as the concentration of dissociable groups of the anionic polyelectrolyte in one litre of solution. A blank titration is also carried out with the difference between the two titres at the same pH being taken as a measure of the concentration of dissociable groups of the cationic polyelectrolyte in solution.

The second method involves the addition of an excess of a standard reagent of opposite charge to the sample, followed by titration of the excess with a standard of the same charge as that of the sample. A blank is carried out between the two standard solutions.

Chitosan in acidic media is a cationic polyelectrolyte due to protonation of the free amine groups and thus will combine with an anionic polymer in solution.

Chitosan in the form of its hydrochloric salt was titrated by Terayana[35] using the potassium salt of poly(vinylsulphonic acid)

(PVSK) as the titre solution. The solution was maintained at an acid pH by addition of hydrochloric acid and the cationic dye Toliudine Blue was used as an indicator. The dye changes from a blue colour to reddish purple in the presence of excess PVSK. At the end of the titration the pH of the solution is recorded and a blank carried out at the same pH value. The amount of PVSK required increases with increase in the extent of de-N-acetylation of the chitosan, in a quantitative manner.

d) Precipitation titrations;

Standard acid-base techniques involving chitosan are concerned with protonation or deprotonation of the free amine groups. However when the amine groups are protonated they are associated with a negative counter ion. For chitosan hydrochloride this counter ion, the concentration of which can be related quantitatively to the amine content, has been determined[34] by titration with silver nitrate using the adsorption indicator dichlorofluorescein to establish the end point. This is an example of a precipitation titration involving a precipitate of silver chloride. The results for the amine group content correspond well to those determined by potentiometric and indicator titrations, using chitosan hydrochloride and sodium hydroxide.

2.2.2.2 Oxidation and hydrolysis methods

a) Introduction;

General methods for the estimation of acetyl groups have been developed for use in polysaccharaide chemistry. Three such techniques

have been investigated for possible use in determining the $-NH_2$/ $-NHCOCH_3$ ratio in chitosan.

b) Oxidation of terminal methyl groups;

A semi-micro scale method for the determination of acetyl groups, developed by Lemieux and Purves[36], involves the oxidation of the terminal methyl group and recovering the acetic acid thus formed. The oxidation is achieved by heating the sample with a 30% aqueous solution of chromium trioxide. The solution is then distilled and the distillate containing the liberated acetic acid is titrated against sodium hydroxide to a phenolphthalein end point. A correction has to be made due to acidity caused by entrapped chromium trioxide. The titrated solution is treated with sodium carbonate and sulphuric acid. The sodium carbonate converts the chromium hydroxide, produced during the titration with sodium hydroxide, to chromium trioxide and carbon dioxide. Addition of potassium iodide causes the liberation of iodine through oxidation by the chromium trioxide. The iodine is titrated with thiosulphate to determine the acidity due to entrapment of chromium trioxide.

c) Hydrolysis of N-acetyl groups;

The method for the microestimation of acetyl groups by hydrolysis was developed by Elek and Harte[37] and applied to chitosan by Sannan et al.[38,39]. The hydrolysis of the acetyl group is achieved by heating with a solution of p-toluene sulphonic acid. Acetic acid is liberated as a result of the hydrolysis together with sulphur dioxide as sulphurous acid. Addition of potassium iodate causes the liberation of iodine which is titrated against sodium thiosulphate

to determine the acid present. Since some of this will inevitably be sulphurous acid allowance for this has to be made. This is achieved by distillation of the titrated solution, which liberates sulphur dioxide into the distillate, into standard iodine and titration of the excess iodine.

d) Oxidation of α-aminoglycol groups

The technique developed by Moore[40] is based upon the oxidation, by sodium periodate, of the anhydro-D-glucose units on cellulose to yield dialdehyde units without the occurrence of any side reactions. Determination of the oxygen consumption enables the percentage of anhydro-D-glucose units to be calculated. When applied to chitosan the deacetylated anhydro-D-glucosamine units would also undergo this oxidation, the α-aminoalcohol group behaving analogously to the α-glycol group in cellulose. However the units containing the acetamido groups are resistant to the oxidant, thus determination of the amount of oxygen consumed enables the percentage of N-acetylated groups to be calculated. Results obtained from this method were consistantly low when compared with results from infrared analysis. This was explained in terms of alkaline hydrolysis of oxidised anhydro-D-glucosamine units by the ammonia liberated as a by-product of the oxidation of the α-aminoglycol group. Hydrolysis will almost certainly lead to the formation of new α-glycol groups resulting in an increase in the periodate consumption and hence an over-estimation of the extent of deacetylation.

2.2.3 Physical methods

2.2.3.1 Infrared techniques

A recent development in the structural analysis of chitosan has

been the use of infrared methods to determine the amide group content in chitosan. Amides may be characterised by means of the absorption bands due to the N-H and C=O stretching vibrations and N-H deformations. The amide I band is essentially due to the C=O stretching and for secondary amides in the solid state occur in the region 1630 cm^{-1} to 1680 cm^{-1}. The amide II band is due to NH_2 deformations in primary amides and due to a mixed vibration of N-H bending and C-N stretching in the case of secondary amides. For secondary amides the band occurs in the region 1510 cm^{-1} to 1570 cm^{-1}. For a typical spectrum of chitosan (Figure 5) the amide I band occurs at 1665 cm^{-1} and the amide II band at 1550 cm^{-1}. Although amides show other characteristic bands in the spectrum most of the infrared techniques use the amide I or amide II bands for their structural assessment. Considerable work has been carried out on infrared methods because they provide a more rapid assay for the acetamido group on chitosan when compared with other available procedures.

Sannan et al.[38] used the absorbance intensity of the amide band at 1550 cm^{-1}, from the spectrum of a chitosan sample incorporated in a KBr disc, and plotted this against the degree of deacetylation as determined by acidimetry. A base line for the measurement of the absorbance was taken from a point at 1900 cm^{-1} to one at 1200 cm^{-1}. These initial results showed a considerable amount of scatter attributed to the effect of the hygroscopic nature of the chitin, on the accuracy of weighing the material. To eliminate such errors the absorbance at 1550 cm^{-1} was divided by that at 2878 cm^{-1} and plotted against the degree of deacetylation. The band at 2878 cm^{-1} due to C-H stretching vibrations was assumed to be unaffected by the extent of deacetylation and was measured using a base line from

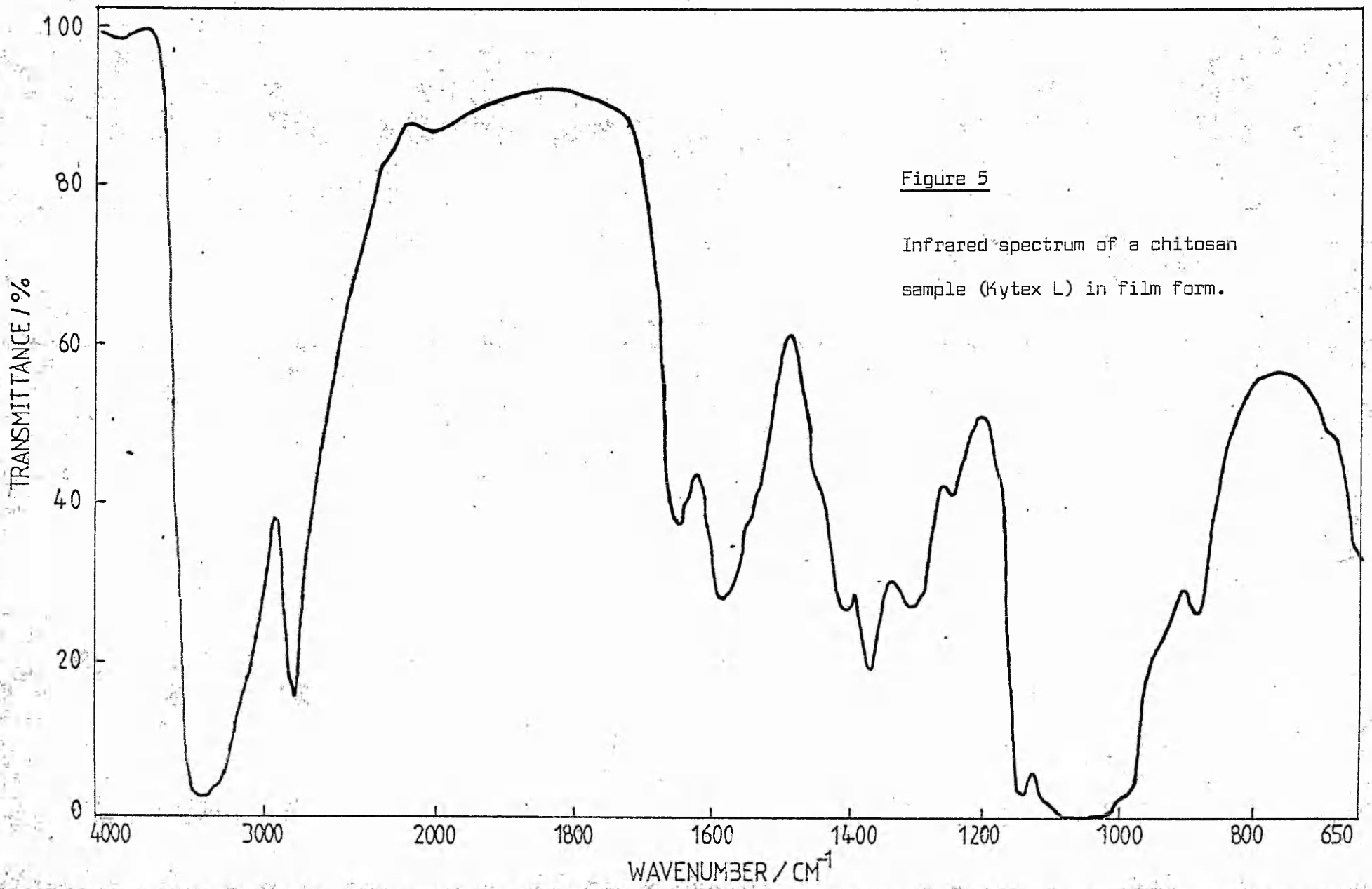


Figure 5

Infrared spectrum of a chitosan sample (Kytex L) in film form.

3800 cm^{-1} to 1900 cm^{-1}. A reasonable linear relationship was found between the ratio A_{1550}/A_{2878} and the degree of deacetylation.

The method of Sannan was applied to chitosans having high degrees of deacetylation by Miya et al.[41], who found that at degrees of deacetylation greater than 90% the infrared results diverge from those obtained by the method of colloid titration[35]. For this reason the amide I band at 1665 cm^{-1}, previously suggested by Moore and Roberts[40], was chosen for the measurement of absorbance, using a base line from the shoulder at 1700 cm^{-1} to the turning point at 1620 cm^{-1}. The second absorbance band at 2878 cm^{-1} was retained but the base line was drawn from the shoulder at 3010 cm^{-1} to the turning point at 2800 cm^{-1}. Using these criteria a plot of the ratio A_{1665}/A_{2878} versus Degree of deacetylation revealed a smooth curve which is nearly linear in the range 90 - 100% deacetylated.

The use of the amide I band at 1665 cm^{-1} was initially suggested by Moore and Roberts[40] who investigated the infrared spectrum of chitosan in both film and KBr disc form with respect to the degree of deacetylation. The band at 1665 cm^{-1} was chosen in preference to the amide II band because at low extents of N-acetylation the amide II band appears at 1595 cm^{-1} and shifts to 1550 cm^{-1} as the extent of N-acetylation increases. This explains the divergence between the infrared and titration results subsequently reported by Miya et al.[41]. This shift was attributed to the gradual disruption of hydrogen bonding involving the amide group. Hydrogen bonding in simple secondary amides has been found [42] to increase the absorption frequency of the amide II band.

As an internal reference to compensate for film thickness the band at 3450 cm^{-1} was chosen. This band is attibuted to absorption

by the hydroxyl groups and was selected for both its prominence and the fact that it is relatively isolated from other bands. A single sloping baseline was used for both absorbance measurements running from the shoulder at 3800 cm^{-1} to the turning point at 800 cm^{-1}. For fully N-acetylated chitosan the ratio A_{1655}/A_{3450} was found to be 1.33 and thus the percentage of the amine groups acetylated in any given sample is given by: $A_{1655}/A_{3450} \times 100/1.33$.

This method requires no calibration against other techniques for determining the extent of N-acetylation. Also the ratio A_{1655}/A_{3450} for fully N-acylated chitosans was also found to be 1.33 regardless of the particular N-acyl group involved. Thus this method allows the extent of any N-acylation of chitosan to be evaluated rather than being specific for N-acetyl groups only. This would not be the case if the absorption band at 2878 cm^{-1} is chosen as the reference band since its intensity will increase, at constant extent of N-acylation, with increase in the hydrocarbon portion of the acyl group.

2.2.3.2 Gas chromatography

Muzzarelli et al.[43] investigated the gas chromatographic behaviour of a number of compounds using chitin and chitosan as the stationary phase. Samples were initially characterised by alkalimetry[12] and by infrared spectroscopy[38]. A linear relationship was established between the retention times of methanol and the degree of N-acetylation of the sample. The greater the degree of N-acetylation the longer the retention time. It has been established that methanol[44] forms a complex with chitin and although the complex is unstable, its presence is sufficient to cause a large difference between the

retention times on chitin and chitosan, and thus is affected by the number of N-acetyl groups present.

2.2.3.3 Mass spectroscopy

The mass spectra of chitin and chitosan were obtained by Hayes and Davies[34] at several different temperatures to determine the degradation temperature. The spectrum of chitosan was found to have relatively complex fragmentation patterns even at low temperatures; however it was possible to assign some of the peaks to specific fragments, including one for the N-acetyl group. Hence the extent of N-acetylation could be estimated, although it was not possible to obtain a direct measure of the $-NH_2/-NHCOCH_3$ ratio.

2.2.3.4 Nuclear magnetic resonance (NMR) spectroscopy

The use of NMR spectroscopy as a technique for the estimation of the N-acetyl content of chitosan was reported by Hirano and Yamaguchi[45]. The extent of N-acetylation was calculated from the ratio of the N-acetyl methyl protons to the methine (C-H) and methylene (CH_2) protons using formic acid as a solvent. However the technique was often applied[45] to polyhydrated chitin gels which contain water and thus will effect the proton ratio in a non-reproducible way. Hence for this method of NMR determination to be effective the sample must be completely dehydrated.

2.3 Chitin, chitosan and paper

2.3.1 Chitosan as a water resistant additive

The use of chitosan as a cementing agent for paper was reported by Rigby[46] who investigated the water resistance of such bonded

papers. A solution of chitosan in the form of its acetate was used to cement together sheets of paper. When dry, the effect of water on the sheets was studied in comparison with other adhesives. The chitosan sheets separated only after several hours of soaking whereas those bonded with other adhesives separated immediately. On such sheets the chitosan exists in the water-soluble acetate form and hence eventual separation must occur. However modification of the chitosan on the sheet yielded a totally water resistant product. This was achieved by either drying at high temperatures, which breaks down the acetate salt to acetic acid and free amine, or converts it to an amide, or by chemical modification. This consists of neutralisation using ammonia or other alkalis to liberate the free amine, or by the use of amine specific reagents e.g. aldehydes.

The water resistant properties of chitosan treated paper was used[47] in the manufacture of lithographic paper plates. A surface coating containing chitosan and other additives was applied to produce a paper which accepts and holds both water and ink.

2.3.2 Chitosan and chitin as strength aids

The use of chitosan as a strength improvement additive for paper was initially investigated by Merrill[48]. He found that treatment of unsized sheets of paper by immersion in aqueous solutions of chitosan acetate, and subsequent high temperature drying resulted in paper having several times the wet and dry bursting strength of the unsized sheets, as well as excellent water resistance, affording a good surface for writing and printing.

A second mode of application involving the addition of a chitosan solution to a water slurry of a pulp was investigated. After

agitation dilute ammonia was added in order to precipitate the chitosan. The resulting pulp was formed into sheets and dried at 100°C. The properties of this paper were compared with those for a similar rosin-sized paper and were found to have higher wet and dry bursting strengths, equal water resistance and a greater tear resistance.

For the treated papers the amount of chitosan taken up was based upon the dry weight of the starting materials. The increases in wet and dry strength properties were dependant on the chitosan content e.g. the wet and dry strength increase of a paper containing 8% chitosan was found to be more than twice as great as a paper containing only 2% chitosan.

Since the initial study by Merrill there have been a number of reports concerning the ability of chitosan to act as a strength aid for cellulosic materials[49-52]. These are all essentially variations on the chitosan solution treatment of paper and add little of fundamental importance or of originality to the initial investigations.

However an extensive study has been carried out by Allen et al.[53-55] to evaluate chitosan and chitin as strength additives for paper with respect to its mode of application and also to determine the mode of bonding between cellulose and chitosan.

Three systems of polymer addition were investigated:

a) Equilibrium adsorption;

This involves the addition of a solution of chitosan acetate to a suspension of fibres, followed by agitation and formation of handsheets in accordance with TAPPI standard T205 m-58.

b) Precipitation;

This is similar to the method of equilibrium adsorption except that after agitation the pH of the system is raised to 10 with sodium hydroxide prior to sheet formation.

c) Spray application;

This involves sheets of untreated fibres being formed in the standard manner and, while still wet, being sprayed with a chitosan solution. Chitosan addition levels were varied by adjusting the duration of spraying.

For this work a high α-cellulose dissolving pulp was used. These materials have some anionic character due to the presence of carboxyl groups from the hemicellulose uronic acid residues or from oxidation reactions associated with bleaching processes.

The equilibrium technique, which corresponds to normal wet end addition in commercial paper making, was found to give rise to a modest increase in the dry breaking length at low chitosan levels of addition, which were determined by micro-kjeldahl analysis, followed by a decrease at higher chitosan levels. The reduction in the dry breaking length was assumed to be caused by increasing fibre flocculation resulting in poor sheet formation and concomitant losses in physical properties.

Precipitation of chitosan onto the fibres prior to sheet formation removes the flocculation problem and allows the effects of a broader range of chitosan retentions to be studied. The dry breaking length of sheets formed in this way were found to increase and then level off with increasing chitosan retention. A similar pattern was

observed for the sprayed sheets.

As a technique spraying was the most convenient in that it eliminated sheet-formation problems and also allowed the chitosan to be added in an ionised form unlike precipitation methods where the chitosan exists as the insoluble free amine. Similar, although not identical results, were observed for a typical unbleached sulphite pulp. The difference was attributed to the considerable natural anionic character of these pulps due to the carboxyl groups of the hemicellulose, and sulphonic acid groups of the residual lignin.

Allan et al.[56] have attempted to explain their results in terms of the bonding forces operating between the fibre and polymer additive. It is assumed that paper is held together by hydrogen bonds which must span the distance between the separate segments of crossing fibres. Their conclusions were that in the presence of water the hydrogen bonding sites on the fibre will be fully occupied by water molecules which are in turn hydrogen bonded to a large excess of water. Hence the fibres can only be regarded as being linked by a layer of water molecules. The weakness of this link is shown by the low wet strength of the paper. Polymeric materials have been used to improve these low strengths by enabling the interfibre bonding areas to remain linked in the presence of water e.g. polyamines improve the wet strength of papers by ionically bonding to the anionic sites on the fibre producing a link that is not readily ruptured by excess water. Chitosan in solution is a cationic polyelectrolyte and therefore should have the ability to ionically bond with the anionic sites on the fibre. This can be shown by the improvement in the wet strength of α-cellulose papers treated by spraying compared to that by precipitation.The latter techniques affords a

non-ionic product whose ability to bond via the sites on the pulp is negligible. There is also the possibility of the amine groups on the chitosan reacting with the carboxyl groups on the pulp to yield a covalent amide link, or with a carbonyl group to yield an imine structure.

However little account has been given to the likelyhood of Van der Waals forces or to hydrophobic bonding in the wet end treatment of the pulps with chitosan.

The dry strength improvements can be more easily attributed to the ability of the chitosan to form hydrogen bonds between itself and the fibres as well as ionic linkages. The strength of a sheet of paper is determined by the fibre and interfibre bond strengths and thus chitosan cannot be expected to significantly improve the physical properties of a well beaten pulp, since fibre breakage is already the dominant factor. This explains the results[53] obtained with chitosan on beaten and unbeaten pulps i.e. for an unbeaten pulp the breaking length of the paper increases significantly before levelling off with increasing chitosan addition, but for beaten pulp there is only a small increase at corresponding addition levels. Beating is a mechanical treatment of a pulp resulting in the modification of the fibre properties e.g. shortening the fibres, altering the fibre flexibility through internal fibrilation (splitting of the fibres internally) and changes in the surface area by external fibrilation.

A modest beating process serves to improve the strength of the paper because of better bonding between fibres brought about by increased fibre flexibility and increase in the surface of the fibres available for bonding.

2.3.2.1 Effect of molecular weight of chitosan on paper strength

Increase in the molecular weight of chitosan samples was found[56] to bring about an increase in both wet and dry strength properties of a softwood groundwood pulp at similar retention levels, the chitosan being applied by spraying onto preformed sheets. The molecular size effect is attributed to the construction of a more extensive interfibre bridging network. The highest molecular weight chitosan sample had a $\bar{M}v$ of 180,000, although in order to optimise the effect it would be desirable to use higher molecular weight materials.

2.3.2.2 Effect of N-deacetylation

Both the insolubility of chitin and the strength of paper can be attributed to the presence of hydrogen bonding. Thus in principle chitin particles should be readily bonded to fibres. However even pulp fibres do not bond well to themselves unless the area of contact has been increased by beating. Due to the relative inefficiencies of the beating process, chitin has been modified by surface alkaline hydrolysis to try and improve the bonding. This operates by exposing free amine groups on the chitin surface by deacetylation, and these are able to form interfibre links. Relative improvements in the dry breaking length of a Western hemlock sulphite pulp, was observed with increase in the deacetylation of chitin. However at high levels of addition (4% and above) there is a decline in the strength improvement caused by disruption of the fibre-fibre interactions.

When the hand sheet stock was beaten prior to addition of the deacetylated chitins, there was a reduction in strength with increasing

chitin addition. This adverse effect with beaten stock was assumed to be related to the dimensions of the deacetylated chitin particles which are large in terms of the individual voids within the structure of the handsheet. Evidence to support this was provided by the use of a paper-making grade clay coated with chtiosan by precipitation. Such clays consist of particles much finer than the chitin derived material. Handsheets prepared from beaten stocks with the clay filler show significant increases in breaking length when the clay particles are surrounded by precipitated chitosan.

2.3.3 Modified derivatives of chitin and chitosan as strength aids

It is possible to carry out after-treatments on chitosan- impregnated papers so as to modify the properties. It is reported[48] that chitosan can be acetylated to obtain regenerated chitin by reaction with acetic anhydride although this claim is highly unlikely without the presence of methanol to swell the polymer[57]. The use of formaldehyde[48] as a precipitant for chitosan-impregnated celluloses was the first example of the formation of carbonyl derivatives of chitosan on paper.

A graft co-polymer of chitosan has been produced[58,59] in an attempt to make an additive to improve the dry strength of paper. It was also designed to operate well in both acidic and alkaline media. The acrylic monomers 2-acrylamido-2-methylpropane sulphonic acid and acrylic acid were used for graft copolymerisation onto a chitosan substrate. The method reported is based upon the ceric salt redox system. It is known that ceric salts form a redox system when coupled with certain reducing agents such as alcohols or amines. The reaction proceeds by a single electron step, resulting in cerous ions and a partially oxidised reducing agent in free radical form.

The free radical is produced on the chitosan macromolecule and, in the presence of a monomer, polymerisation occurs. Since the free radical is on the substrate only graft polymers are formed. However no mention is made of the chelating ability of chitosan with cerium salts[60] and its potential effect on the polymerisation.

Nevertheless, using a polymer containing 40% chitosan, 54% acrylic acid and 6% 2-acrylamido-2-methylpropane sulphonic acid, as an additive, hand sheets were prepared with addition values ranging from 0.1% to 5% on dry weight of pulp, with a preferred range between 0.2% and 1%. The sheets were formed under both acidic and alkaline conditions. Improvements in both burst and tensile strengths were observed in the acidic mode, compared to untreated paper and also to chitosan treated paper. Alkaline conditions tended to produce a decline in strength relative to chitosan treated papers, but still showed an improvement over untreated stock.

2.3.4 Other applications of chitosan

2.3.4.1 Effect on board

The effect of chitosan on paper has been extended to the manufacture of cardboard. Feldman et al.[61] used chitosan acetate to size pulp for making board, at high levels of addition of between 15% and 20%. A more quantitative approach[62] involved the measurement of the flat crush strength of cardboard manufactured using chitosan as a wet end additive. An increase in the flat crush strength was observed compared to similar boards prepared without chitosan.

2.3.4.2 Electrical properties

The adhesive properties of chitosan[46] was found to be poor for

conducting materials such as metallic surfaces but excellent for relatively non-conducting materials such as paper. Use of this property has led to the development of a capacitor paper[63] containing chitosan with an improved electrical resistance. The use of a chitosan derivative, cyanoethyl chitosan, afforded a paper[64] with enhanced electrical insulation and mechanical properties.

2.3.4.3 Chitosan as a flocculant

The possibility of the use of chitosan as a flocculant in paper making to improve retention and water drainage has been mentioned[65] in terms of its ability to act as a cationic polyelectrolyte in solution. The water-soluble polymers that are used as retention aids, to improve the retention of fibre fines, fillers and other particulate matter, generally operate by flocculating the small matter in the system to the large fibres. The retention of starch in papers containing up to 34% starch was improved by the use of chitosan[51].

Flocculants tend also to improve drainage of water by redistributing the fines throughout the sheet, preventing them from being retained via a filtration mechanism, whereby they tend to plug the pores. In addition collapse of the hydration shell around the fibres due to action of the flocculant may be involved.

There is a problem, when using chitosan, of over-flocculation causing the small particulate matter to bind to each other rather than to the longer fibres or even causing fibre flocculation. This results in poor sheet formation and reduction in physical properties at higher chitosan addition levels[53].

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Determination of the viscometric constants for chitosan

3.1.1 Introduction

One of the simplest and most rapid methods for the determination of the molecular weights of polymers is viscometry, although this is not an absolute method and requires the determination of constants through correlation of limiting viscosity numbers (LVN) with molecular weights (see Section 2.1.6.1).

There is only one report[26] in the literature dealing with the determination of the viscometric constants for chitosan. The values obtained are $\underline{a}$ = 0.71 and $\underline{K}$ = 8.93×10^{-2} $cm^3 g^{-1}$ using 0.2M acetic acid - 0.1M sodium chloride - 4M urea as the solvent. The value for $\underline{a}$ is very low when compared with the values in the literature for other ionic β-(1→4)- linked polysaccharides e.g. sodium carboxymethyl cellulose[66] and sodium cellulose sulphate[67] (see Table 1), whilst the value for $\underline{K}$ is rather high. Furthermore only three fractions of chitosan were used in the determination and far from having narrow molecular weight distributions, as required for evaluation of the viscometric constants by the methods used, one fraction had $\bar{M}w/\bar{M}n$ = 1.76 and another had $\bar{M}w/\bar{M}n$ = 3.51. In view of these facts it was considered useful to redetermine the values of $\underline{K}$ and $\underline{a}$.

The non-absolute nature of viscometric techniques requires the molecular weight of the samples to be determined by means of a second, absolute technique. In this thesis number average molecular weight values ($\bar{M}n$), as determined by a method of spectroscopic end-

Table 1

Viscometric constants (K and a) for β-(1→4)- linked polysaccharides.

Polymer	Salt concentration/M	$K/cm^3\ g^{-1}$	a
Chitosan acetate	0.1	8.93×10^{-2}	0.71
Sodium carboxy methyl cellulose	0.1	1.23×10^{-4}	0.91
Sodium cellulose sulphate	0.5	7.91×10^{-2}	0.93

group analysis, are used to determine K and a.

3.1.2. End group analysis of chitosan

3.1.2.1 Introduction

The most common method for determining $\bar{M}n$ values of polymers is osmometry but this technique is difficult to apply to polyelectrolytes because of the decreased activity of the counter ions relative to their activity in solutions of simple salts. It was therefore decided to use the technique of end group analysis and to measure the concentration of end groups through the formation of the phenylosazone derivative by reaction of the reducing chain ends with phenylhydrazine.

The reaction between phenylhydrazine and cellulose has previously been studied[68]. The product was found to be similar to D-glucose phenylosazone in spectral properties and chemical behaviour. This and related reactions were extensively studied by Blair et al.[69-73] who showed that there was a correlation between the molecular weights of the hydrocellulose samples used, as determined by viscometry, and the absorption intensity of the phenylosazone derivatives[69]. The reaction between phenylhydrazine and the reducing end groups are outlined in Figure 6. In solution the cyclic hemi-acetal form of the end group is able to mutarotate to the open chain aldehydo form. The phenylhydrazine reacts with the carbonyl group producing initially the phenylhydrazone. A second phenylhydrazine molecule then oxidises the C(2) hydroxyl to a ketone which in turn reacts with a third phenylhydrazine molecule to yield the phenylosazone. The reaction stops at this point. A more detailed discussion of the

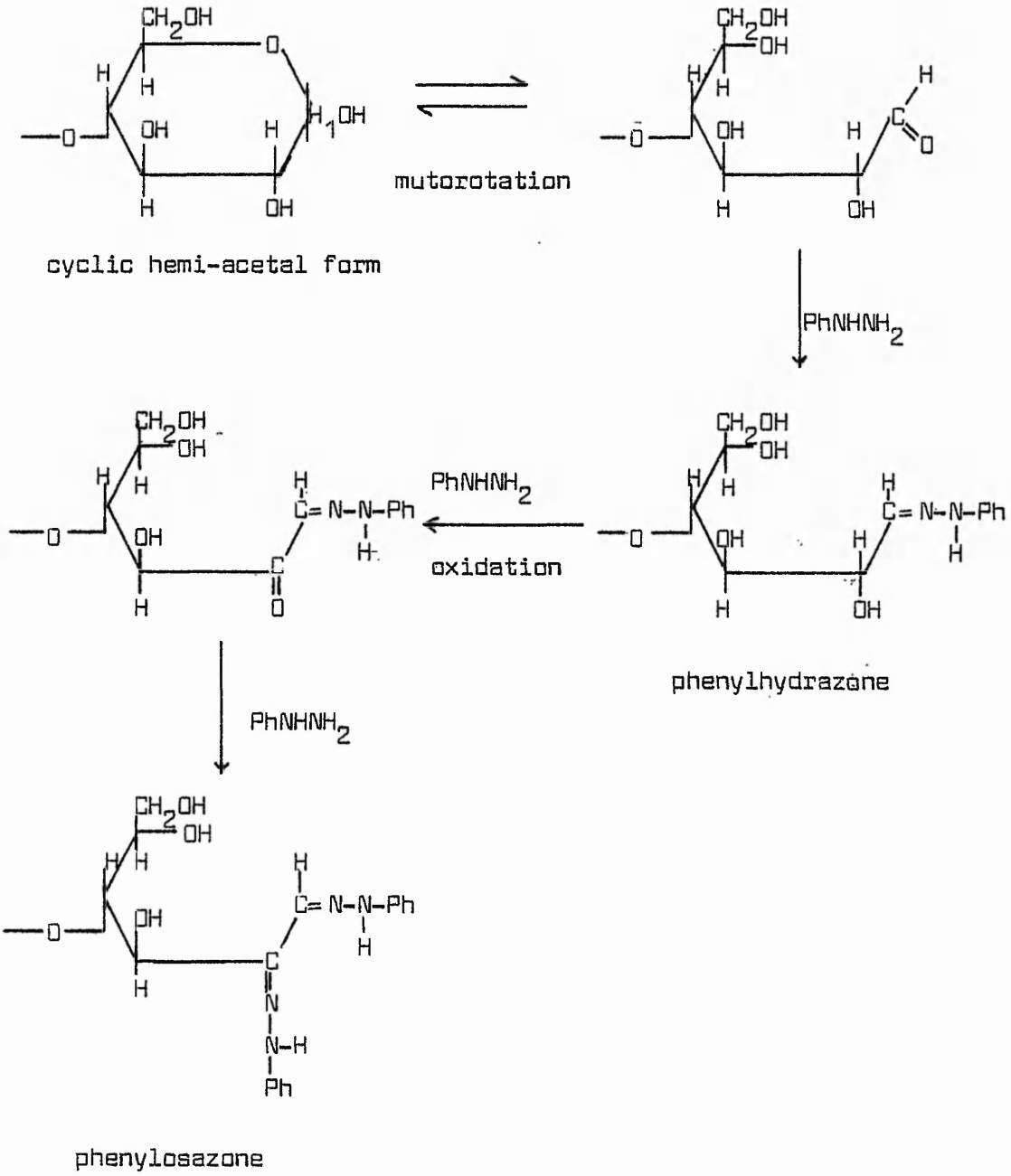


Figure 6

Phenylosazone formation from cellulose reducing end groups.

properties and reactions of arylosazones is given in Section 3.1.4. The phenylosazones are yellow in colour and have a high extinction coefficient and thus lend themselves well to uv spectroscopic analysis.

The situation with respect to chitosan is slightly different. The C(2) hydroxyl group on cellulose is replaced with either an amine or an acetamido group. Thus for complete end group phenylosazone formation both these species must undergo the reaction with phenylhydrazine. Tieman[74] has shown that D-glucosamine will form the same phenylosazone as D-glucose, the only difference between the starting materials being the replacement of the C(2) hydroxyl in D-glucose with an amine group in D-glucosamine. Thus the end group on chitosan in the free amine form should undergo phenylosazone formation.

Micheel and Dallmann[75] investigated the reactions of N-acetyl-D-glucosamine with phenylhydrazine in the absence of oxygen. They found that the reaction stopped at the phenylhydrazone stage and that the acetyl group on the nitrogen was removed. From this stage, in theory, it should be possible to complete the phenylosazone formation by including atmospheric oxygen.

3.1.2.2 Phenylosazone formation from N-acetyl-D-glucosamine

To confirm the theory that N-acetyl-D-glucosamine should yield D-glucosephenylosazone, N-acetyl-D-glucosamine, prepared by the method of Inouye et al.[76], was reacted with a three-fold excess of phenylhydrazine in the presence of acetic acid and sodium acetate. The yellow crystalline product obtained was compared to D-glucose phenylosazone, prepared from D-glucose, using thin layer chromatography (with 50/50 ethanol/ether as eluant), infrared spectroscopy and melting point data. The comparison showed that the two products

were identical, although the yield for D-glucosephenylosazone formation from N-acetyl-D-glucosamine was low (10%) compared to that from D-glucose.

A comparative study of the rate of phenylosazone formation from N-acetyl-D-glucosamine and D-glucose, set up under identical molar concentrations of reagents and temperature, showed that the rate of phenylosazone formation from N-acetyl-D-glucosamine is slower (Table 2). The extent of reaction was followed by monitoring the intensity of the uv/visible absorbance band at approximately 390 nm which is characteristic of phenylosazones[77,78]. The slower rate may be explained due to the extra stages involved in the reaction compared to that for D-glucose i.e. hydrolysis of the N-acetyl group and subsequent dehydrogenation and ammonia loss before the second phenylhydrazine residue can enter.

When the osazone formation reaction was applied to chitosan the slower rate of reaction was taken into account by increasing the reaction time (days rather than hours).

3.1.2.3 Phenylosazone formation from chitosan

Initial attempts to produce chitosan phenylosazone involved the reaction of a high molecular weight chitosan (Kytex H), dissolved in a stoichiometric quantity of acetic acid, based on the free amine content, with phenylhydrazine in the presence of sodium acetate at 70°C. The product obtained on reprecipitation resembled that of the starting material. However modification of the reaction by the use of an acid-degraded chitosan, which has more reducing end groups, together with the use of excess acetic acid, afforded a product which was pale yellow in appearance. Infrared analysis of the

Table 2

Relative rate study on phenylosazone formation between N-acetyl-D-glucosamine and D-glucose by monitoring uv absorbance at 395 nm in 1 cm cells.

N-acetyl-D-glucosamine		D-glucose	
Time/Minutes	Absorbance	Time/minutes	Absorbance
15	0.085	15	0.16
30	0.135	25	0.24
50	0.185	40	0.45
90	0.284	85	1.0
200	0.65	195	2.7
300	0.83	300	3.7

solid showed no sign of the aromaticity one would expect due to the phenylhydrazine residues. However infrared spectroscopy is a rather insensitive technique, and would require at least 1% of the total weight of the material to exist as a phenylosazone end group. This corresponds approximately to a molecular weight of 30,000. The chitosan samples used were of considerably higher molecular weights and thus it is unlikely that the phenylosazone absorption bands would be observable in the infrared.

Uv/visible spectroscopic analysis of the product afforded better results. In 1% acetic acid a broad band was observed in the 400 nm region. However using 1% hydrobromic acid as the solvent gave a well defined spectrum and a sharp absorption band (Figure 7) was found at 395 nm. This peak is characteristic of sugar phenylosazones[78] and confirms the formation of the phenylosazone of the reducing end groups on chitosan.

The extent of phenylosazone formation was followed by the removal of aliquots from the reaction mixture at different time intervals, reprecipitation of the polymer by addition of alkali and uv/visible analysis of the redissolved product. A plot of Relative absorbance (absorbance per g of material) versus Reaction time (Figure 8) showed that the reaction was complete after 48 hours. Thus all further phenylosazone formation reactions were carried out for 48 hours. During the reaction there is the likelyhood that the acetic acid will also degrade the polymer, particularly at the reaction temperature used. However acetic acid is only a weak acid and thus the degradation should be slow in relation to the osazone formation of the new reducing end groups produced by this degradation.

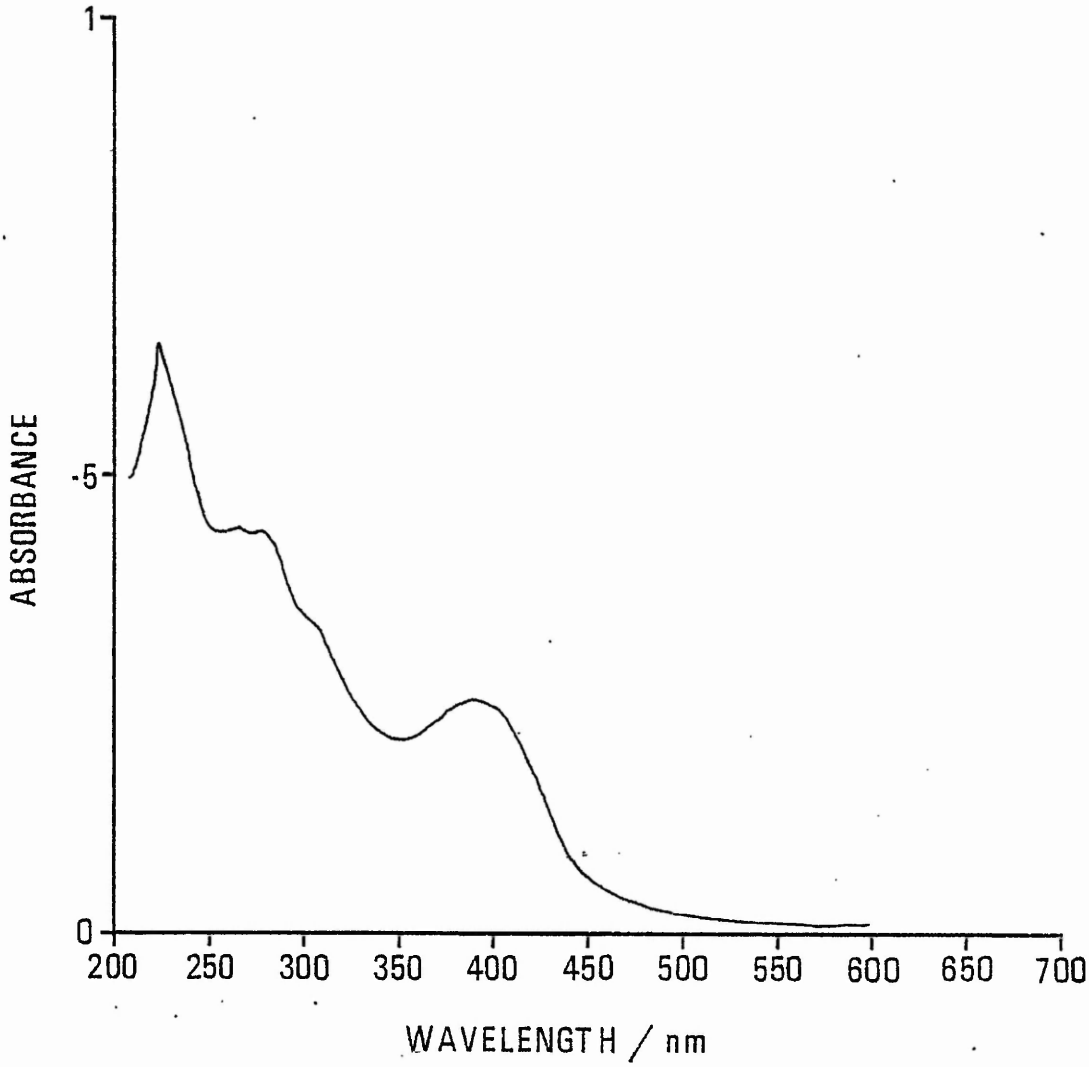


Figure 7

Uv/visible spectrum of chitosan phenylosazone in 1% hydrobromic acid.

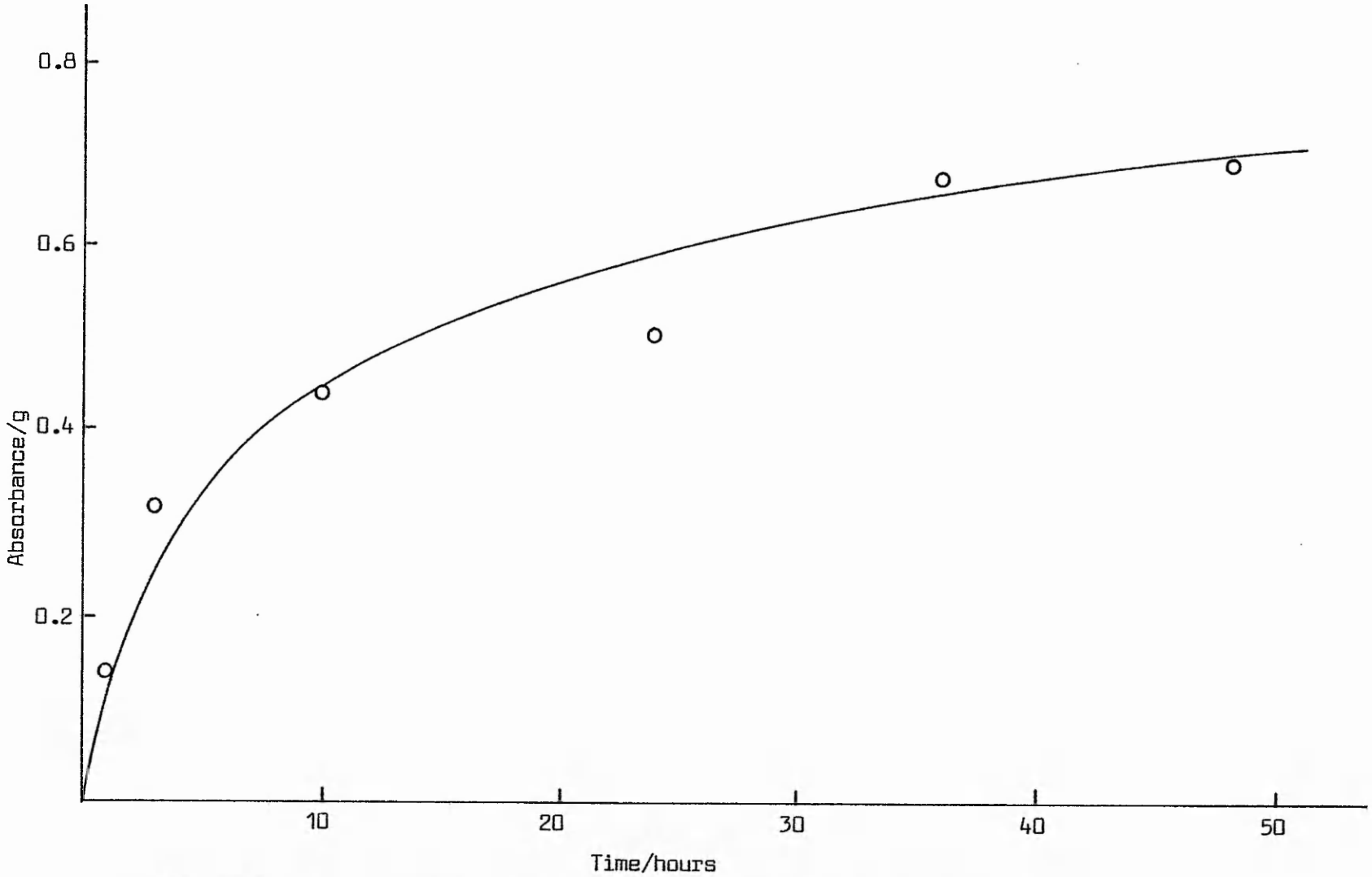


Figure 8 Absorbance per g of chitosan phenylosazone versus Reaction time

3.1.2.4 Determination of the extinction coefficient for D-glucose phenylosazone in acid media

In order to determine the molecular weight of chitosan by spectroscopic end group analysis, the extinction coefficent of the phenylosazone residues must be known. Barry et al.[79] have reported that the extinction coefficients of a large number of monosaccharide and disaccharide phenylosazones, measured for fresh solutions in methanol, are very similar and are independant of molecular weight. The average value obtained for the extinction coefficient (ε) was 20,200. However chitosan is not soluble in methanol and thus the extinction coefficient of the phenylosazone must be measured in an aqueous system. The majority of sugar phenylosazones are water insoluble and thus a water-soluble phenylosazone was prepared by reaction of the sodium salt of D-glucuronic acid with phenylhydrazine. The product obtained was moderately soluble in water due to the solubilising effect of the carboxyl group. Unfortunately the determination of an accurate extinction coefficient was hindered by the ease of hydration of the product, so that the exact molecular weight of the hydrate was not known.

However by the use of a mixed solvent system containing methanol and aqueous hydrobromic acid it was possible to determine the extinction coefficient of D-glucose phenylosazone. The choice of the solvent system was based on the fact that solutions of chitosan in dilute hydrobromic acid can tolerate dilution with considerable volumes of methanol. The solvent must contain sufficient methanol to prevent the water-insoluble D-glucose phenylosazone precipitiating out of solution and enough acid to render the chitosan phenylosazone soluble.

Thus a solvent system of methanol/0.1M hydrobromic acid (7/3) was chosen as the standard solvent for adsorption measurements, and used to prepare a series of solutions of D-glucose phenylosazones at different concentrations. The absorbance values were measured at 395 nm (λ max) and a plot of Absorbance *versus* Concentration (Figure 9) gave an extinction coefficient of 20,200, identical to that for D-glucose phenylosazone in methanol alone.

3.1.2.5 Stability of chitosan phenylosazone in acid media

One of the experimental difficulties encountered by Blair and Cromie[70], namely the gradual oxidative degradation of the phenylosazone chromophore in the strongly alkaline Cadoxen solution used as a solvent for the hydrocellulose phenylosazones, would not apply to chitosan because it is acid-soluble. However phenylosazones are susceptible to acid hydrolysis and so the stability of the chitosan phenylosazone chromophore in the methanol/0.1M HBr solvent at 25^{o}C was assessed. No change in the absorbance was observed over a period of 20 hours, indicating that the phenylosazone is sufficiently stable under these conditions of acidity and temperature to be used for molecular weight determinations.

3.1.2.6 Determination of $\bar{M}n$ values of chitosan phenylosazones

Using the methanol/0.1M HBr mixed solvent system and measuring the uv/visible absorbance at 395 nm enables the number average molecular weight of a sample to be calculated using the relationship[72]:

$$\bar{M}n = \frac{20,200 \times c \times l}{A} \quad \text{.....(7)}$$

where $\bar{M}n$ = number average molecular weight, c = concentration in g dm^{-3}, l = path length in cm and A = absorbance. All measurements

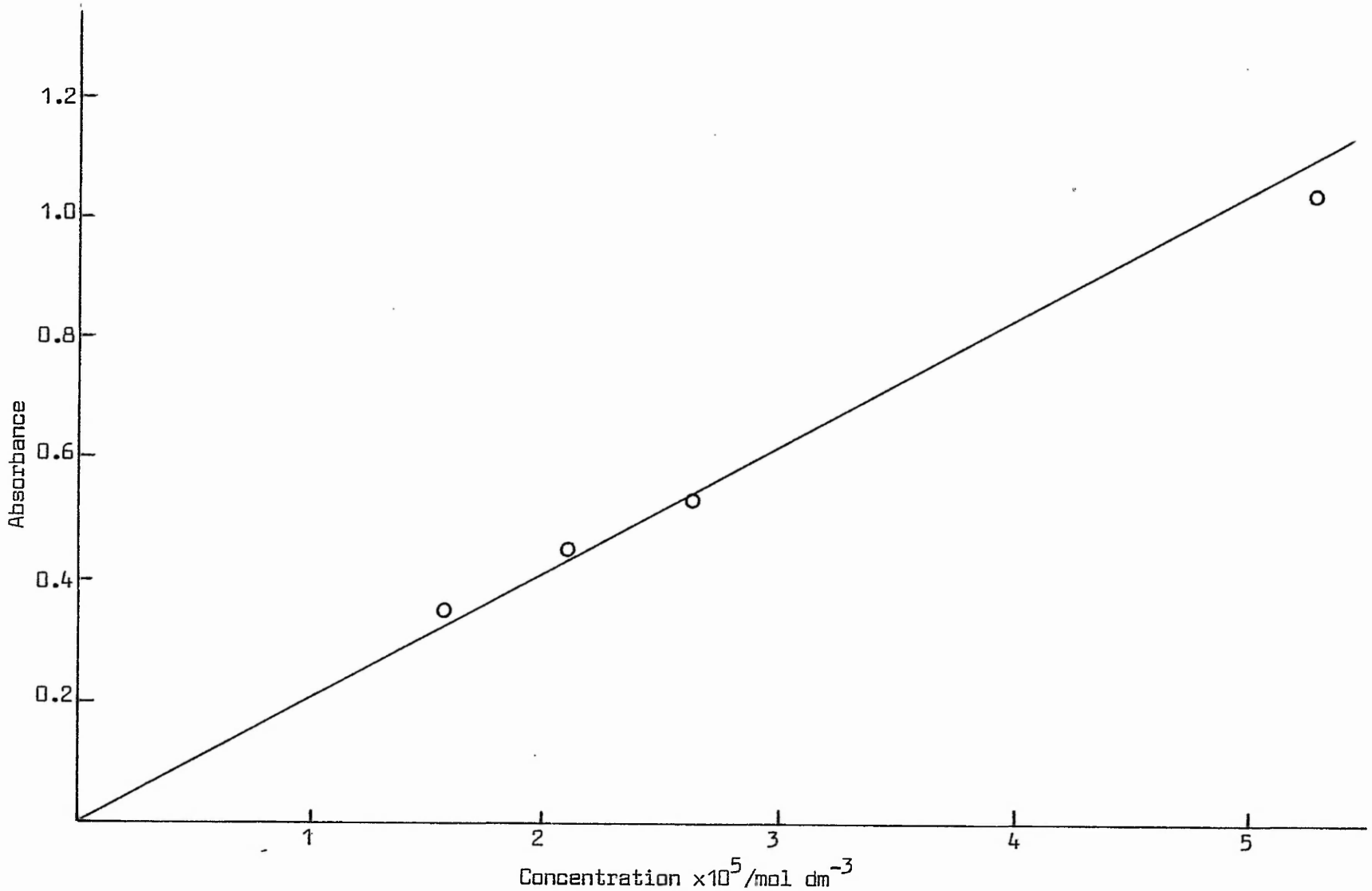


Figure 9 Absorbance versus Concentration for D-glucose phenylosazone in 7/3 (v/v) methanol/1N HBr

were made in matched 1 cm cells.

Two series (1 and 2) of progressively degraded chitosan phenylosazones were prepared. The first used a commercial high molecular weight chitosan (Kytex H) as starting material and was degraded by preparing and heating a 1% solution of the chitosan in 10% acetic acid. Aliquots were removed after various time intervals and treated by dilution with water prior to reaction with phenylhydrazine. The dilution was necessary to minimise the side reaction that can occur between the phenylhydrazine and the acetic acid to yield N-acetyl-N-phenylhydrazine. At high acetic acid concentrations this product tends to crystallise out of solution and was identified by infrared, NMR and melting point determinations. A total of 6 successively degraded samples of chitosan phenylosazones were produced in this way. The intensity of the yellow colour of the products increased visibly with the extent of degradation, and thus with decrease in molecular weight. The samples were labelled 1-6.

The second series of chitosan phenylosazones were prepared in a similar manner except the starting material was chemically treated prior to the hydrolysis step. This pretreatment involves the reaction of a solution of Kytex H in dilute hydrobromic acid with sodium borohydride at $0^{o}C$. Further acid is added during the reaction to maintain the pH of the system at about 3 and thus keep the chitosan in solution. Sodium borohydride is a powerful reducing agent and in this case serves to convert any of the end groups on the chitosan that exist in either the lactone or sodium carboxylate forms to reducing end groups. In view of the strongly alkaline conditions normally used in the production of chitosan from chitin it is probable that many, if not all, of the reducing end groups originally present in

the chitin are converted to either lactone (IV) or sodium carboxylate groups (III), neither of which would form a phenylosazone derivative. The use of sodium borohydride to convert these groups into their reducing forms has been studied for sugars by Wolfrom and Blair[80] who found that the reduction could be prevented from continuing to the alcohol derivative if it was carried out at $0^{o}C$. Subsequent acid degradation of the chitosan yields new end groups in the correct reducing forms.

A second series of 7 degraded chitosan phenylosazones were thus prepared all labelled 7-13.

The number average molecular weights of all 13 samples were determined by uv/visible spectroscopy using equation (7) and the results are given in Table 3. From this the molecular weight is seen to decrease with increase in time of hydrolysis, as would be expected. The extensive times of hydrolysis are a consequence of using the weakly acidic acetic acid, causing a slow rate of degradation of chitosan. An attempt was made to increase the rate of degradation by the use of hydrobromic acid, but subsequent osazone formation in this acidic media was not possible, probably due to the hydrolysis of the product by the stronger hydrobromic acid at the elevated reaction temperature.

3.1.3 Viscosity measurements on chitosan

3.1.3.1 Introduction

In acidic solution chitosan is a polyelectrolyte in that it has ionisable groups along the chain. Polyelectrolytes normally exhibit properties in solution which are quite different from those exhibited

Table 3

Number average molecular weight ($\bar{M}n$) for degraded chitosan phenylosazones (series 1 and 2).

Sample number	Hydrolysis time/hour	Sample weight/g	Solvent volume/cm^3	Polymer conc./gl^{-1}	Absorbance	$\bar{M}n$/g
1	75	0.1396	50	2.792	0.145	388,954
2	100	0.0864	50	1.728	0.104	336,439
3	125	0.1337	100	1.337	0.091	295,971
4	150	0.0603	50	1.206	0.095	256,423
5	200	0.0875	50	1.750	0.270	130,926
6	250	0.1079	50	2.158	0.430	101,376
7	40	0.1774	50	3.548	0.164	630,062
8	64	0.2901	100	2.901	0.130	450,771
9	136	0.2063	50	4.126	0.345	241,580
10	164	0.1807	50	3.614	0.405	180,253
11	200	0.1415	50	2.830	0.365	156,619
12	250	0.2628	50	5.254	1.090	96,585
13	380	0.0145	50	0.290	0.123	47,820

by non-ionic polymers. When they are soluble in non-ionising solvents polyelectrolytes behave in completely normal fashion but in aqueous solution they are ionised, with three major consequences. First the mutual repulsion of their charges causes expansions of the coiled polymer chain. The size of the polyelectrolyte random coil is, moreover, a function of the concentrations of polymer and added salt since both influence the degree of ionisation. Secondly, the ionisation of the electrolyte groups leads to several unusual effects in the presence of small amounts of added salts e.g. the intensity of light scattering decreases because of the ordering of the molecules in solution. Finally the ionic charges attached to the chains create regions of high local charge density affecting the activity coefficients and properties of small ions in these localities.

Although the various effects cannot be completely separated the results of chain expansion are of primary interest for the measurement of molecular weight and size. Those properties dependant on the size of the chain, such as viscosity, are strongly affected by chain expansion. The effect of chain expansion for solutions of polyelectrolytes in the absence of added salt is shown in the non-linear relationship between the Viscosity Number and polymer concentration. However addition of low molecular weight electrolyte (salt) to the aqueous solution increases the ionic strength of the solution outside the polymer coil relative to that inside and also reduces the thickness of the layer of "bound" counterions around the chain. Both effects cause the chain to contract and at a certain concentration of added salt the solution behaviour will normalise resulting in a linear relationship between Viscosity Number and polymer concentration.

For chitosan the applicability of a 0.1M acetic acid - 0.2M sodium chloride solvent was tested on the linearity of the Viscosity Number *versus* Concentration plot, using an undegraded sample of chitosan. The solvent system was found to give the necessary straight line plot (Figure 10) and was therefore used in preference to the one reported by Lee[26], of 0.2M acetic acid - 0.1M sodium chloride - 4M urea, because of the greater simplicity in working without large concentrations of urea. The applicability of a second solvent system containing 0.1M acetic acid - 0.02M sodium chloride, was also tested and found to have a linear Viscosity Number *versus* Concentration relationship (Figure 11). This second solvent was chosen in order to study the effects of the concentration of salt on the viscometric constants.

3.1.3.2 Measurements of Limiting Viscosity Number (LVN) values for chitosan phenylosazones

Viscosity measurements were carried out on both series of degraded chitosan phenylosazones using a capillary viscometer at 25°C and the solvent system of 0.1M acetic acid - 0.2M sodium chloride. The Viscosity Number *versus* Concentration plots for samples 1-6 are shown in Figure 12 and those for samples 7-13 in Figure 13. Tabulated data for individual sample flow times are included in Appendix I. Also the viscosities of samples 7, 9, 11, and 12 were redetermined in the 0.1M acetic acid - 0.02M sodium chloride solvent (Figure 14). From the linear Viscosity Number *versus* Concentration plots the LVN for each chitosan sample in the particular solvent system used was found by extrapolating to zero concentration (Tables 4, 5 and 6).

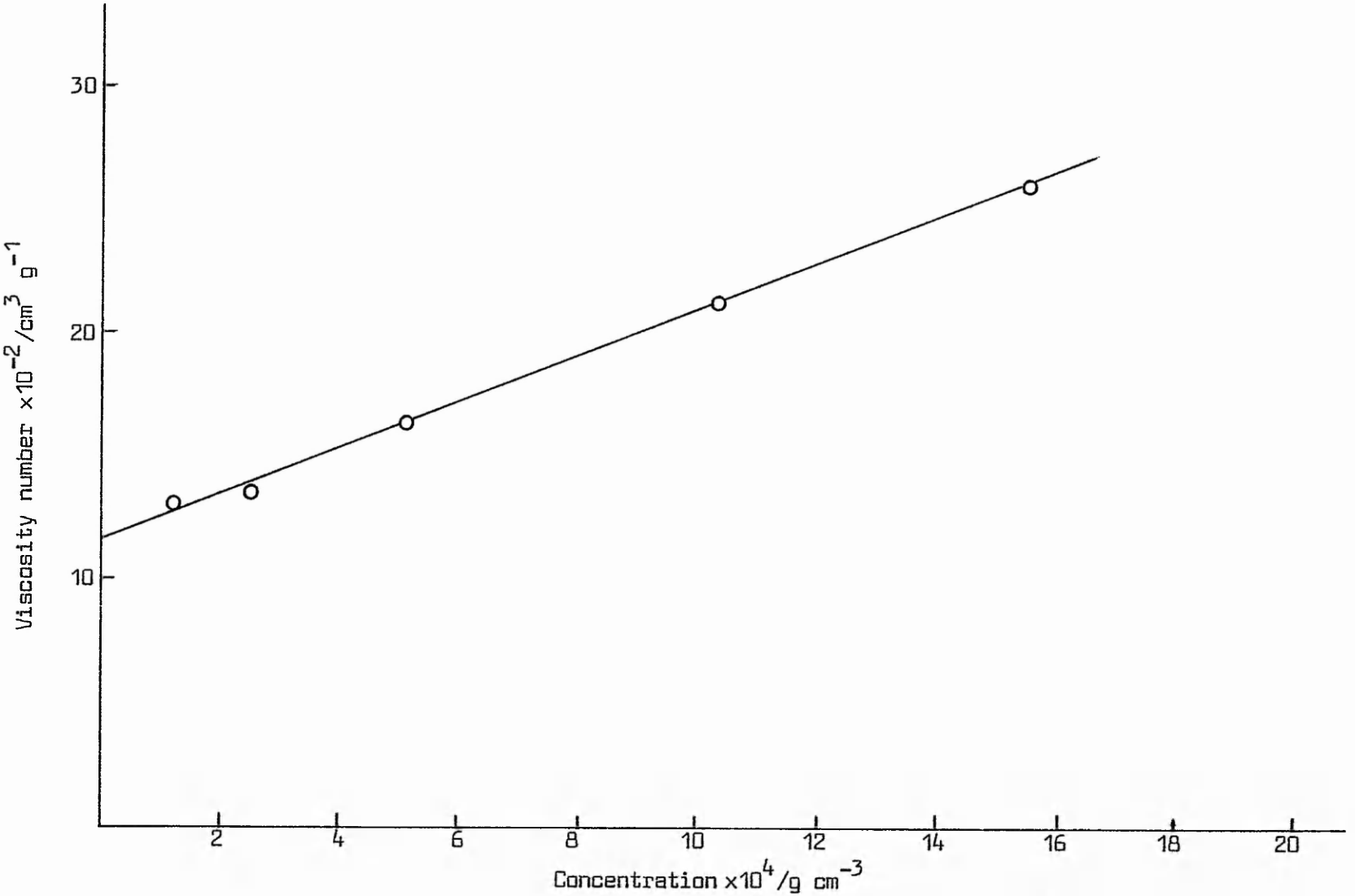


Figure 10 Viscosity number versus Concentration expressed in g cm^{-3} for Kytex H in 0.1M acetic acid - 0.2M sodium chloride.

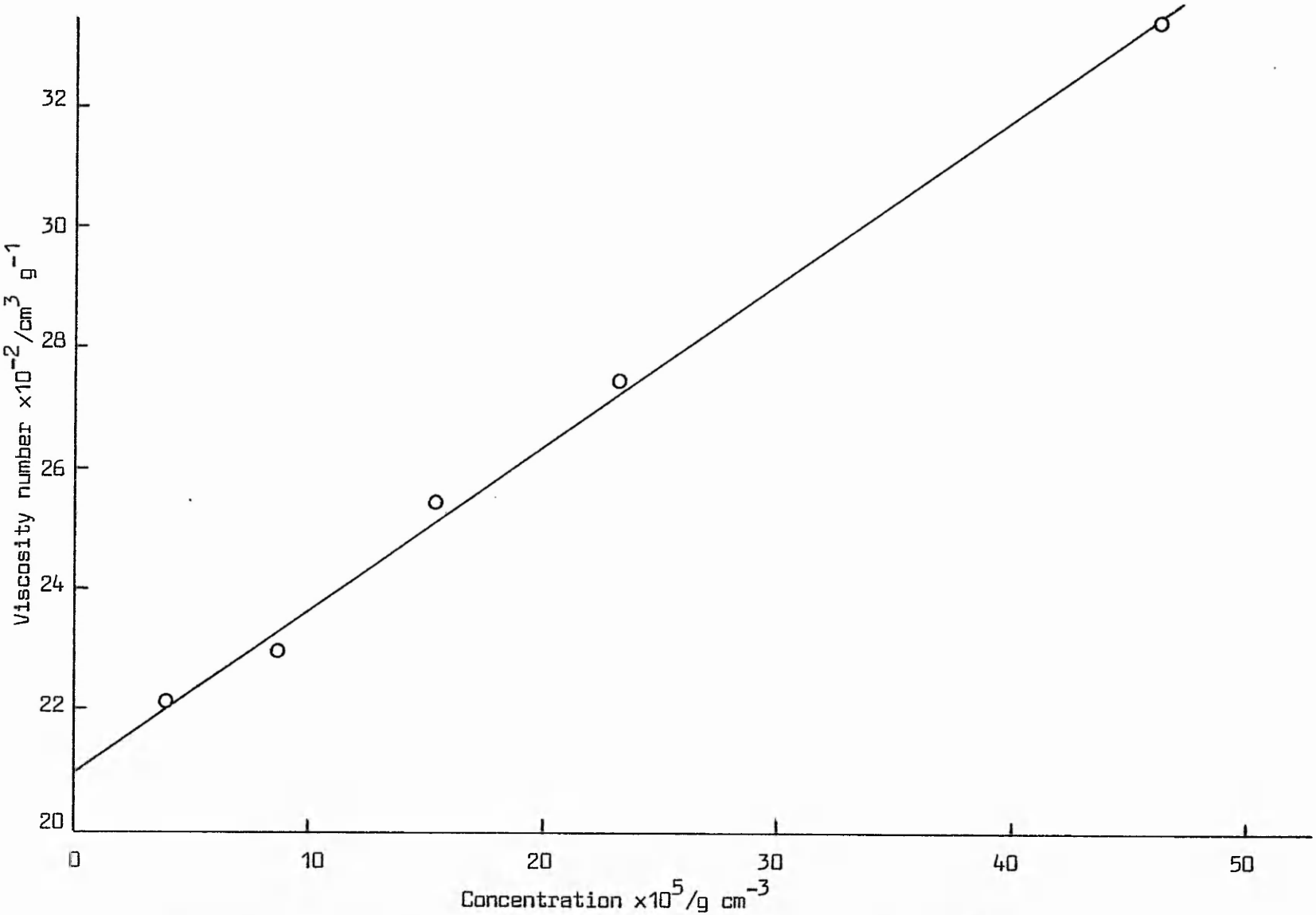


Figure 11 Viscosity number versus Concentration expressed in g cm^{-3} for Kytex H in 0.1M acetic acid - 0.02M sodium chloride.

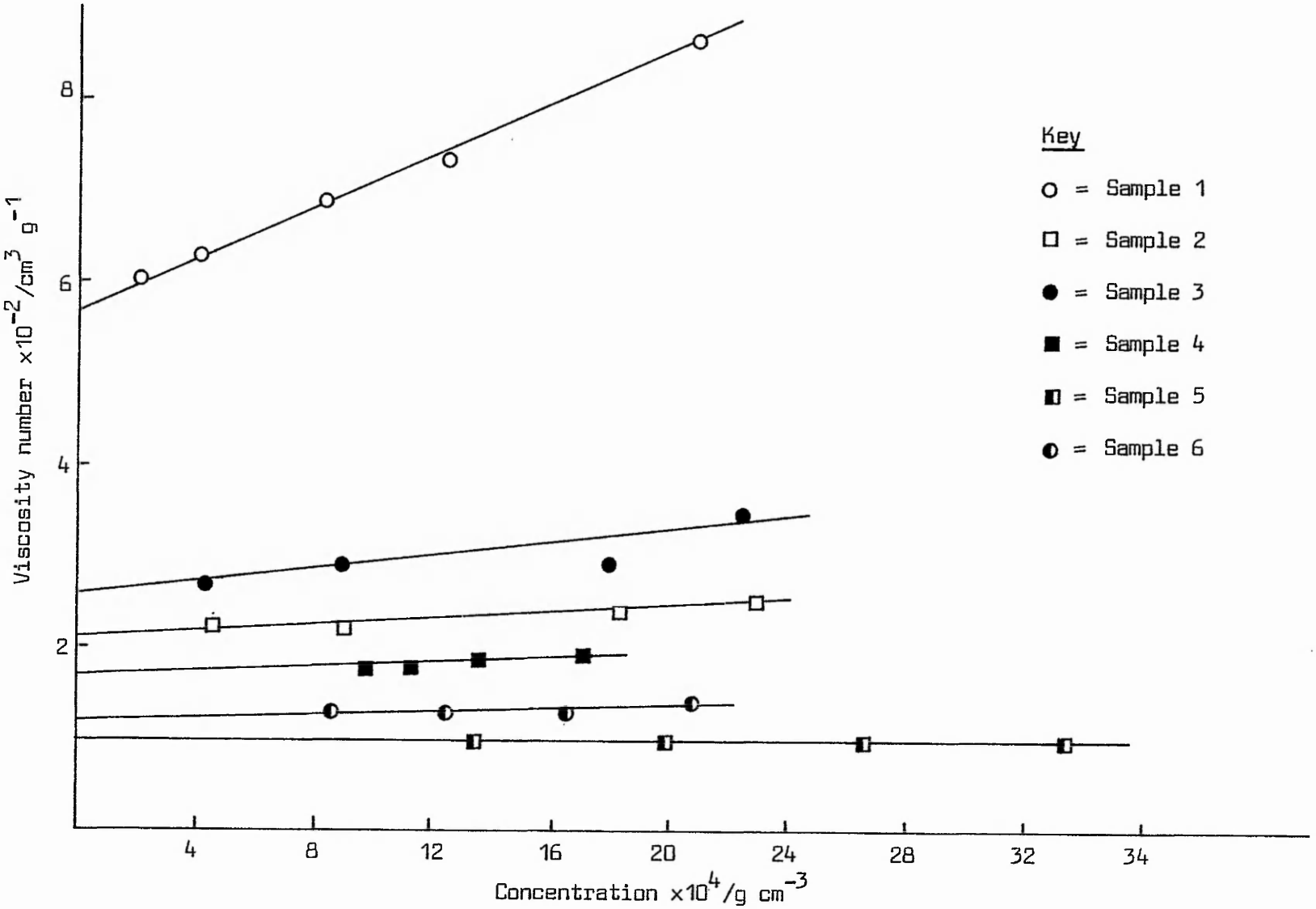


Figure 12 Viscosity number versus Concentration for degraded chitosan phenylosazone samples 1 to 6 in 0.1M acetic acid - 0.2M sodium chloride.

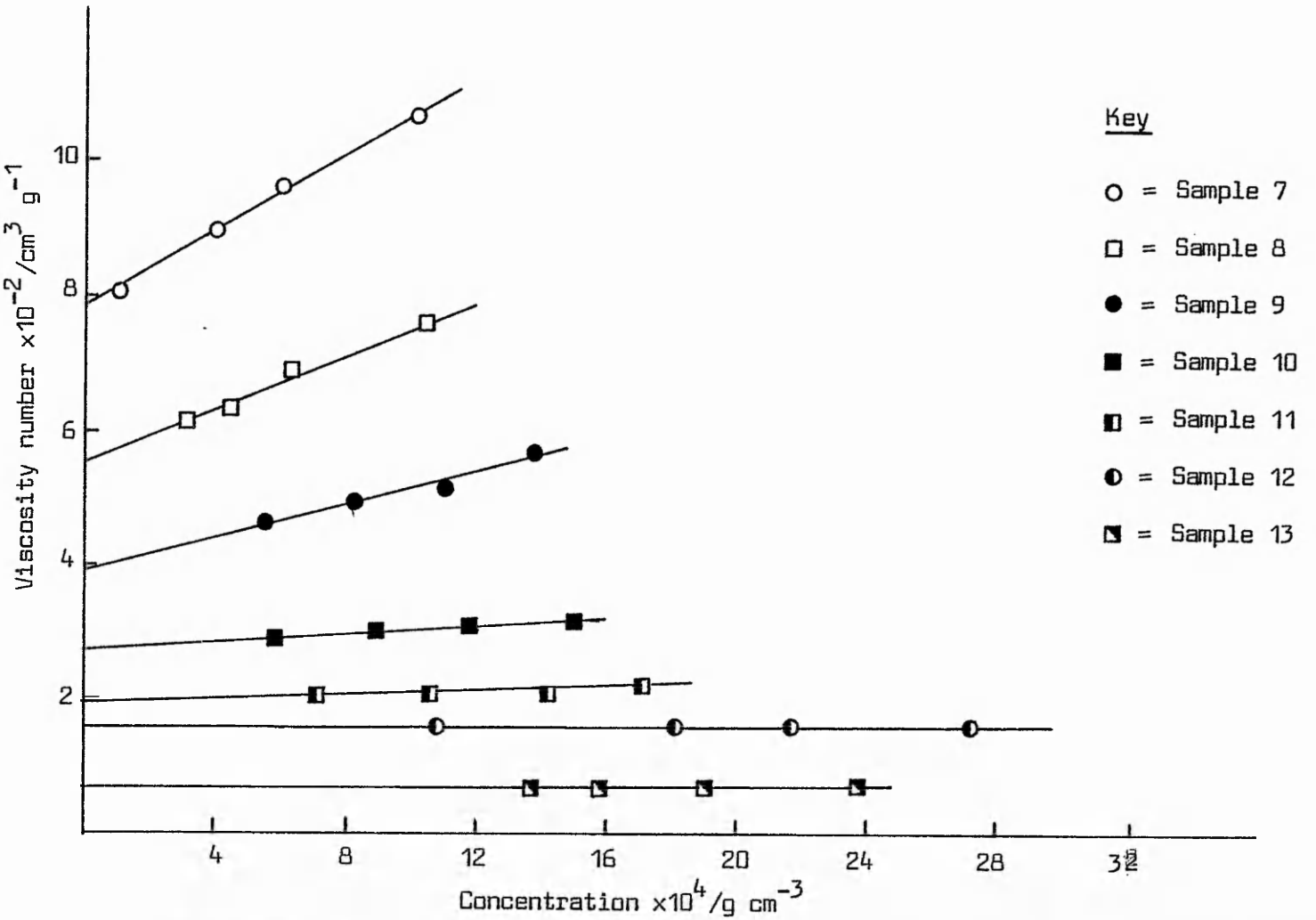


Figure 13 Viscosity number versus Concentration for degraded chitosan phenylosazone samples 7 to 13 in 0.1M acetic acid - 0.2M sodium chloride.

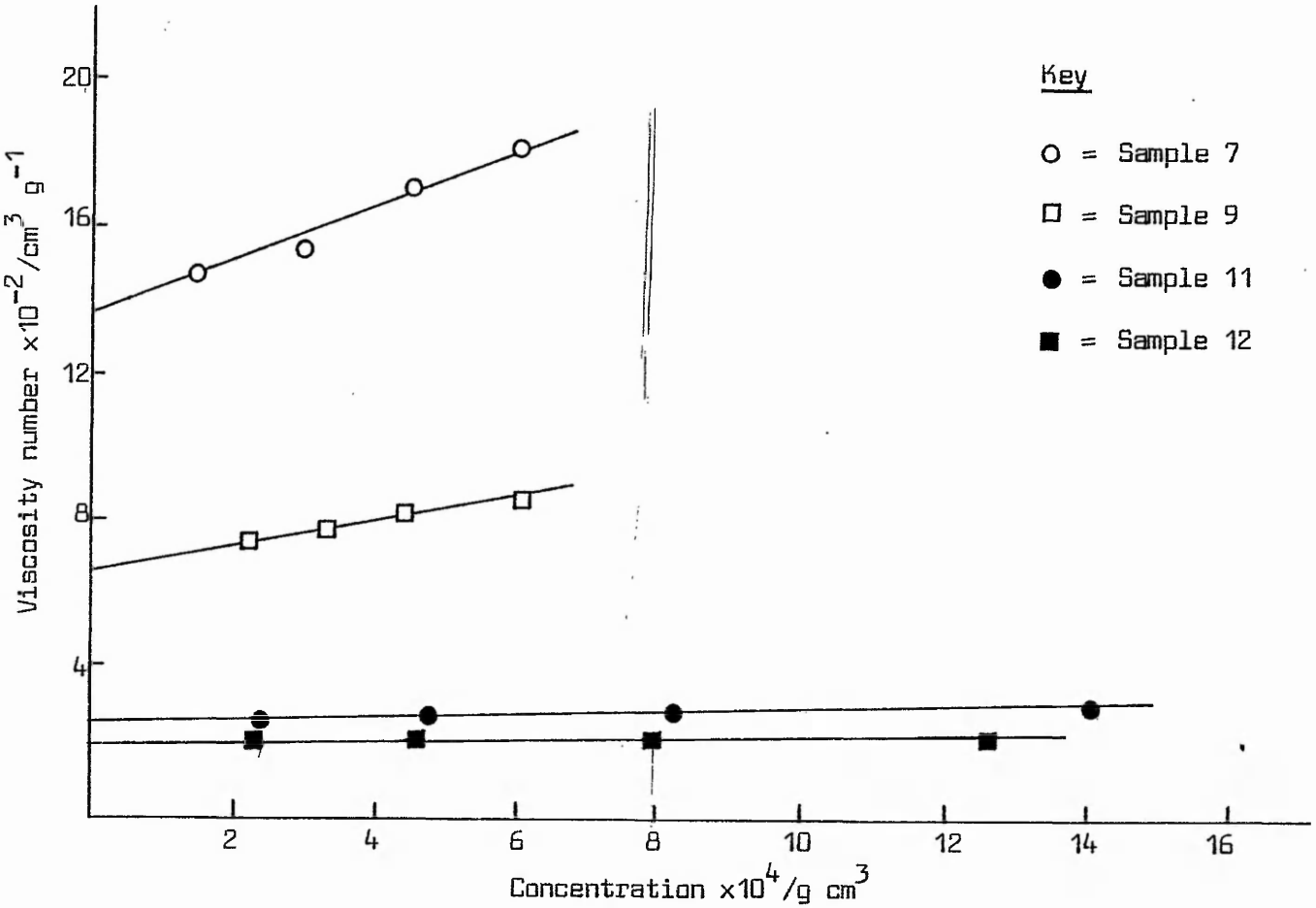


Figure 14 Viscosity number versus Concentration for degraded chitosan phenylosazone samples 7, 9, 11 and 12 in 0.1M acetic acid - 0.02M sodium chloride.

Table 4

Limiting Viscosity Number (LVN) data for chitosan phenylosazones (series 1) in 0.1M acetic acid/0.2M sodium chloride.

Sample number	LVN
1	570.4
2	215.8
3	262.9
4	170.7
5	102.1
6	119.4

Table 5

LVN data for chitosan phenylosazones (series 2) in 0.1M acetic acid/ 0.2M sodium chloride.

Sample number	LVN
7	780
8	549
9	389
10	272
11	198
12	156
13	75

Table 6

LVN data for chitosan phenylosazones (series 2) in 0.1M acetic acid/ 0.02M sodium chloride.

Sample number	LVN
7	1360
9	653
11	248
13	182

3.1.3.3 Treatment of data

It is possible to use the data obtained, namely $\bar{M}n$ and the corresponding LVN's to obtain the viscometric constants for chitosan using the modified Staudinger equation (6). However this would provide constants that could only be applied accurately to chitosan samples with the same molecular weight distributions as the degraded samples used in the evaluation of the LVN's since the samples are not narrow fractions and cannot be considered to be monodisperse. Attempts to fractionate chitosan have been tried[81] using the method of Doczi[82] but proved unsuccessful in that only polydisperse products were obtained.

However an alternative approach to the molecular weight/viscosity relationship has been introduced by Charlesby[83]. Use is made of the properties of distributions which are likely to be encountered in practice rather than for theoretical monodisperse systems. One example is that resulting from the random degradation of linear high polymers. Modification of the method of Charlseby by Sharples and Major[84] enables both $\underline{K}$ and $\underline{a}$ to be evaluated.

Equation (8) was derived[85] relating the viscosity average degree of polymerisation, $\bar{P}v$, with the number average degree of polymerisation, $\bar{P}n$, based on the probability, b, of bond breakage resulting from random degradation[86]

$$\bar{P}v = \bar{P}n[\Gamma\ (2+a)]^{1/a} \ldots\ldots\ldots\ldots\ldots (8)$$

where Γ is the Gamma function. The equation is valid providing that the viscometric constant $\underline{a} \geqslant 0$ and $1/b = \bar{P}n >> 1$.

The original modified Staudinger equation (6) can be expressed

in terms of the degree of polymerisation.

$$\bar{P}v = K^1[\eta]^{1/a} \quad \text{.....(9)}$$

Combination of equations (8) and (9) leads to the expression of Sharples and Major

$$\log \bar{P}n = (1/a) \log[\eta] + \log K^1 - (1/a) \log[\Gamma(2+a)] \quad \text{....(10)}$$

We have modified equations (8) and (9) to take into account molecular weight rather than degree of polymerisation, and on combination they lead to

$$\log \bar{M}n = (1/a) \log[\eta] + \log K^{11} - (1/a) \log[\Gamma(2+a)] \quad \text{...(11)}$$

Hence a plot of Log $\bar{M}n$ versus Log $[\eta]$ should give a straight line of slope 1/a and intercept equal to $\log K^{11} - (1/a) \log[\Gamma(2+a)]$. From the intercept and knowledge of a, K^{11} can be found and related to the viscometric constant K by equation (12).

$$K^{1/a} = 1/K^{11} \quad \text{.....(12)}$$

Acid hydrolysis of chitosan in solution under relatively mild conditions should give rise to random degradation leading to products having the required molecular weight distributions for use with equation (11). Thus equation (11) is applicable for use with the acid degraded chitosan phenylosazones.

For the first series of chitosan phenylosazones (Table 7), a plot of Log $\bar{M}n$ versus Log$[\eta]$ did not give a straight line, (Figure 15), with the points showing a considerable degree of scatter. This was attibuted to the fact that the original starting material possessed non-reducing end groups, as a consequence of the alkaline treatment in its production, which are incapable of phenylosazone formation.

Table 7

Limiting Viscosity Number (LVN) and molecular weight data for chitosan phenylosazones (series 1).

Sample number	Solvent	LVN/cm^{-} g^{-1}	Log LVN	$\bar{M}n$/g	Log $\bar{M}n$
1	0.1M acetic	570.4	2.75618	388954	5.58990
2	acid/0.2M NaCl	215.8	2.33405	336439	5.52691
3	"	262.9	2.40979	295971	5.47125
4	"	170.7	2.23223	256423	5.40896
5	"	102.1	2.00902	130926	5.11706
6	"	119.4	2.07700	101376	5.00594

Table 8

Limiting Viscosity Number (LVN) and molecular weight data for chitosan phenylosazones (series 2).

Sample number	Solvent	LVN/cm^{3} g^{-1}	Log LVN	$\bar{M}n$/g	Log $\bar{M}n$
7	0.1M acetic	780	2.89709	630062	5.79938
8	acid/0.2M NaCl	549	2.73597	450771	5.65396
9	"	389	2.58995	241580	5.38306
10	"	272	2.43457	180253	5.25588
11	"	198	2.29667	156619	5.19484
12	"	156	2.19313	96585	4.98491
13	"	74	1.86923	47820	4.67961
7	0.1M acetic	1360	3.13354	630062	5.79938
9	acid/0.02M NaCl	652	2.81458	241580	5.38306
11	"	248	2.39410	156619	5.19484
12	"	182	2.26007	96585	4.98491

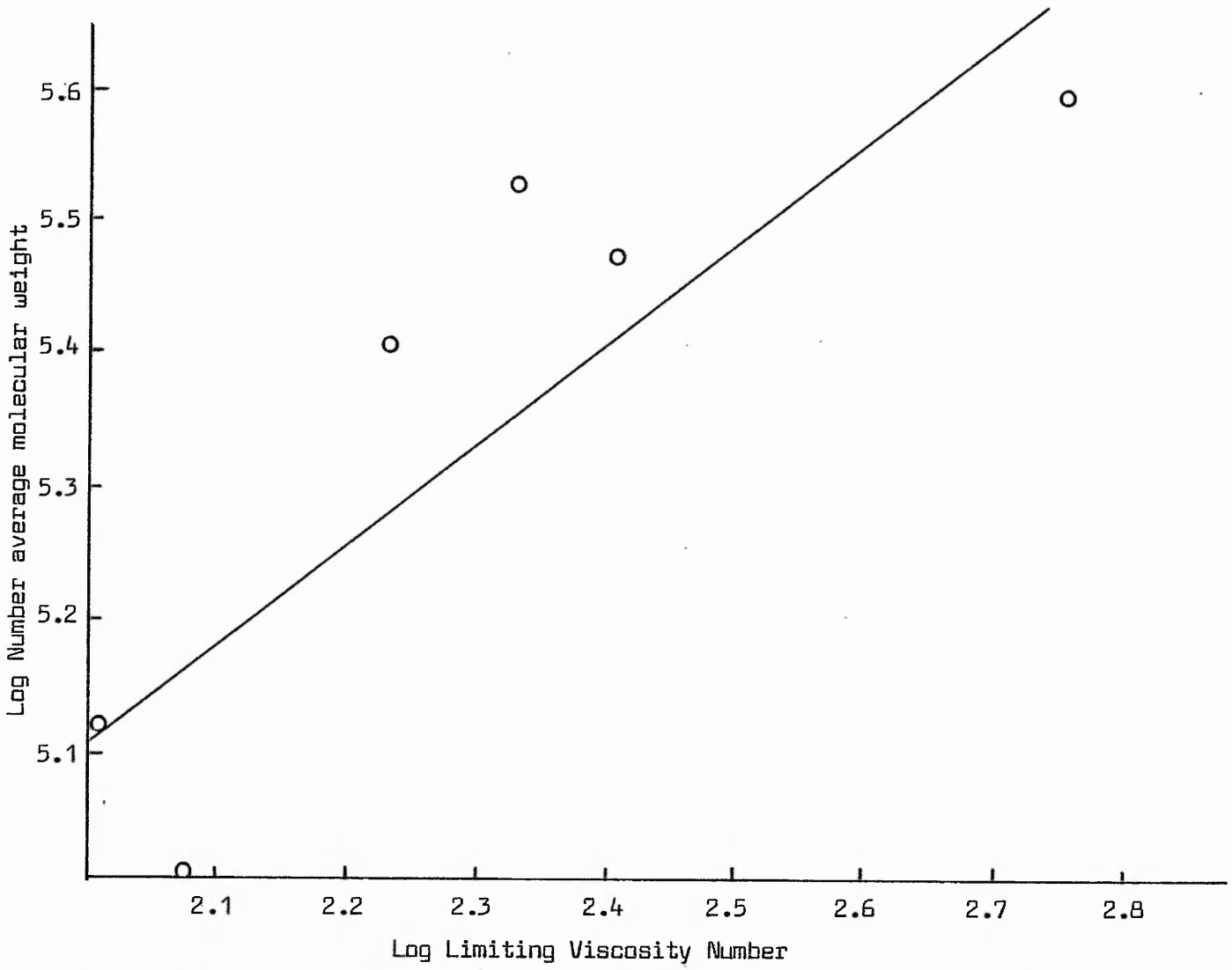


Figure 15 Plot of Log $\bar{M}n$ versus Log LVN expressed in terms of chitosan concentration of g cm^{-3} for degraded chitosan phenylosazone samples 1 to 6 in 0.1M acetic acid/0.2M sodium chloride.

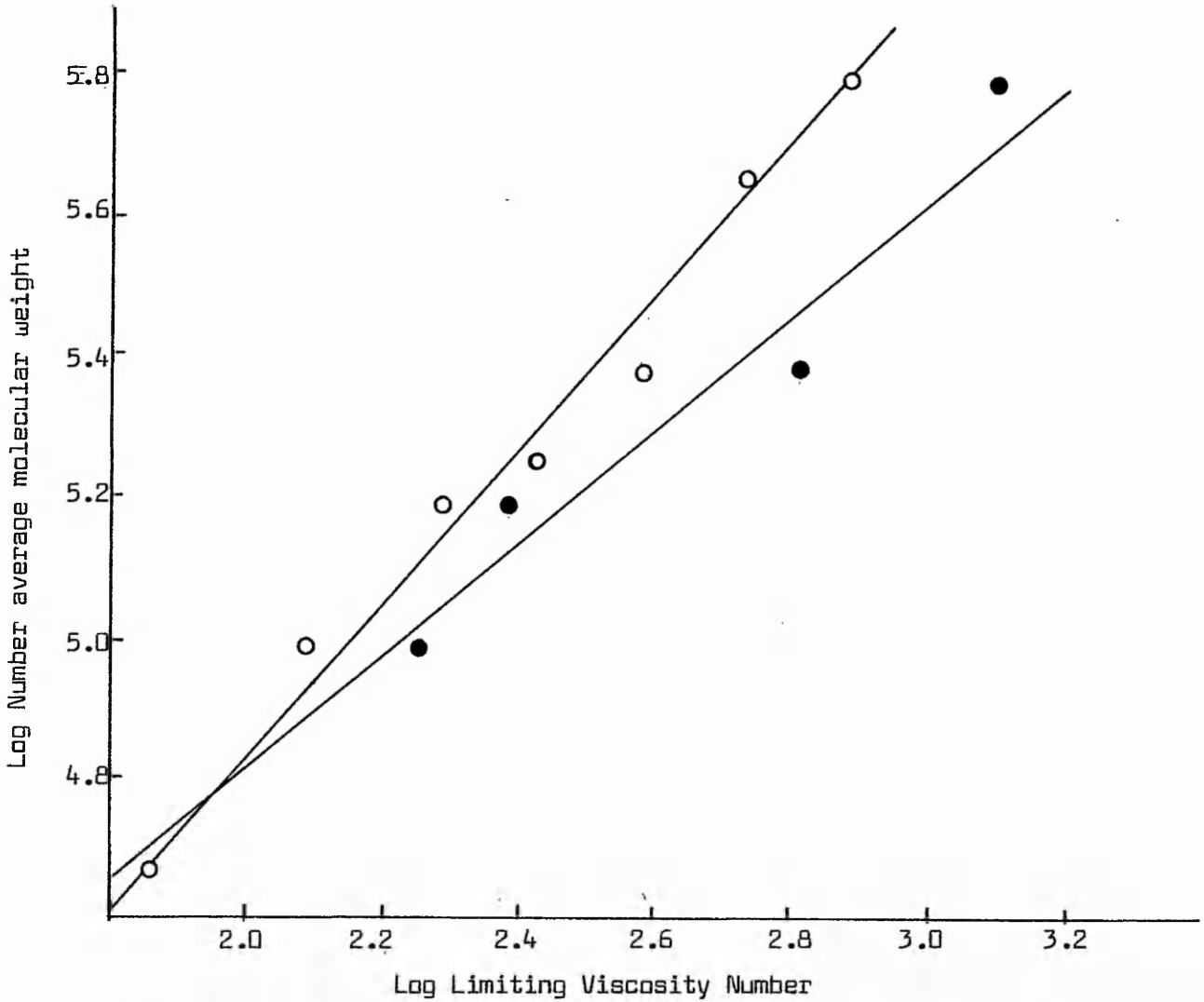


Figure 16 Plot of Log $\bar{M}n$ versus Log LVN for degraded chitosan phenylosazone samples 7 to 13 in 0.1M acetic acid/0.2M sodium chloride and samples 7, 9, 11 and 12 in 0.1M acetic acid/0.02M sodium chloride.

This will lead to erroneous values for the number average molecular weight. However for the second series (Table 8) a good linear relationship between log $\bar{M}n$ and log[η] (Figure 16) was observed, as one would expect due to the sodium borohydride reduction of the non-reducing end groups into a form, suitable for reaction with phenylhydrazine. Thus for the second series all of the end groups, both original and new ones produced during degradation, are able to form a phenylosazone derivative, resulting in a more reliable value for the number average molecular weight. Regression analysis of the plot afforded a slope of 1.075 and an intercept of 2.655. Thus:

$$1/a = 1.075 \text{ and } a = 0.93$$

Using this value of $\underline{a}$ the Gamma function, Γ, of $(2+\underline{a})$ was found by a graphical method. This was achieved through use of the following relationship:

$$\Gamma(x) = (x-1)! \quad \ldots\ldots\ldots\ldots\ldots\ldots (13)$$

From this a plot of the Γ values for the interger points can be constructed and intermediate non-interger values can be read off. Thus the intercept value of 2.655 leads to a value for K^{11} of 888 and through equation (12) to a value for K of 1.81×10^{-3} cm^3 g^{-1}. These values were for chitosan in the 0.1M acetic acid - 0.2M sodium chloride solvent. The Limiting Viscosity Number data (Table 8), determined in 0.1M acetic acid - 0.02M sodium chloride were treated in a similar way (Figure 16) to give a slope of 0.7925 and an intercept of 3.262, leading to an $\underline{a}$ of 1.26 and $\underline{K}$ of 3.04×10^{-5} cm^3 g^{-1}.

The values obtained for the constants differ considerably from those reported by Lee[26] however they are far closer to the values

obtained for other related β-(1→4)- linked ionic polysaccharides[66,67] under salt concentrations of a similar order (Table 9). The effect on the viscometric constants of reduction in the salt concentration follows a similar trend for chitosan and sodium carboxymethylcellulose[66], in that the value of a increases whilst that of K decreases. The increase in the value of a is as expected from the theories relating to the expansion of the polyelectrolyte polymer coil. The constant a is a measure of the size of this coil and increases the more expanded the coil becomes. As the salt concentration is reduced chain expansion (see Section 3.1.3.1) increases and thus a will also increase.

3.1.4 Arylhydrazine derivatives of chitosan

3.1.4.1 Introduction

In theory it should be possible to use different absorbing end groups on chitosan to obtain its number average molecular weight spectroscopically. Because of this other arylhydrazine derivatives as well as phenylosazones were investigated.

3.1.4.2 Phenylhydrazone and phenylosazone derivatives

The phenylosazone of chitosan has been readily produced (see Section 3.1.2.3) by homogeneous reaction of chitosan, in aqueous acetic acid, with phenylhydrazine. However it is difficult to stop the reaction at the initial phenylhydrazone stage in such an homogeneous system, because of the presence of acid. Thus the phenylhydrazone was produced by heterogeneous reaction at room temperature between a chitosan film, and a solution of phenylhydrazine in methanol. Uv/visible spectroscopic analysis of the colourless product in both

Table 9

Comparison of the new viscometric constants for chitosan with other β-(1→4)- linked polysaccharides, showing the effect of salt concentration.

Polymer	Salt concentration	$K/cm^3\ g^{-1}$	a
Chitosan acetate	0.2	1.81×10^{-3}	0.93
Chitosan acetate	0.02	3.04×10^{-5}	1.26
Sodium carboxy-methyl cellulose	0.1	1.23×10^{-4}	0.91
Sodium carboxy-methyl cellulose	0.01	6.46×10^{-6}	1.2
Sodium cellulose sulphate	0.5	7.91×10^{-2}	0.93

film form and dissolved in methanol/0.1M HBr (7/3 v/v) revealed a band at 280 nm (Figure 17). This maximum is in agreement with the uv/visible spectra of phenylhydrazone derivatives of low molecular weight sugar compounds[87]. The methanol/aqueous hydrobromic acid solvent system was used for uv/visible analysis of the chitosan derivatives in order to obtain a comparison with the spectra of the low molecular weight analogues, which were recorded on solutions in methanol.

3.1.4.3 p-Nitrophenylhydrazine derivatives

The p-nitrophenylosazone of chitosan was produced in a similar way to the phenylosazone, by homogeneous reaction with p-nitrophenylhydrazine in the presence of acetic acid, and precipitation of the product by the addition of alkali. p-Nitrophenylhydrazine is less soluble than phenylhdyrazine and requires to be dissolved in a relatively large amount of methanol before addition to the chitosan solution. Analysis of the uv/visible spectrum (Figure 18) of the deep red product in the methanol/0.1M HBr solvent revealed a small shoulder in the region of 430 nm and a maxima at 385 nm. These two bands are in reasonable agreement with the uv/visible characteristics of D-glucose-p-nitrophenylosazone[88], although the fact that there is only a shoulder rather than a well defined peak at 430 nm suggests the product exists as a mixture of the p-nitrophenylhydrazone as well as the p-nitrophenylosazone.

The p-nitrophenylhydrazone of chitosan was produced in a similar way to the phenylhydrazone by heterogeneous reaction on chitosan film. The uv/visible spectrum of the yellow product revealed a maximum at 395 nm in film form and at 385 nm in the mixed methanol/

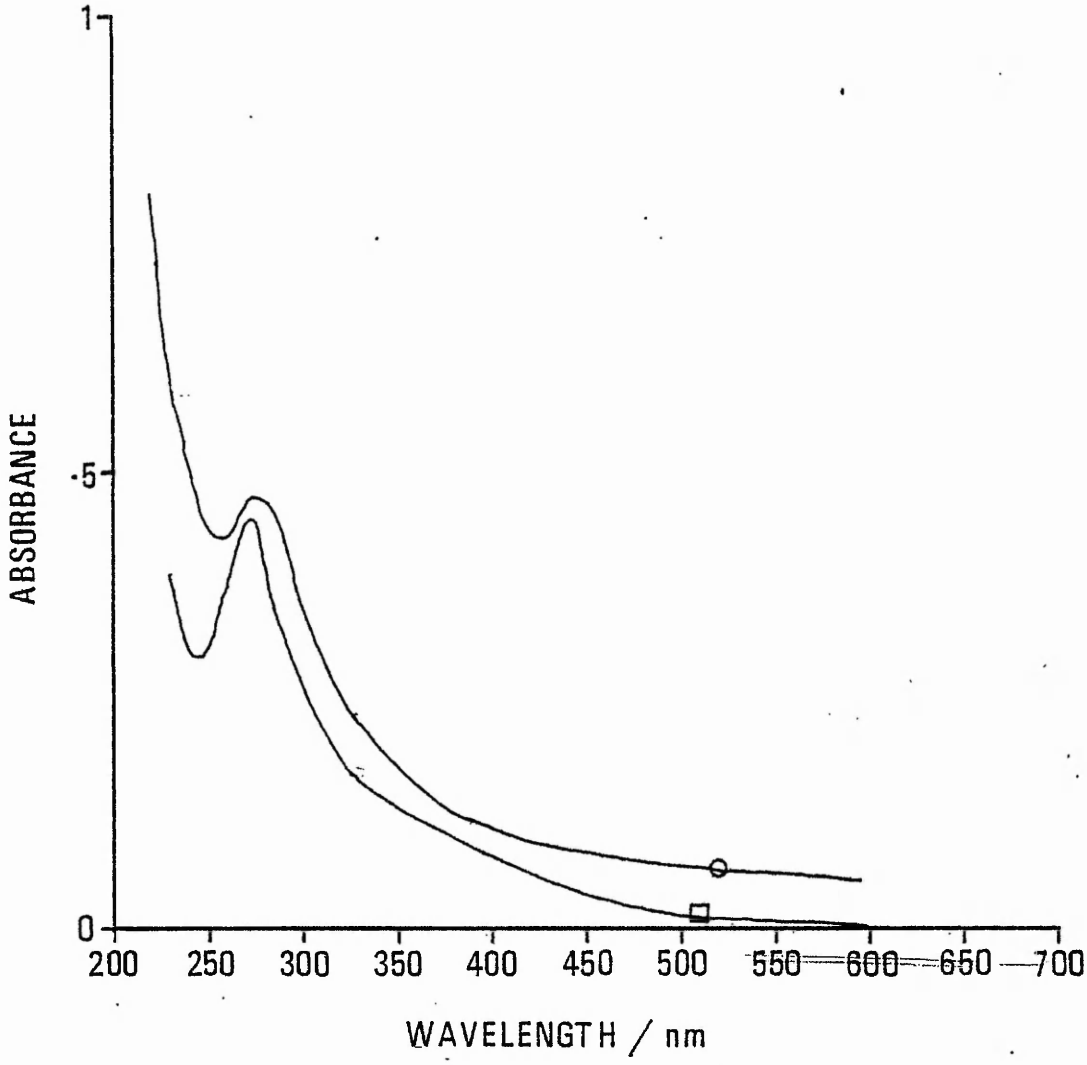


Figure 17

Uv/visible spectrum of chitosan phenylhydrazone in film form (O) and in 7/3 (v/v) methanol/1% HBr (□).

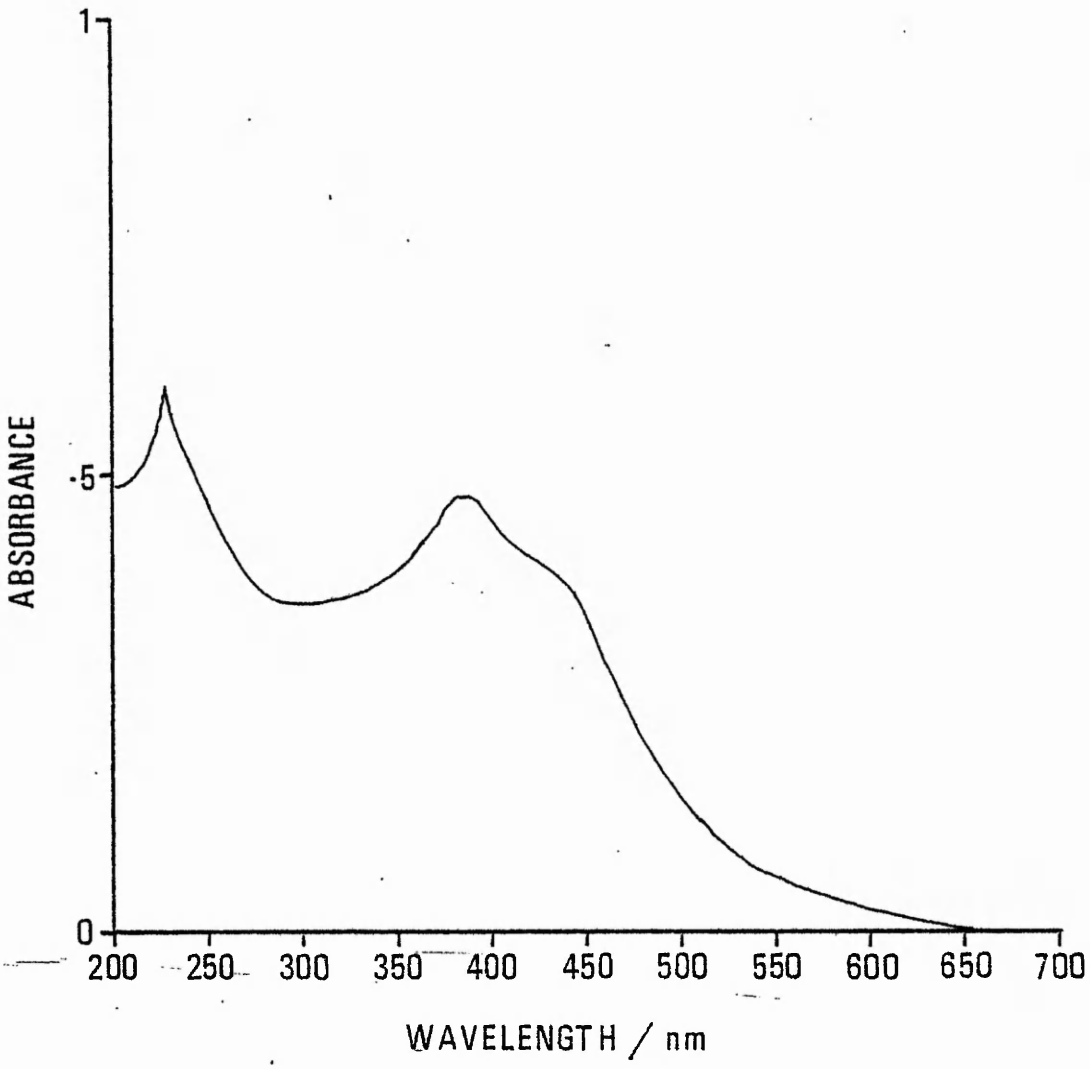


Figure 18

Uv/visible spectrum of chitosan p-nitrophenylosazone in 70/30 (v/v) methanol/1% HBr.

0.1M HBr solvent (Figure 19). These again were in good agreement with the uv/visible spectrum of D-glucose-p-nitrophenylhydrazone[89]. The shift in the spectrum of 10 nm from film form to solution form, must be a consequence of the interaction of the polar solvent with the nitro group since no shift is observed in the absence of the nitro group i.e. in the phenylhydrazone derivative.

The reluctance of chitosan to undergo complete p-nitrophenylosazone formation in the homogeneous system is also manifested in the heterogeneous reaction. The extension of the reaction between chitosan film and phenylhydrazine in methanol eventually results in the formation of some phenylosazone as indicated visually by the change in colour of the film to yellow and spectroscopically by the gradual appearance of the characteristic phenylosazone band at 395 nm. However for the corresponding reaction with p-nitrophenylhydrazine no osazone formation is observed even after considerable times of reaction (up to a week). It is difficult as yet to explain the effect of the nitro group on the reaction because the mechanism of osazone formation has not been completely established. However the considerably lower solubility of the p-nitrophenylhydrazine reagent compared to phenylhydrazine may have some bearing on the relative extent of the two reactions.

3.1.4.4 Structural investigations of arylhydrazine derivatives of chitosan

One of the important properties of arylhydrazones and arylosazones is their ability under certain conditions to undergo formazan formation. This reaction has been extensively used in the investigation of the structural arrangements of arylosazones and arylhydrazones[90].

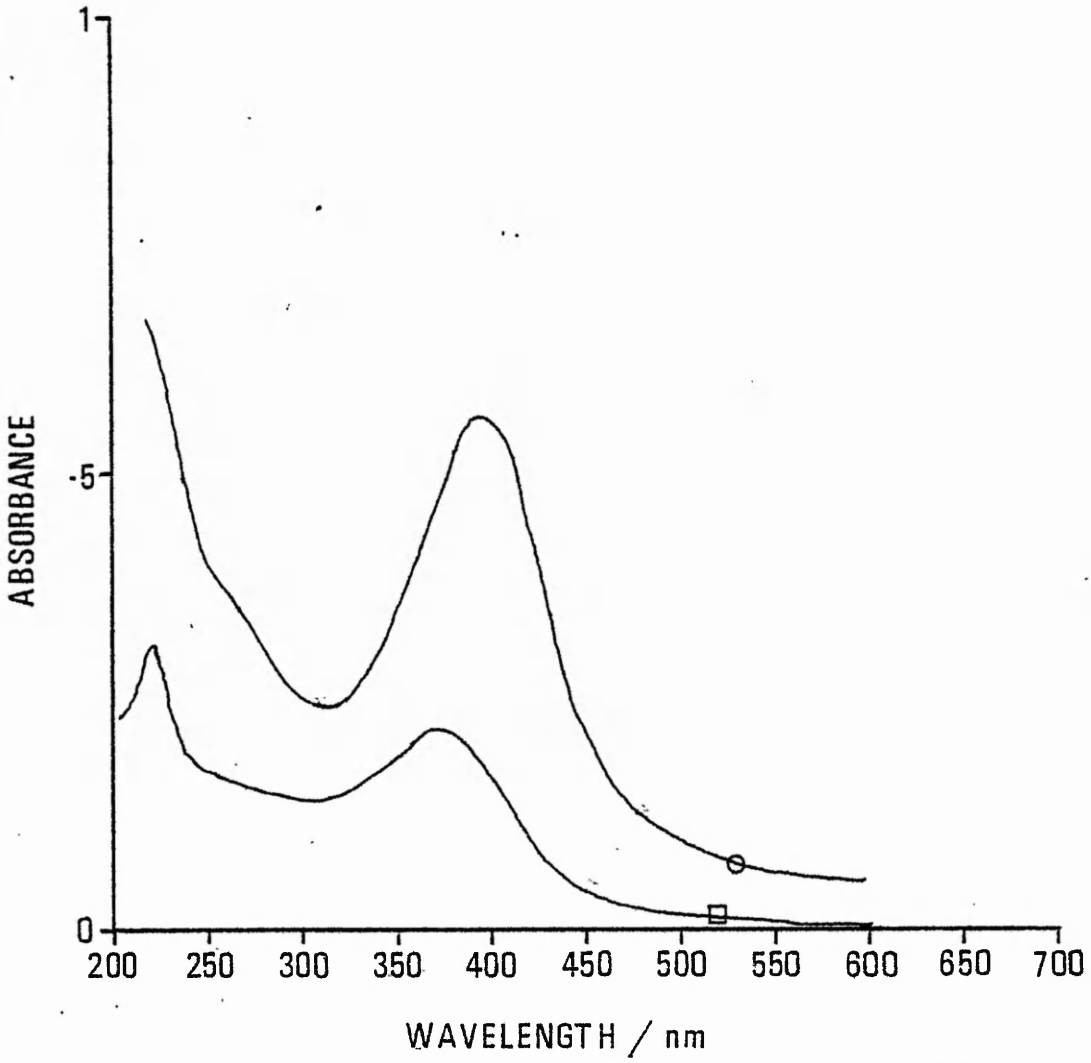
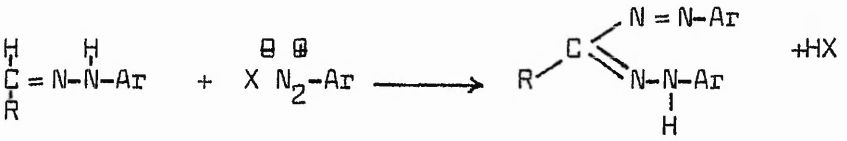


Figure 19

Uv/visible spectrum of chitosan p-nitrophenylhydrazone in film form (○) and in 7/3 (v/v) methanol/1% HBr (□).

Formazan formation occurs by reaction between the hydrazone group and a diazotised arylamine:



aryl hydrazone diazonium salt formazan

For the reaction to proceed two structural features are needed;

i) a Schiff's base structure, -CH = N-;

ii) a free imino hydrogen on the arylhydrazone group.

Both chitosan phenylhydrazone and chitosan p-nitrophenylhydrazone satisfy these requirements and should undergo the formazan reaction. The reaction was carried out using diazotised aniline and the aryl-hydrazones of chitosan in film form. The solvent medium used was methanol/pyridine, 80/20, and the reaction mixture was kept at $0^{o}C$ for 24 hours, the length of time used reflecting the heterogeneous conditions involved. For the chitosan phenylhydrazone formazan uv/visible analyses were carried out in film form and in the mixed methanol/aqueous hydrobromic acid solvent. The spectrum of the product in film form showed a small shoulder at 420 nm and a large band at 280 nm. This main band was similar to the one in the starting material, except its intensity was much greater (Figure 20). In the mixed solvent the main band occured at 265 nm with a shoulder at 420 nm (Figure 21) which is similar to the spectrum of D-glucose-diphenylformazan[91], indicating that the formazan reaction has taken place. The spectroscopic evidence for the production of the chitosan p-nitrophenylhydrazone formazan was much clearer due to the more pronounced shifts in the absorption bands, between starting material and product.The uv/visible spectrum in the mixed solvent showed a

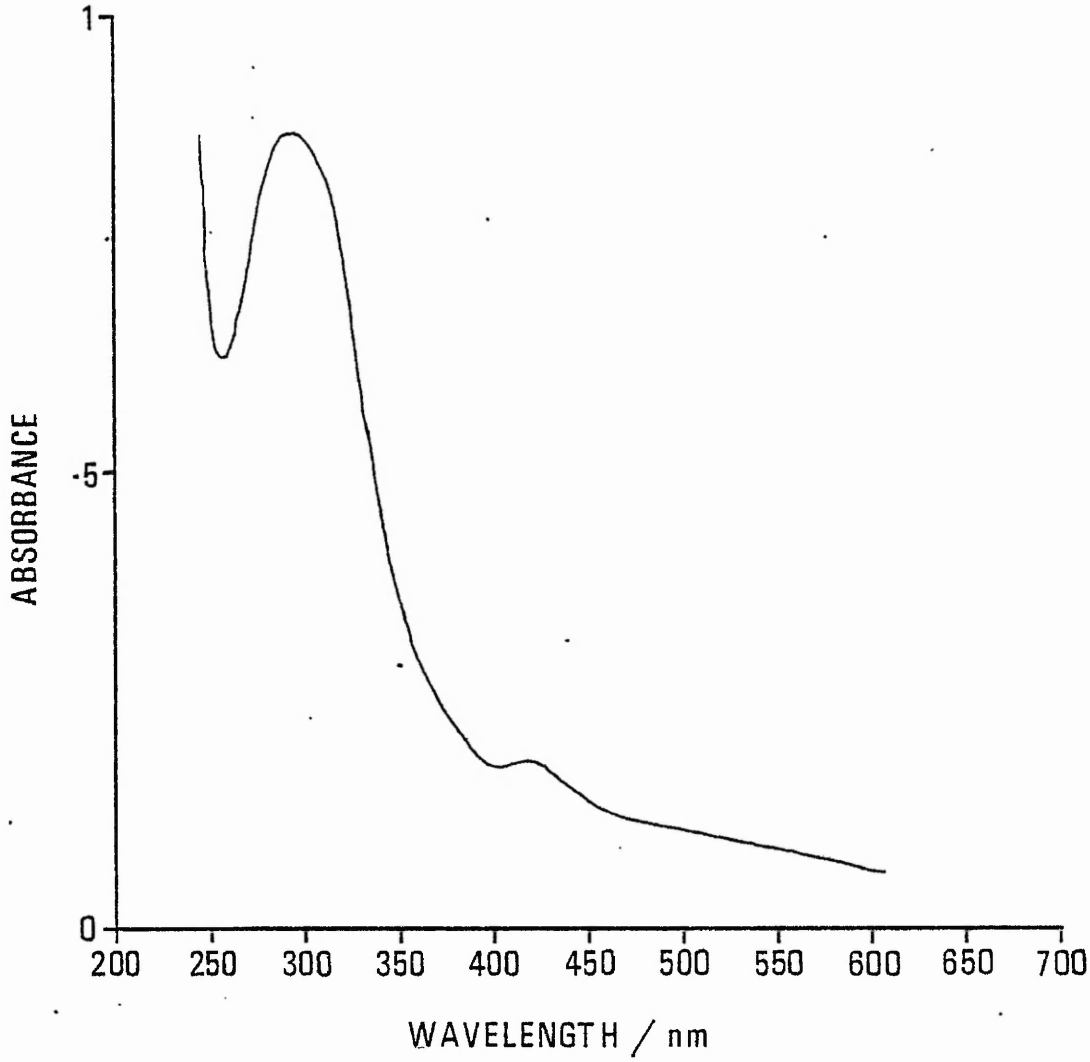


Figure 20

Formazan of chitosan phenylhydrazone in film form.

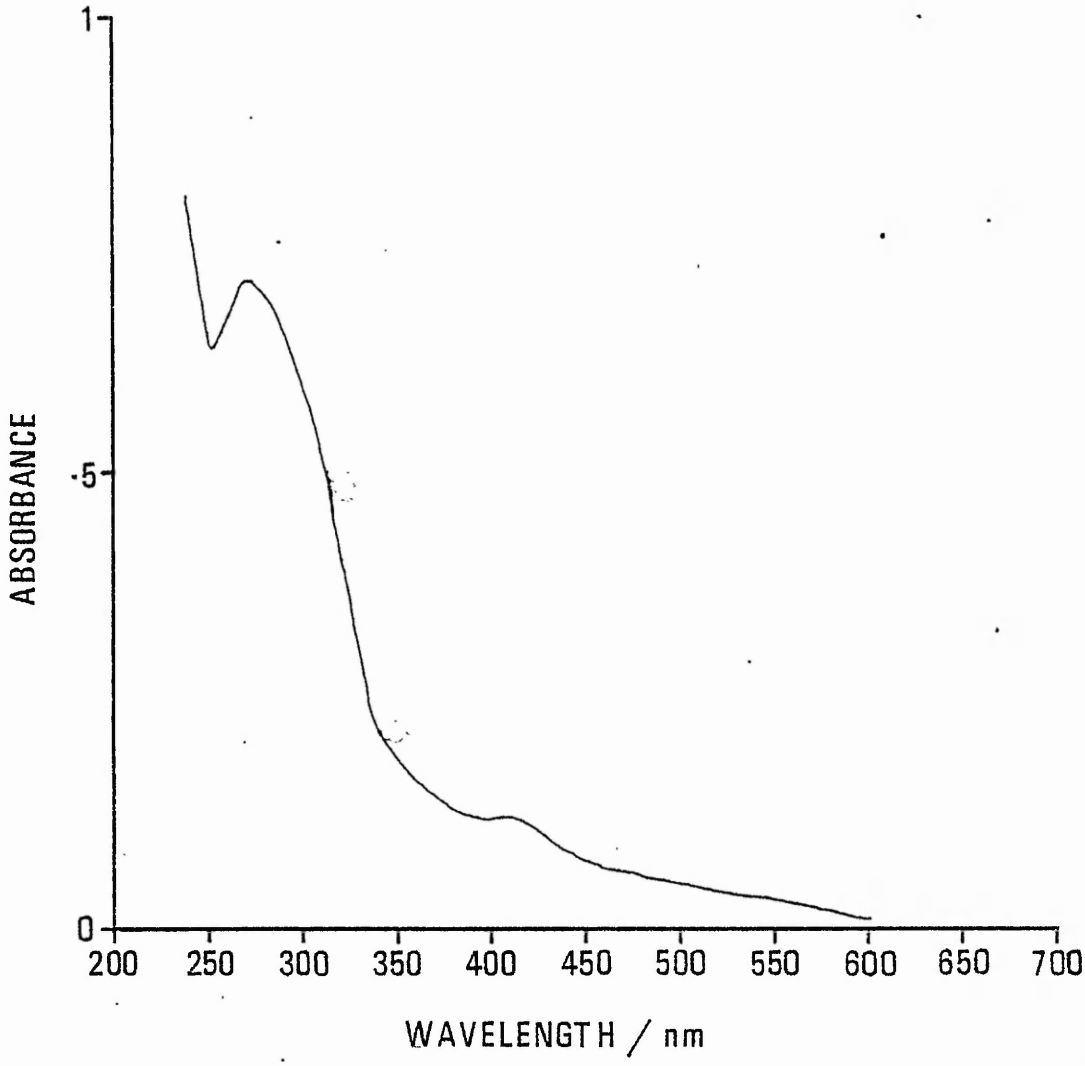
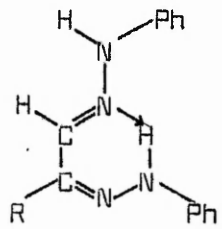


Figure 21

Formazan of chitosan phenylhydrazone in 7/3 (v/v) methanol/1% HBr.

shift for the main band at 385 nm in the starting material to one at 265 nm for the formazan (Figure 22). A second smaller shoulder was observed at about 400 nm. Both these peaks are in reasonable agreement with the uv/visible spectrum of D-glucose-p-nitrophenyl-hydrazone formazan, produced by the reaction between diazotised aniline and D-glucose-p-nitrophenylhydrazone.

The application of the formazan reaction to arylosazone derivatives is of great significance. Mester[90] has shown that D-glucosephenyl-osazone will only undergo the formazan reaction in strongly alkaline media. He concluded that the reaction does not proceed in non-alkaline systems because one of the requirements for formazan formation, namely the presence of an imino-hydrogen is not satisfied. He explained this in terms of the involvement of this hydrogen in the formation of an internal chelate structure (V) via hydrogen bonding.



(V)

Strongly alkaline conditions will rupture the chelate ring and thus allow the formazan reaction to take place.

The heterogeneous reaction of chitosan phenylosazone with diazotised aniline in methanol/pyridine showed a similar trend in that no formazan formation was observed, as monitored by uv/visible spectroscopy. This gives support to a chelate structure being present in

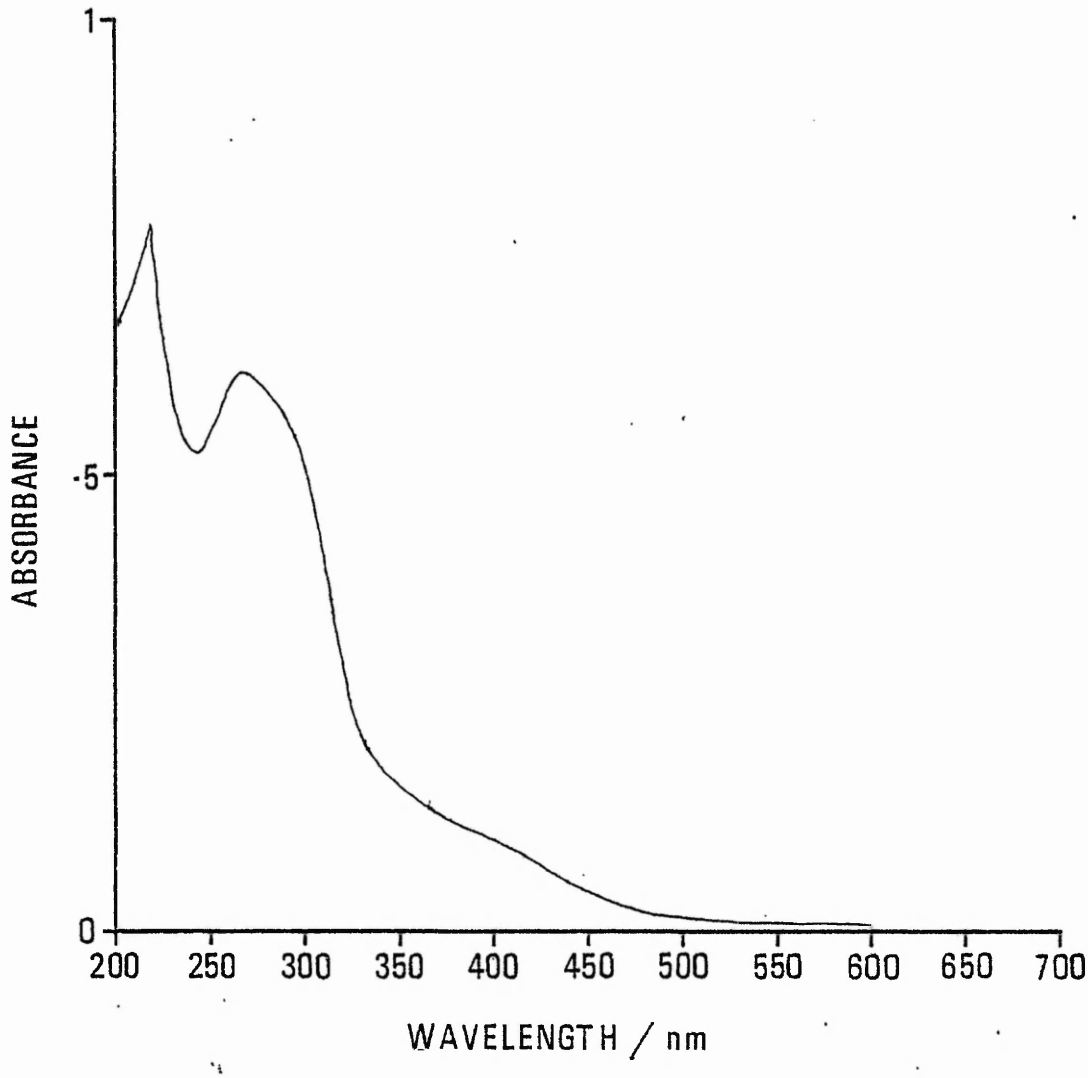
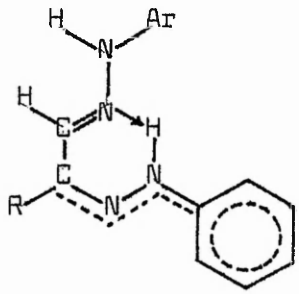


Figure 22

Formazan of chitosan p-nitrophenylhydrazone in 7/3 (v/v) methanol/1% HBr.

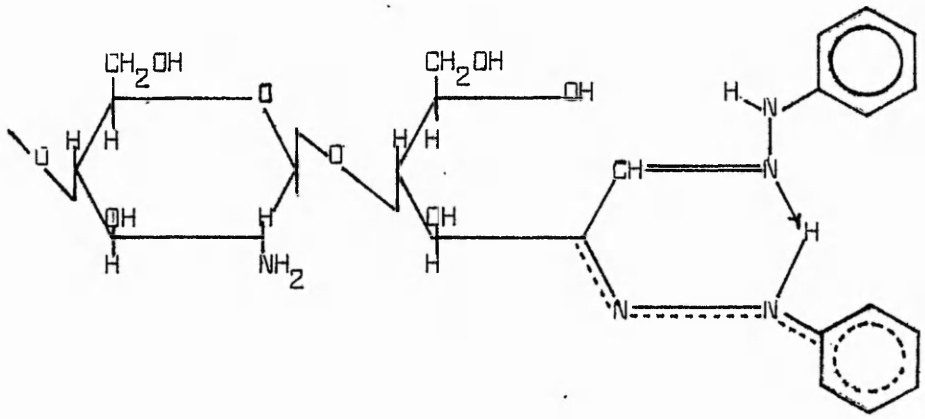
the chitosan phenylosazone. Unfortunately when the reaction was carried out under alkaline conditions, this and the length of the reaction time caused the gradual oxidative degradation of the phenylosazone system, to yield inconclusive spectral results.

The presence of an internal chelate ring in the structure of chitosan phenylosazone is in agreement with its uv/visible characteristics according to the interpretation of Roberts[88]. He represented the structure of arylosazones as (VI)



(VI)

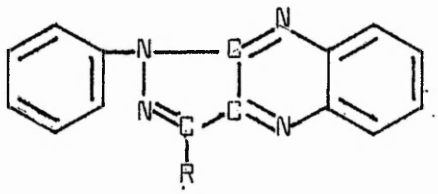
where the electrons of the C(2) arylhydrazone group are highly delocalised and form a mesomeric bond system. It is this delocalisation that causes the characteristic absorption band around 390 nm, whereas the C(1) arylhydrazone is not delocalised and gives the normal downfield absorption of an arylhydrazone group at about 280 nm. Thus the chitosan phenylosazone end group can be represented by (VII).



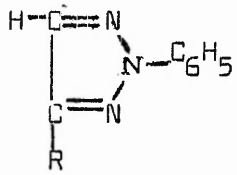
(VII)

3.1.4.5 Other end group derivatives

Two other end group derivatives of chitosan were investigated, the phenylflavazole (VIII) and the phenylosotriazole (IX).



(VIII)



(IX)

The phenylflavazole derivatives of hydrocellulose has been prepared[72] and its uv/visible spectrum investigated. They are generally prepared by the reaction of the reducing sugar end group with o-phenylenediamine and phenylhydrazine in the presence of acetic acid. The reaction was attempted for chitosan both heterogeneously and homogeneously, however analysis of the product gave an absorption

spectrum consistant only with phenylosazone formation.

Phenylosotriazoles can usually be prepared by the oxidation of phenylosazones with aqueous cupric sulphate[92], and have also been produced on hydrocellulose end groups[68]. Due to the chelating ability of chitosan with copper ions[93], the oxidising agent may be changed to bromine which has been used in the preparation of phenylosotriazoles. Chitosan phenylosazone in film form was reacted with a solution of bromine water, which caused the film to dissolve. Unfortunately reprecipitation of the product and spectroscopic analysis indicated the presence of only the starting material. Bromine absorption[18] by the chitosan may be responsible for the lack of reaction.

3.2 Determination of the free amine group content of chitosan

3.2.1 Introduction

Variations in the degree of N-acetylation of different chitosan samples is one of the consequences of the production of chitosan from chitin. Thus for accurate quantitative work it is essential for the chitosan sample to be characterised. Several techniques have been reported previously (see Section 2.2), but the work carried out and reported in this thesis involves the application of novel methods to the validation of the technique of Moore and Roberts[40] (see Section 2.2.3.1). This is one of the infrared methods and is of special interest because it requires no prior calibration and can be applied to any N-acyl derivative of chitosan. These unique characteristics allied with its simplicity and the relatively short time required, make it an ideal technique for the evaluation of the degree of N-acetylation. Unfortunately no successful comparative

study into its accuracy has been carried out until now. Several different and novel approaches to the determination of the free amine group content have been investigated for comparison with the values given by infrared spectroscopy, with varying degrees of success.

3.2.2 NMR studies

Previous NMR work[36] has been carried out using concentrated deuterated formic acid as a solvent, however it would be desirable to use a more established organic solvent for this purpose. Although chitosan is not soluble in such solvents, it is possible to prepare organosoluble derivatives of chitosan. Two such derivatives were prepared according to the method of Moore and Roberts[94] namely di-O-phenylcarbamate-N-phenylureidochitosan and di-O-phenylcarbamate-N-benzylidenechitosan. These are the phenylisocyanate derivatives of chitosan and N-benzylidene chitosan (a Schiff's base derivative of chitosan) respectively. It was intended to carry out NMR studies on these derivatives to determine the ratio of acetyl protons to other protons. Unfortunately the samples thus prepared were not soluble enough to give resolvable NMR spectra, using deuterated DMSO as a solvent. In order to try and improve the solubility the polymer was dissolved in THF and perchloric acid added to degrade the polymer chain. The resultant product, on reprecipitation, yielded little improvement in the NMR spectrum. This relative lack of solubility allied to the detrimental effect of the polymeric material on expensive NMR tubes severly limits the use of NMR in the proposed manner. It should however be possible to produce samples of increased solubility but the extent of polymer degradation involved would be so severe, resulting in a product bearing little relation to the original high molecular weight chitosan.

3.2.3 Use of 2,4-dinitrofluorobenzene as an amine specific reagent

3.2.3.1 Introduction

This method utilises the reactive properties of the free amine group of chitosan to yield a product capable of undergoing spectroscopic analysis. The reagent 2,4-dinitrofluorobenzene (DNFB) has previously been used for the estimation of amine groups of low molecular weight compounds[95]. The reagent is particularly reactive towards nucleophiles due to the electron withdrawing ability of the fluorine atom, and thus will readily undergo reaction with a nucleophilic amine group, to form a bond between the amino nitrogen and the C(1) carbon of the benzene ring. It has also been used with chitosan[96] to estimate the extent of enzymic deacetylation of chitin, by monitoring the production of free amine groups. This was achieved by reaction with DNFB, hydrolysis of the product to the monomer, chromatographic separation and then uv/visible spectroscopic analysis of the N-(2,4-dinitrophenyl)-D-glucosamine (DNP-D-glucosamine) formed. In the present study a method for direct spectroscopic determination was investigated.

3.2.3.2 Spectroscopic properties of DNFB- derivatives of amines

In order to apply spectroscopic analysis to the derivated chitosan the extinction coefficient of the R-NH DNP system must be known. Thus low molecular weight analogues were prepared using the chitosan precursor D-glucosamine and cyclohexylamine as examples of primary amines. DNFB will also react with secondary amines and thus the products of reaction with diethanolamine and morpholine were made to study their uv/visible characteristics. The results are summarised in Table 10. The extinction coefficient for DNP-D-glucos-

Table 10

Extinction coefficient data for various N-dinitrobenzene derivatives.

Sample	Weight/g	Volume of solvent/cm^3	Solvent	Dilution factor	λmax/nm	Absorbance	ε max
N-(2,4-DNP)-cyclohexylamine	0.023	250	methanol	5	349	1.2	16,370
N-(2,4-DNP)-cyclohexylamine	0.016	250	methanol	10	349	0.39	16,353
N-(2,4-DNP)-morpholine	0.0132	250	acetone/ H_2O 4/6	5	380	0.56	13,417
N-(2,4-DNP)-diethanolamine	0.0202	250	acetone/ H_2O 0.5/ 9.5	5	390	0.81	13,684
N-(2,4-DNP)-D-glucosamine	0.0105	500	1% HBr	1	365	1.0	
"	"	"	"	2	"	0.5	
"	"	"	"	3	"	0.33	
"	"	"	"	4	"	0.25	
"	0.0142	"	"	1	"	1.36	16,500
"	"	"	"	2	"	0.68	
"	"	"	"	3	"	0.453	
"	"	"	"	4	"	0.335	

amine was found graphically to be 16,500 (Figure 23) from the data in Table 10. Although the position of λ max varies according to the solvent, the products from the primary amines generally have λ max at lower wavelengths than do those from secondary amines. The values for the extinction coefficient seem to be unaffected by the nature of the solvent, but are dependant on the type of amine from which the product is derived e.g. those from secondary amines have values around 13,000 whilst those from primary amines have extinction coefficients around 16,500.

This value of 16,500 found for the model compound DNP-D- glucosamine was used for applications with the related chitosan systems.

3.2.3.3 Studies on the reaction between DNFB and chitosan

The reaction between chitosan and DNFB was carried out both heterogeneously, on chitosan in film form, and homogeneously in ~~methanol/aqueous~~ acetic acid solutions.

The heterogeneous reaction was carried out in methanolic solution containing an excess of reagent, and followed by monitoring the infrared spectra of the films at various time intervals. The gradual change of colour of the film to yellow, along with the appearance of characteristic aromatic and nitro bands in the infrared spectrum indicated that reaction was occuring. The reaction appeared to have stopped after 72 hours, as shown by the stability of the infrared absorption bands for reaction times in excess of 72 hours. However the reaction had not gone to completion as indicated by the ability of the partially reacted chitosan film to undergo N-acetylation with acetic anhydride in methanol. The N-acetylation was observed

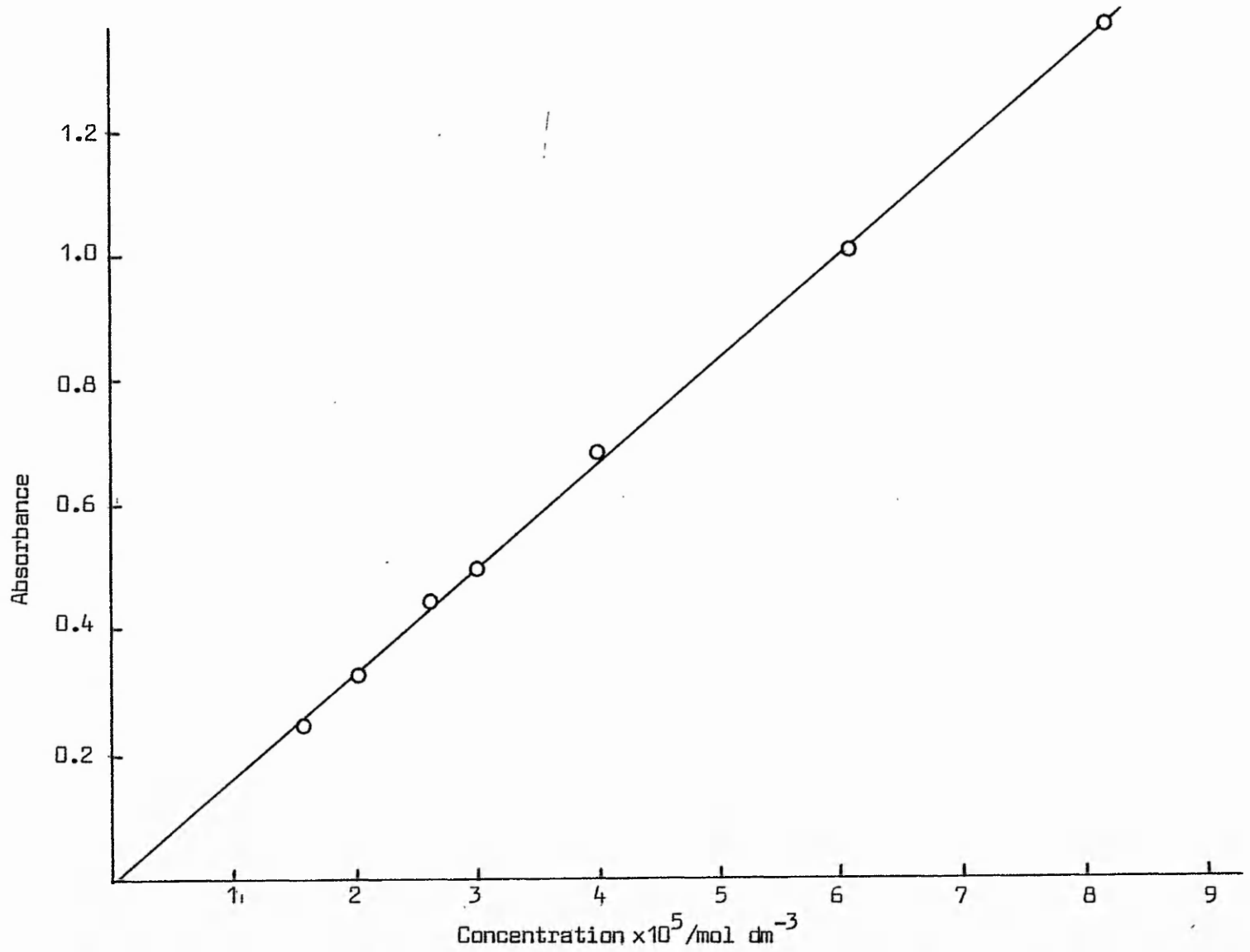


Figure 23 Absorbance versus Concentration for N-(2,4-DNP)-D-glucosamine in 1% HBr at 365 nm.

by monitoring the increase in the amide I band, in the infrared at 1665 cm^{-1}, after acetylation (compare Figures 24 and 25). The increase in absorbance of the amide I band can be attributed to the N-acetylation of unreacted free amine groups rather than to displacement of the N-dinitrophenyl residues by acetic anhydride, prior to acetylation. This was shown by a spectroscopic study of the model compound DNP-D-glucosamine whose absorbance at 360 nm in a methanol/acetic anhydride solution was measured as a function of time and found to be constant for a period of 72 hours, indicating the stability of the N-dinitrophenyl residues toward acetic anhydride. Attempts to improve the extent of reaction by refluxing chitosan films in a methanolic solution of DNFB again yielded a product which contained unreacted amine groups.

As the N-dinitrophenyl residues are relatively large when compared to the N-acetyl group it is likely that steric hinderance is responsible for incomplete reaction. Chitosan is a β-(1→4)- linked polysaccharide and thus successive N-dinitrophenyl groups on the same side of the polymer chain would be separated by distances of approximately 1.03 nm and would appear to be unlikely to interact with each other or with an approaching DNFB molecule. However Moore and Roberts[97] have shown that in the O-acetylation of N-acyl chitosans and Schiff's base derivatives of chitosan, the imino hydrogen of the amide groups in the former derivatives offers sufficient steric hinderance to prevent more than approximately 50% of the hydroxyl groups being esterified.

Another fact that may explain the incomplete reaction is the effect of the N-dinitrophenyl residues on the solubility parameter of the polymer and hence its accessibility to the DNFB reagent. Moore[33,57,98] has shown that the extent of N-acetylation of chitosan

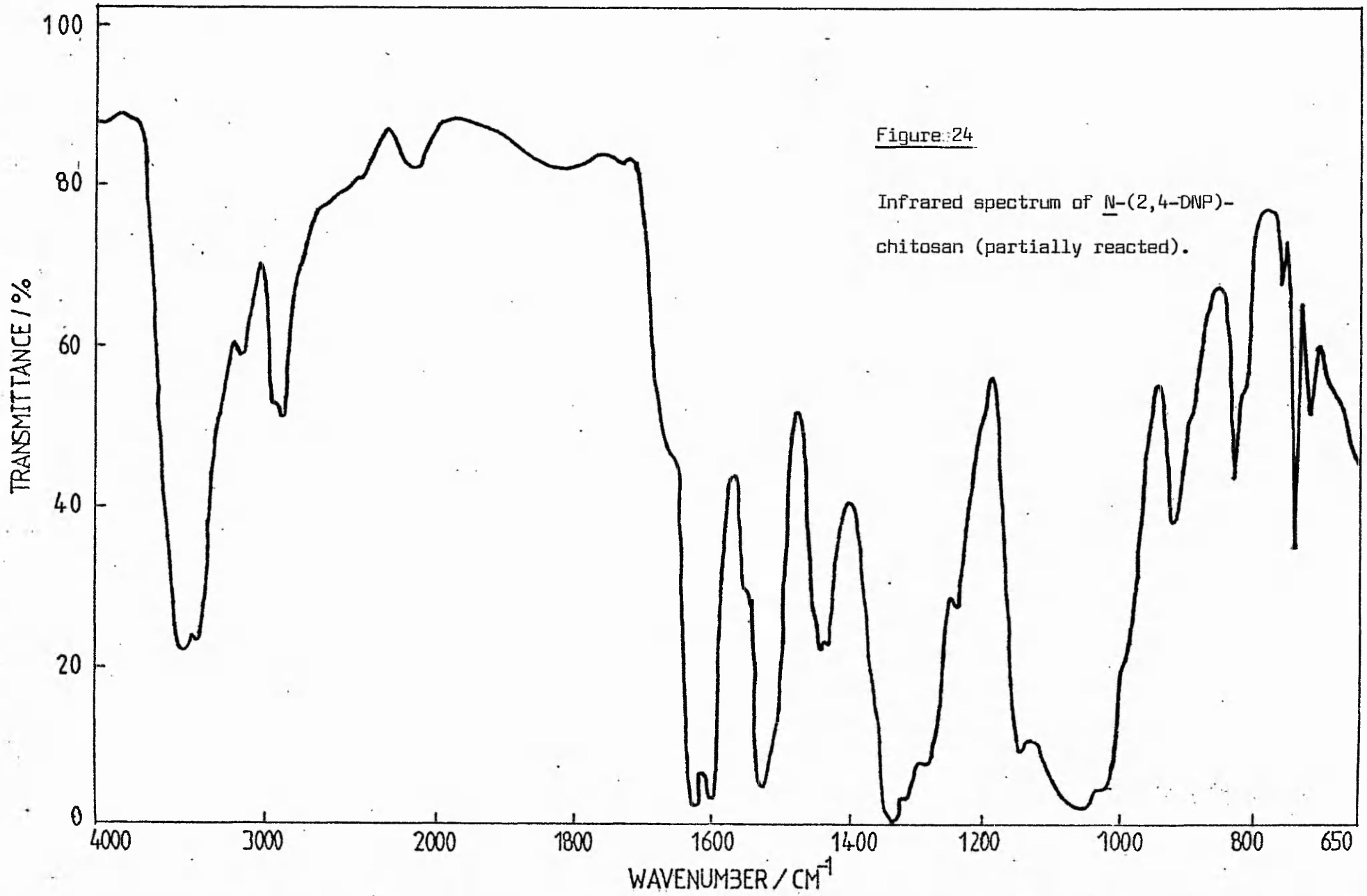


Figure 24

Infrared spectrum of N-(2,4-DNP)-chitosan (partially reacted).

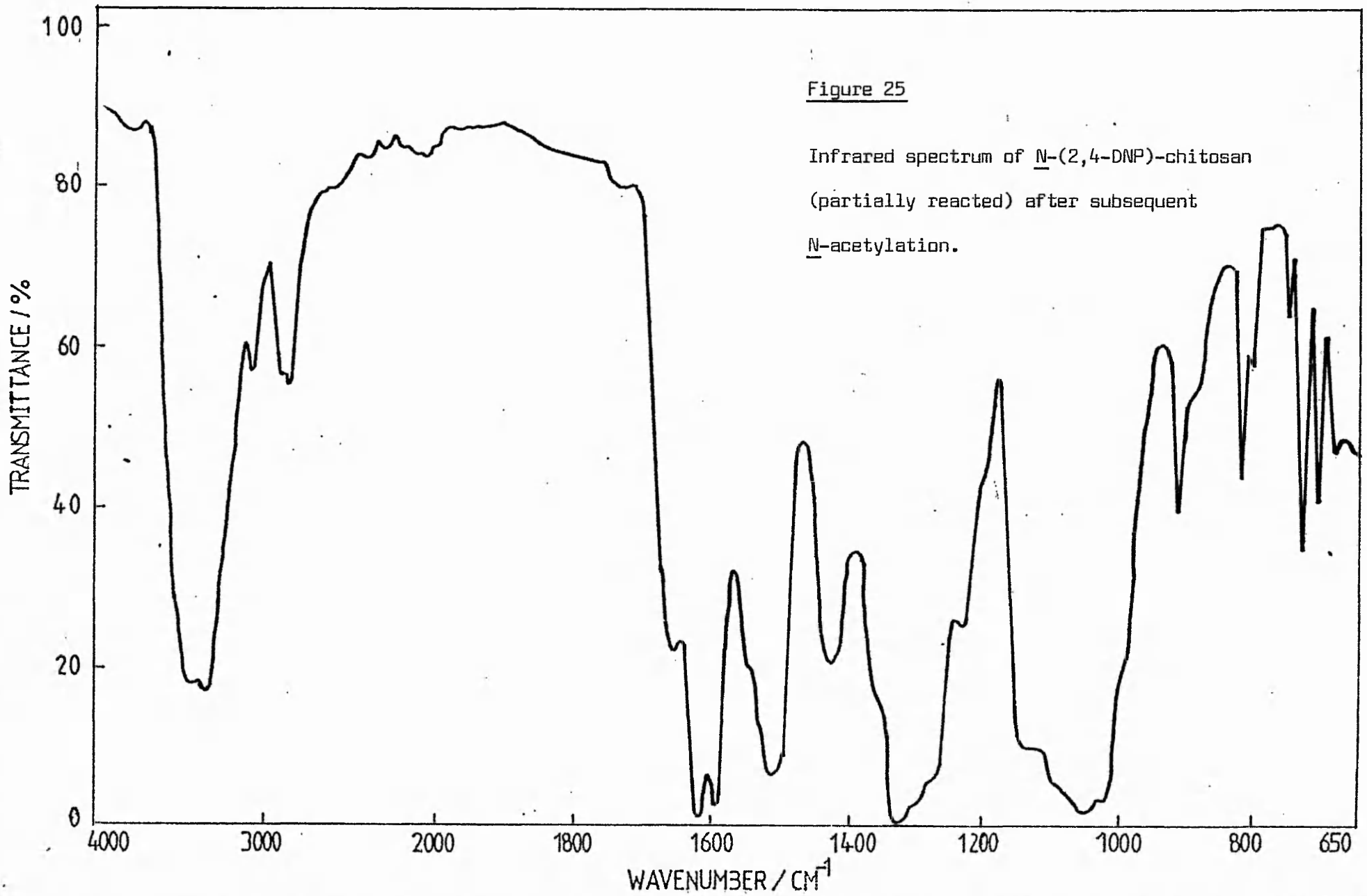


Figure 25

Infrared spectrum of N-(2,4-DNP)-chitosan (partially reacted) after subsequent N-acetylation.

is dependant upon the solubility parameter of the solvent used. It is assumed that the most favourable conditions for N-acetylation, with acetic anhydride, occur when the solubility parameter of the solvent resembles that of the polymer. Thus changes in the solubility parameter of the polymer may make the solvent a less suitable reaction medium. The relationship between the solubility parameter of the polymer and the reagent may also be of importance. It is difficult to predict the exact affect of the N-dinitrophenyl groups except that it has been found[99] for disperse dyes that the presence of a nitro group tends to increase the solubility parameter for these dyes.

Although due to imcomplete heterogeneous reaction with DNFB it is not possible to determine the residual free amine content of a chitosan sample, the actual extent of reaction can still be found by spectroscopic means. The partially reacted N-(2,4-DNP)-chitosan film was found to be insoluble in organic solvents, as well as in dilute acidic media. However it could be dissolved by refluxing in concentrated hydrobromic acid (9N) to give a yellow solution with λ max in the uv at about 360 nm, similar to those for low molecular weight analogues. It is likely that under these drastic conditions of acid concentration and temperature the polymer has become severely degraded and exists in the form of very low molecular weight species. Continued refluxing of the solution caused a considerable decrease in the absorbance of the system and a small downfield shift in the position of the absorption band to 355 nm. Thus under the extreme conditions used to dissolve the polymer, chromophore destruction is taking place and therefore for a quantitative spectroscopic analysis

the conditions of dissolution must be modified. (The destruction of the chromophore is more extensively studied in Section 3.2.4). The effect of temperature variation on the stability and position of the uv maximum for the model compound DNP-D-glucosamine has been investigated in a solvent system of 50/50 9N HBr/H_2O. The results are summarised in Table 11 and show that chromophore destruction is temperature dependant and that at 50°C the system appears to be stable for up to 66 hours.

These conditions were then applied to a sample of DNP-chitosan film. Dissolution was complete after 5 hours of heating at 50°C. From the uv absorbance and dilution data (Table 12) it is possible to determine the extent of reaction between chitosan and DNFB:

Absorbance, A at λ max $= .485$

From the Beer Lambert Law $A = \varepsilon \times c \times l$

$l = 1$

$\varepsilon = 16,500$

Thus $c = A/\varepsilon$

$= .485/16,500$

$= 2.94 \times 10^{-5}$ mol dm^{-3}

However taking into account the initial 10 fold dilution

$$c = 10 \times 2.94 \times 10^{-5}$$

$$= 2.94 \times 10^{-4} \text{ mol } dm^{-3}$$

Therefore in the initial volume of 250 cm^3 this is

$$\frac{2.94 \times 10^{-4} \times 250}{1000}$$

$$= 7.35 \times 10^{-5} \text{ moles of DNP residues}$$

Table 11

Effect of temperature on stability of DNP-D-glucosamine chromophore system in 50/50 (v/v) 9N HBr/H_2O.

Temp./^oC	Time/hour	Initial λmax/nm	Final λmax/nm	Initial absorbance	Final absorbance
80	8	365	350	0.79	0.66
60	8	365	360	0.79	0.76
50	8	365	365	0.79	0.79
50	24	365	365	1.16	1.16
50	66	365	365	1.16	1.10

Table 12

Spectroscopic data for DNP-chitosan samples in 50/50 (v/v) 9N HBr/H_2O at a dissolution temperature of 50^oC. Absorbance (Abs.) measurements were carried out at 395 nm in 1 cm cells.

Sample source	Weight/g	Volume of solvent/cm^3	Dilution factor	Dissolution time/hours	Abs.	% amine reacted
Hetero-geneous reaction	0.0509	250	10	8	0.485	41
Homogeneous synerised gel	0.0327	100	10	48	1.0	54
Homogeneous gel	0.0407	100	10	48	1.2	51

In 0.0509 g of material there is 7.35×10^{-5} moles of DNP groups

These contribute $7.35 \times 10^{-5} \times 168$ g to the total weight

$= 1.235 \times 10^{-2}$ g

Thus weight of starting material before reaction

$= 0.0509 - 0.01235 + 7.35 \times 10^{-5}$

This last term takes into account the loss of a proton during the reaction.

Therefore weight of starting material

$= 0.0386$ g.

The degree of N-acetylation of the original starting material was determined by the method of Moore and Roberts[40] and found to be 21%. The repeat unit molecular weight of an N-acetyl chitin unit is 203 g while that of the amine unit is 161 g. Thus the equivalent weight of the amine group is given by:

$$\frac{21 \times 203 + 79 \times 161}{79} = 215 \text{ g/mole of free amine}$$

Therefore in 0.0386 g we have $\frac{0.0386}{215}$

$= 1.795 \times 10^{-4}$ moles.

Therefore percentage of free amines reacted

$$= \frac{7.35 \times 10^{-5}}{1.795 \times 10^{-4}} \times 100$$

$$= \underline{41\%}$$

Thus only 41% of the available free amine groups have undergone

reaction with the DNFB reagent under the heterogeneous conditions applied.

In order to try and improve the extent of reaction an homogeneous system was devised along similar lines to the homogeneous N-acetylation of chitosan[57]. An aqueous acetic acid/methanol solution of chitosan was reacted with a 1½- fold excess of DNFB reagent. On standing for several days the solution was found to have gelled. The ability of chitosan solutions to undergo gelation has previously been studied by Moore and Roberts[100,101] and by Hirano et al.[45,102]. Gelation is usually brought about through acylation of the free amino group on the chitosan and occurs when about 70% of the amine groups have reacted[100]. A portion of the gel was treated by blending it with methanol and then adding ether to aid polymer precipitation. The product thus obtained, in a suitably washed form, had an intense yellow colour and a powdery appearance. A second portion of the gel was allowed to undergo syneresis before being treated. For the homogeneous reaction a low molecular weight commercial chitosan (Kytex L) with a degree of N-acetylation of 16% was used to try and obtain a product with better solubility characteristics. The two products from the gelation reaction were dissolved in the 50/50 9N HBr/H_2O solvent at 50°C. Dissolution was much slower than for the film form and required 48 hours for completion. The uv data of the resulting solutions are contained in Table 12 and indicate that 51 and 54% of the available amine groups had reacted for the gelled and syneresed products respectively. Taking into account the initial residual N-acetylation of the starting material, 16%, these figures correspond to a total extent of reaction of the amine groups (acetylation and 2,4-dinitrophenylation) of 59% for the gelled system

and 61% for the syneresed product. From these results it appears that little further reaction is taking place after gelation occurs, unlike the case for N-acetylation[101] where the reaction goes to completion although gelation occurs when approximately 80% of the amine groups have been acetylated. As the polymer in the gel is still in a highly swollen and accessible state, the failure to go to completion supports the idea that steric hinderance is a major factor in this.

Gelation[101] is assumed to be caused by the decrease in the solubility of the polymer chains due to increasing extent of reaction of the amine groups. The extent of reaction in relation to the onset of gelation depends upon the desolubilising ability of the reacting species e.g. for N-acylation Moore and Roberts[100] have found that the extent of N-acetylation at onset of gelation decreases with the increase in molecular weight of the hydrophobic acyl group. For N-acetylation gelation occurs at a N-acetyl content of 80%, however for the N-2,4-dinitrophenylation, gelation occurs after only 59% of the total amine groups have reacted. This indicates the pronounced desolubilising ability of the N-DNP- residues. Thus although the homogeneous reaction results in an increase in the extent of the reaction of DNFB compared with heterogeneous techniques, reaction is still incomplete.

3.2.4 Stability of the N-DNP- amine chromophore system

3.2.4.1 Introduction

During the investigation into the use of 2,4-dinitrofluorobenzene

as a reagent for the quantitative determination of the amine groups on chitosan it was found that the action of heat, to aid polymer solubility, had a detrimental effect on the absorbance intensity of the chromophore system (see Section 3.2.3.3).. Although the effect can be negated by the use of milder conditions it may explain the different values for the extent of deacetylation of chitosan obtained by Araki and Ito[103] when using different analytical techniques. One method was based on the use of ^{3}H labelled acetic anhydride and the second technique was based on hydrolysis of N-DNP-chitosan followed by uv/visible absorption spectroscopy to determine the concentration of N-DNP-D-glucosamine produced. The values for the free amine content were always lower; in some instances up to 50% lower, when determined by the latter method. Thus an investigation was carried out to try and determine the causes of the apparent destruction of the chromophore system of N-DNP-D-glucosamine.

3.2.4.2 Solvent effects

From experiments on the model compound N-DNP-D-glucosamine it is clear that aqueous acidic conditions, allied to high temperatures, cause chromophore destruction (see Section 3.2.3.3 Table 11). However the system was also further investigated in non-acidic media. The effect of refluxing solutions of N-DNP-D-glucosamine in water, aqueous acid and methanol on the absorbance intensity of λmax was studied. The results in Table 13 show an almost identical loss in absorbance intensity for the aqueous and the acidic system but complete stability in methanol. Because the reflux temperatures of the aqueous and the methanolic systems are different due to differences in their boiling points, heating was also carried out in methanol at 100^{o}C using a

Table 13

Effect of solvent on uv/visible absorbance intensity of N-DNP-D-glucosamine, at 360 nm in 1 cm cells, on heating for 4 hours.

Solvent	Solution conc./g dm^{-3}	Initial Abs.	Final Abs. after reflux	Final Abs. after heating at 100°C
H_2O	0.0157	0.775	0.44	-----
HBr/H_2O 20/80 (v/v)	"	0.750	0.42	-----
MeOH	0.0074	0.355	0.355	-----
MeOH	0.0162	0.770	-----	0.770
H_2O	"	0.775	-----	0.336

Table 14

Effect of temperature on uv/visible absorbance intensity of N-DNP-D-glucosamine in water at 360 nm in 1 cm cells.

Temp./°C	Solution conc./g dm^{-3}	Reaction time/hours	Initial Abs.	Final Abs.
80	0.0165	8	0.79	0.66
60	"	8	"	0.76
50	"	8	"	0.79

sealed autoclave to prevent the lower boiling methanol from evaporating. However even at this elevated temperature the chromophore was completely stable in methanol. Therefore it is apparent that the presence of water rather than acid is responsible for the breakdown of the chromophore system as shown by the loss in the absorbance intensity within the temperature ranges studied.

The temperature dependance of the breakdown can be seen from the effect of reduction in reaction temperature on the absorbance intensity of an aqueous N-DNP-D-glucosamine solution (Table 14). At 50°C the system appears to be quite stable under the conditions applied.

A suggested explanation for the breakdown of the chromophore is hydrolysis by the water. However it is likely that the presence of acid would also accelerate this process, which is not the case since from Table 13 it can be seen that the effects of an aqueous system and of a highly acidic system are almost identical. Furthermore the N-2,4-dinitrophenyl derivatives of amino acids are stable in hot aqueous solutions. The potential reaction product from such a proposed hydrolysis would be 2,4-dinitrophenol. To test this hypothesis a comparative TLC study was carried out using N-DNP-D-glucosamine, 2,4-dinitrophenol and the N-DNP-D-glucosamine product after refluxing in water. This revealed that the original material had changed into a two component system on refluxing, one component corresponding to the starting N-DNP-D-glucosamine and the second to the reaction product. However this product was shown not to be 2,4-dinitrophenol by TLC using a 50/50 (v/v) petroleum ether/ether solvent. The aromatic residues on the TLC plate were developed

using iodine, whilst the yellow N-DNP-D-glucosamine spot was clearly visible.

3.2.4.3 Effects of amine structure on the chromophore stability

The TLC evidence indicates that hydrolysis of the N-DNP- residues is not occuring and thus in order to see if the effect is due to the specific nature of the N-DNP-D-glucosamine, N-DNP derivatives of other amines were investigated. Aqueous solutions of N-DNP-cyclohexylamine and N-DNP-diethanolamine were heated at 90°C and the characteristics of their uv/visible spectra monitored. The diethanolamine analogue was sufficiently soluble in water to form a reasonably concentrated solution (for absorbance measurement), but the cyclohexylamine derivative required a 90/10 (v/v) methanol/H_2O solvent for dissolution. Results of the thermal treatment of these solutions (Table 15) indicate that the chromophore is stable in these compounds, in complete contrast to the N-DNP-D-glucosamine system. Therefore the breakdown of the chromophore must be a specific consequence of the structural arrangement of N-DNP-D-glucosamine in aqueous media.

3.2.4.4 Detailed spectroscopic analysis

A more detailed spectroscopic analysis of N-DNP-D-glucosamine in water was studied by monitoring its complete uv/visible absorbance spectrum from 200 nm to 500 nm, after several periods of heating at 90°C. The combined spectra are shown in Figure 26. They show the usual decrease in the absorbance intensity of λmax with time, and a slight hypsochromic shift of this band. However in conjunction with this is the appearance of new bands in the region around 250 nm that increase in intensity with time of heating. Also, after prolonged

Table 15

Effect of heat (90°C) for 5 hours on the uv/visible absorbance intensity of N-DNP-cyclohexylamine and N-DNP-diethanolamine.

Sample	Solvent	Solution conc./g dm^{-3}	λ max	Initial Abs.	Final Abs.
N-DNP-cyclohexylamine	MeOH/H_2O 90/10 (v/v)	0.016	249	0.803	0.798
N-DNP-diethanolamine	H_2O	0.017	287	0.865	0.860

Table 16

Effect of thermal treatment (90°C) on the uv/visible absorbance intensity of N-DNP-2-amino-2-deoxy sorbitol in 1 cm cells.

Time of heat/hours	λ max	Absorbance
0	360	0.343
1	"	0.342
2	"	0.341
4	"	0.340
6	"	0.341

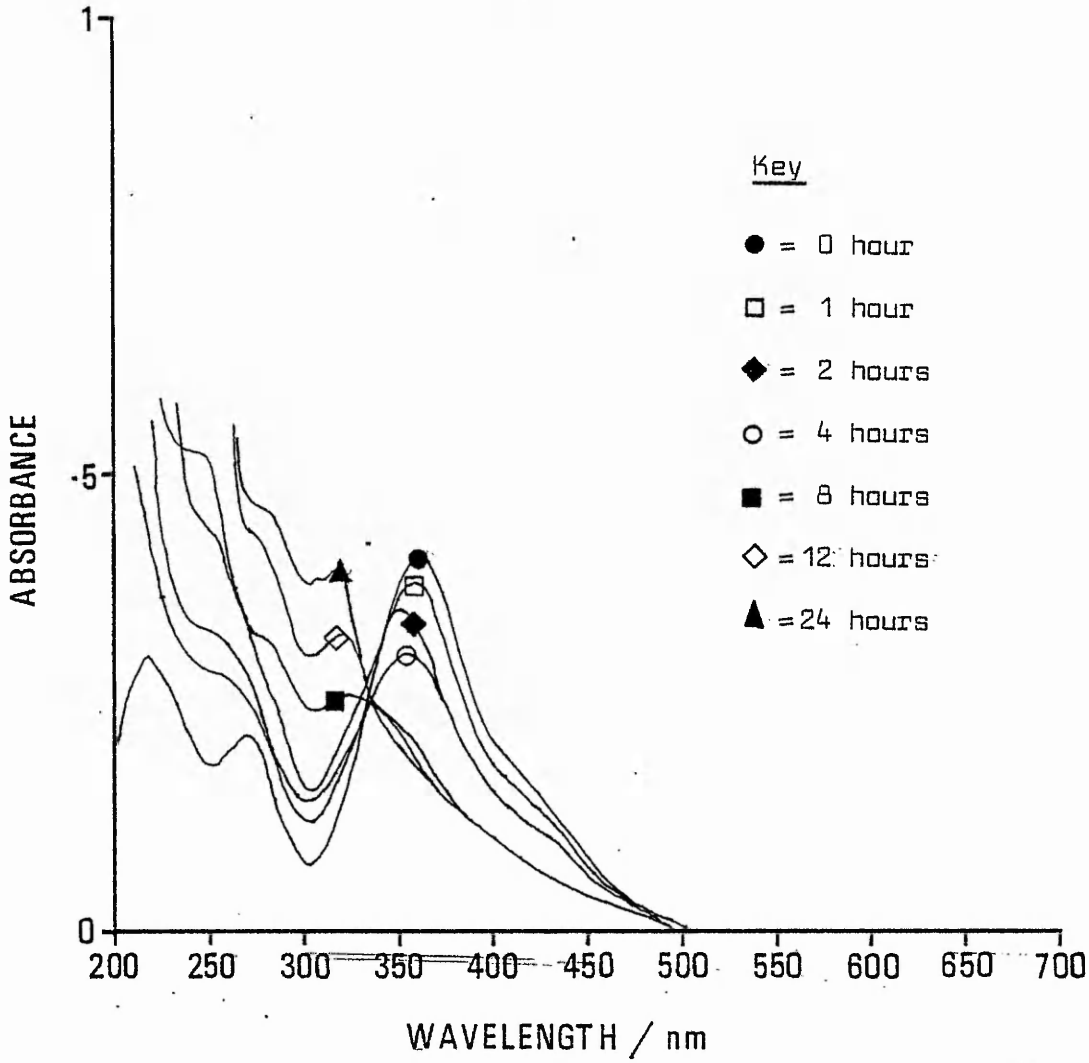
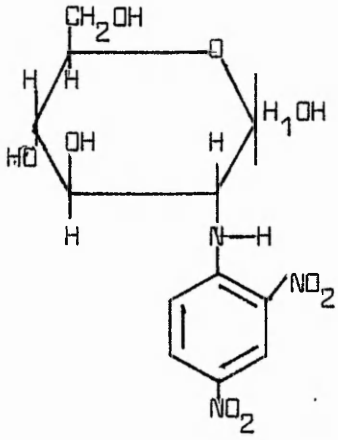


Figure 26

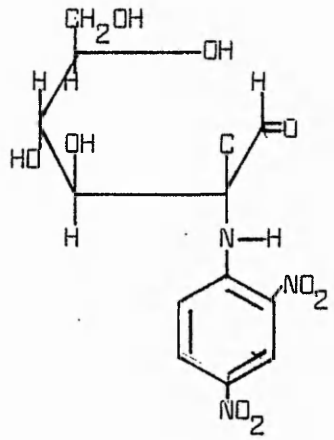
Uv/visible spectrum of DNP-D-glucosamine after various periods of heating at 90°C.

periods of heating a band in the 320 nm region appeared, which had developed into a double peak at 310 nm and 300 nm after the longest period of treatment (24 hours). The spectrum after 24 hours revealed no sign of the band at 360 nm assigned to the original N-DNP-chromophore.

We have postulated that the reason for the decrease in the absorbance intensity of the system is due to chemical reduction of the nitro groups on the benzene ring by the aldehyde on C(1). The reduction of a nitro group would eliminate its electron withdrawing properties and thus effect the conjugated electron system responsible for the absorption band. Hence the new bands in the spectrum may be a consequence of the reaction products. This theory of chemical reduction is consistant with the data on the solvent effects (see Section 3.2.4.2) in that no reaction is observed in a methanolic system. In the solid state N-DNP-D-glucosamine exists in the cyclic hemiacetal form (X) as indicated by the absence of the carbonyl band in the infrared spectrum.



(X)



(XI)

However in the presence of water, mutarotation[104] can occur to to open chain structure (XI), which provides a reducing aldehyde group. Mutarotation requires the presence of an amphiprotic solvent, capable of acting as both an acid and a base, such as water. Thus in methanol, which is not such a solvent, no mutarotation can occur and hence no aldehyde groups will be available for reduction.

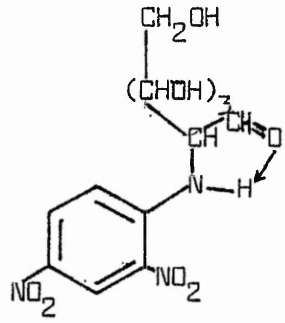
3.2.4.5 Spectroscopic analysis of N-(2,4-DNP)-2-amino-2-deoxysorbitol

The uv/visible absorbance spectrum of an aqueous solution of N-(2,4-DNP)-2-amino-2-deoxysorbitol, on heating at 90°C was investigated because it provides a comparison with N-DNP-D-glucosamine, since they are structurally identical except for the presence of a primary hydroxyl group on the C(1) carbon in the sorbitol derivative rather than a carbonyl group. The absence of the carbonyl group will provide a non-reducing system. N-(2,4-DNP)-2-amino-2-deoxysorbitol was prepared by initial chemical reduction of the carbonyl group on D-glucosamine with sodium borohydride according to the method of Wolfrom and Wood[80]. The 2-amino-2-deoxysorbitol produced was reacted with 2,4-dinitrofluorobenzene to yield a yellow product, N-(2,4-DNP)-2-amino-2-deoxysorbitol.

The uv/visible absorbance intensity at 360 nm of an aqueous solution of this product was found to be stable on heating at 90°C for up to 6 hours (Table 16), nor was there any shift in the position of λ max. This seems to support the theory that the aldehyde group is involved in the breakdown of the chromophore in N-DNP-D-glucosamine.

Further aqueous solutions of N-DNP-2-amino-2-deoxysorbitol were heated in the presence of both stoichiometric and excess amounts

of D-glucose, and also in the presence of excess formaldehyde, these compounds being added as possible sources of reducing groups. The uv/visible absorbance intensity and the position of λ max remained unaltered even after prolonged treatment. One would expect the reducing ability of the added reagents to cause a similar effect to that observed for the N-DNP-D-glucosamine system in water. The lack of a similar effect may be explained in terms of the structure of the N-DNP-D-glucosamine molecule. Chemical reduction by the aldehyde group may only be occuring by steric acceleration due to the close proximity of this group to the N-DNP residues on the C(2) carbon atom (see Structure XI). Alternatively the aldehyde group may have a completely different effect, through the formation of an internal chelate structure by means of hydrogen bonding between the hydrogen of the imino group and the oxygen of the carbonyl group. As mutarotation occurs the open chain form may exist as an internal chelate (XII) thus preventing ring closure. The concentration of the chelate form would increase, as mutarotation continues, resulting in the gradual shift of the uv absorption band due to its effect on the electronic nature of the chromophore.



(XII)

As yet there is no experimental evidence to suggest which if any of the two proposed mechanisms (steric acceleration or chelate formation) is the correct one, and further work is required to do this.

3.2.5 Use of salicylaldehyde as an amine-specific reagent

3.2.5.1 Introduction

The result of a reaction between a primary amine and an aldehyde is a Schiff's base which is a product that contains an azomethine group, -CH=N-. The free amine groups on chitosan will also undergo reaction with aldehydes[97,105-107] to yield such products. The reaction between chitosan and the aromatic aldehyde salicylaldehyde has been extensively investigated in this report, for use in the determination of the degree of N-acetylation of chitosan. Both heterogeneous and homogeneous studies have been carried out.

3.2.5.2 Heterogeneous studies

A method based on the reaction between chitosan and salicylaldehyde has been developed to quantitatively determine the primary amine content of the chitosan. Salicylaldehyde was chosen as the reagent because of its previous use by Milum[108] for the estimation of low molecular weight primary amines. Moore and Roberts[97] have shown that the heterogeneous reaction of chitosan with salicylaldehyde in methanol goes to completion to yield the yellow N-salicylidene chitosan. The basis of the present method is the reaction of a known excess of salicylaldehyde with chitosan followed by uv spectroscopic determination of the reagent remaining after reaction.

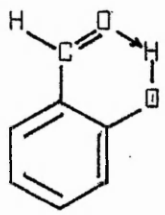
Salicylaldehyde in methanol has two main absorption bands in the uv spectrum. One is at 325 nm and is the result of intramolecular

chelation between the hydroxyl group and the aldehyde group on the molecule. The second band is at 255 nm and is typical of an aromatic absorption band. For spectroscopic analysis it was decided to use the lower wavelength band as its intensity is three times that of the 325 nm band. The heterogeneous reaction between chitosan and salicylaldehyde was found by Moore and Roberts[94] to have gone to completion after 16 hours at room temperature using a three molar equivalent of reagent to amine groups. Because of the relatively long time involved for full reaction the stability of the salicylaldehyde reagent in methanol was investigated by monitoring its uv absorption spectrum as a function of time. It was found that at concentrations of the order of 10^{-6} mol dm^{-3} (the concentration range required for on-scale uv measurement) the absorbing chromophore remained stable for greater than 7 days. However in more concentrated solutions of the order of 10^{-2} mol dm^{-3} (the concentrations required for reaction with chitosan) the absorbance intensity, on dilution, of the salicylaldehyde decreased steadily over a period of days (see Table 17). A similar decrease in the absorbance at 255 nm was observed (Table 17) for benzaldehyde in methanol, indicating that some change in the chromophore was occuring through interaction of the aldehyde group. From Table 17 it can be seen that, at the same molar reagent concentrations, the relative rate of chromophore loss is faster for benzaldehyde than for salicylaldehyde. This can be explained in terms of the equilibrium that exists between the intramolecularly chelated and the non-chelated forms of salicylaldehyde. Chelation effectively ties up a proportion of the aldehyde groups as shown by structure (XIII).

Table 17

Uv/visible absorbance stability of salicylaldehyde and benzaldehyde in methanol, in 1 cm cells at 255 nm.

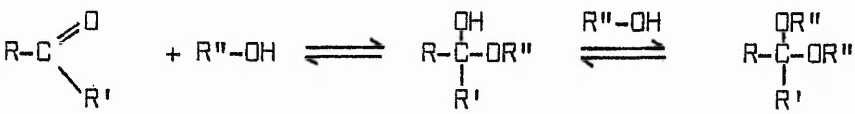
Salicylaldehyde				Benzaldehyde			
Conc./mol dm^{-3}	Dilution factor	Time/ hours	Abs.	Conc./mol dm^{-3}	Dilution factor	Time/ hours	Abs.
0.0341	400	0	0.926	0.034	400	0	0.977
"	"	24	0.856	"	"	24	0.802
"	"	48	0.813	"	"	48	0.535



(XIII)

Intramolecular chelation in salicylaldehyde.

The decrease in the uv absorbance with time, at the concentrations studied, can be explained in terms of the reaction between the aldehyde and the solvent, methanol. Alcohols add to the carbonyl groups of aldehydes and ketones to form initially a hemiacetal and then an acetal.



hemiacetal acetal

When considering the rate of reaction for the system used in the uv stability experiments, the large excess of methanol relative to the salicylaldehyde effectively makes any methanol concentration term, in the rate equation for the reaction, a constant and thus the rate will be dependant upon the salicylaldehyde concentration. This explains why at high concentrations the acetal formation is observable, shown by decrease in absorbance, whereas at concentrations 400 times lower the reaction is so slow as not to be noticeable over several days.

For accurate spectroscopic analysis of salicylaldehyde in methanol it is essential that the acetal formation reaction be reversed. It is known that water and aqueous acid systems have the ability to aid

the reversal of acetal and hemiacetal formation through hydrolysis of these products. The uv absorbance stability in the following solvent systems were therefore investigated: 90/10 (v/v) methanol/water, 50/50 (v/v) methanol/water and 80/20 (v/v) methanol/1% acetic acid. The results are summerised in Table 18. From these it can be seen that the presence of water aids the acetal hydrolysis although over 50% of water in the system would be required to completely reverse the acetal formation. However at too high a water/methanol ratio the solubility of the hydrophobic salicylaldehyde is impaired and the reactivity of the reagent toward chitosan may be less due to a change in the solubility parameter of the reaction medium. This has been found[97] to be important for complete reaction. However the hydrolysis of the acetal or hemiacetal was more effectively achieved by use of a dilute solution of a weak acid, acetic acid. The presence of 1% acetic acid at a 20% level to 80% methanol was sufficient to maintain the uv absorbance of the salicylaldehyde stable for up to 48 hours. In such a system there is a sufficiently high proportion of methanol to ensure complete Schiff's base formation between the reagent and chitosan, although it is assumed that the acetic acid is weak enough and dilute enough not to cause hydrolysis of the Schiff's base under the heterogeneous conditions used.

The extinction coefficient for salicylaldehyde in the aqueous acetic acid/methanol solvent was determined by preparation of three separate solutions and measuring their absorbance at 255 nm. The average of the three extinction coefficients was taken (data in Table 19) and found to be 10,200.

Table 18

Uv/visible absorbance stability of salicylaldehyde in various aqueous methanol systems, in 1 cm cells at 255 nm.

Solvent	Solution conc./mol dm^{-3}	Time/ hours	Dilution factor	Absorbance
90/10 MeOH/H_2O	0.035	0	400	0.935
"	"	24	"	0.889
"	"	48	"	0.840
50/50 MeOH/H_2O	"	0	"	0.935
"	"	24	"	0.925
"	"	48	"	0.910
80/20 MeOH/1% acetic acid	"	0	"	0.935
"	"	24	"	0.935
"	"	48	"	0.935

Table 19

Uv/visible spectroscopic data for salicylaldehyde in 80/20 (v/v) methanol/1% acetic acid at 255 nm in 1 cm cells.

Weight of salicylaldehyde/g	Volume of solvent/cm^3	Solution conc./mol dm^{-3}	Dilution factor	Abs.	Extinction coefficient
0.1381	50	0.0226	400	0.575	10,169
0.2664	100	0.0218	400	0.551	10,103
0.3703	150	0.0202	400	0.523	10,349

For application of salicylaldehyde to chitosan a series of chitosan samples were prepared of varying degrees of N-acetylation, by means of homogeneous reaction[101] with acetic anhydride in aqueous acetic acid/methanol. The system was prevented from gelling by the use of low concentrations of acetic anhydride, and by precipitation of the product after a reaction time of one hour. The products were filtered immediately after precipitation, and quickly washed and dried to prevent heterogeneous acetylation. The extent of N-acetylation was varied by altering the volume of acetic anhydride added. Five samples were produced, labelled 14-18, and along with the commercial chitosans, Kytex H and Kytex L, were characterised with respect to their degree of N-acetylation using the infrared method of Moore and Roberts[40] (see Section 2.2.3.1). The results of the infrared analysis of the samples are included in Table 20.

These samples were reacted using an approximate two-fold molar excess of salicylaldehyde in methanol/acetic acid, based on their amine group contents as measured by infrared spectroscopy. A reaction time of 48 hours was used to ensure complete reaction. Uv analysis of the original and the residual solutions enables the amount of salicylaldehyde that has reacted to be determined. The ratio of the weight of chitosan starting material to the weight of salicylaldehyde reacted is used to evaluate the extent of N-acetylation from a theoretical calibration plot (Figure 27) of this ratio against the degree of N-acetylation. The results given in Table 21 show reasonable agreement with the values determined by infrared spectroscopy. This is demonstrated by the linearity of the plot of Degree of N-acetylation by infrared spectroscopy versus Degree of N-acetylation by residual salicylaldehyde analysis (Figure 28) which has a slope of 0.99 and

Table 20

Infrared data for determination of degree of N-acetylation.

Sample	A_{1665}	A_{3450}	A_{1665}/A_{3450}	% N-acetylation = $\frac{A_{1665}/A_{3450}}{0.0133}$
Kytex H	0.28	1.0	0.28	21
Kytex L	0.183	0.86	0.213	16
14	0.838	0.877	0.956	72
15	0.741	0.864	0.859	64.5
16	0.610	0.866	0.704	53
17	0.290	0.540	0.537	40.5
18	0.185	0.425	0.435	33

Table 21

Uv/visible spectroscopic data for determination of degree of N-acetylation via residual analysis of salicylaldehyde reagent, at 255 nm in 1 cm cells.

Sample	Weight/g	Volume of reagent/cm^3	Dilution factor	Initial Abs.	Final Abs.	% N-acetyl groups
Kytex L	0.1020	40	400	0.551	0.219	14
14	0.0554	10	"	0.523	0.316	72.5
16	0.1214	30	"	"	0.241	51
17	0.0919	25	"	"	0.216	39
18	0.1025	35	"	"	0.216	30

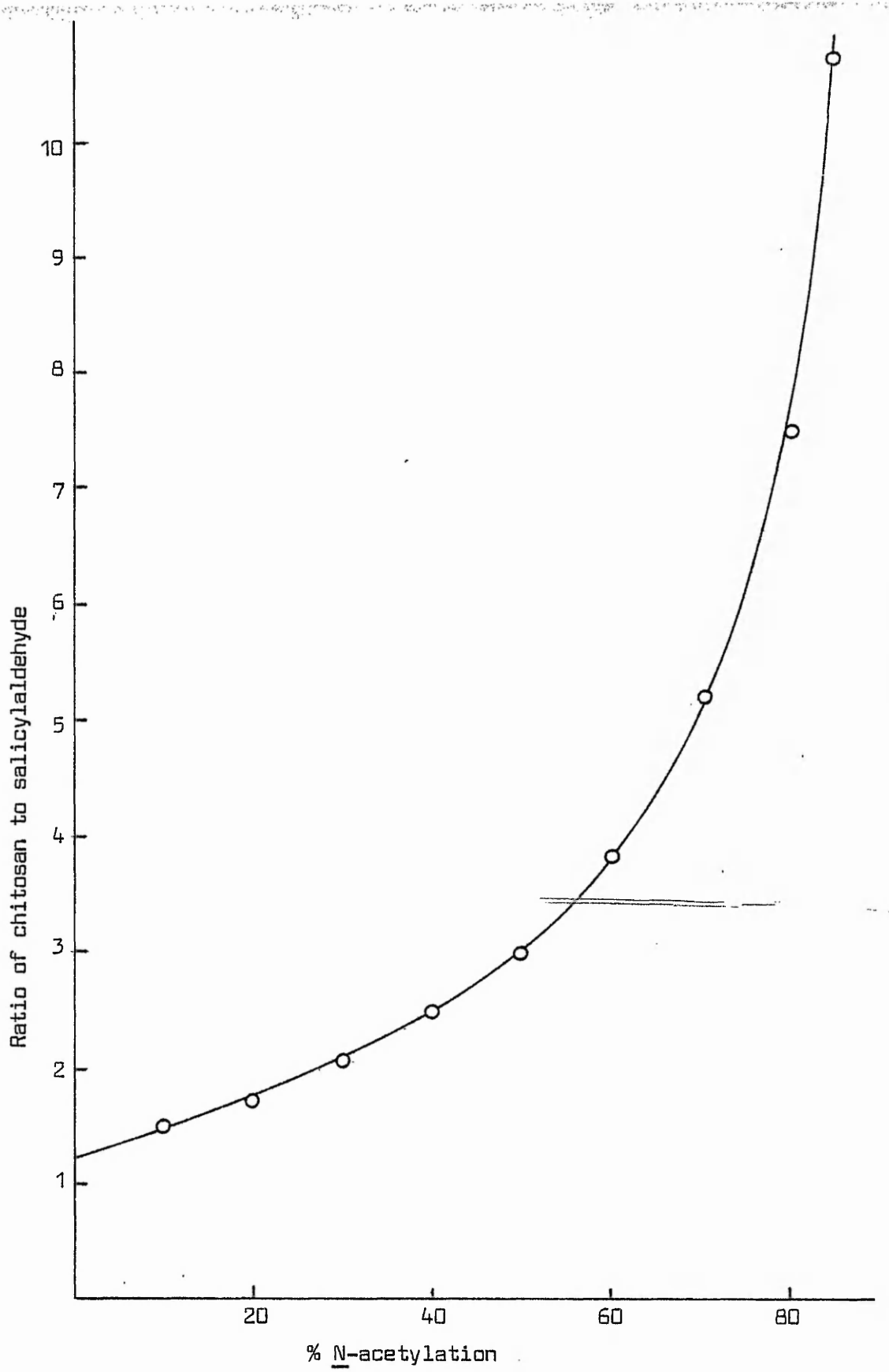


Figure 27 Theoretical calibration plot of Weight of chitosan to weight of salicylaldehyde versus Degree of N-acetylation.

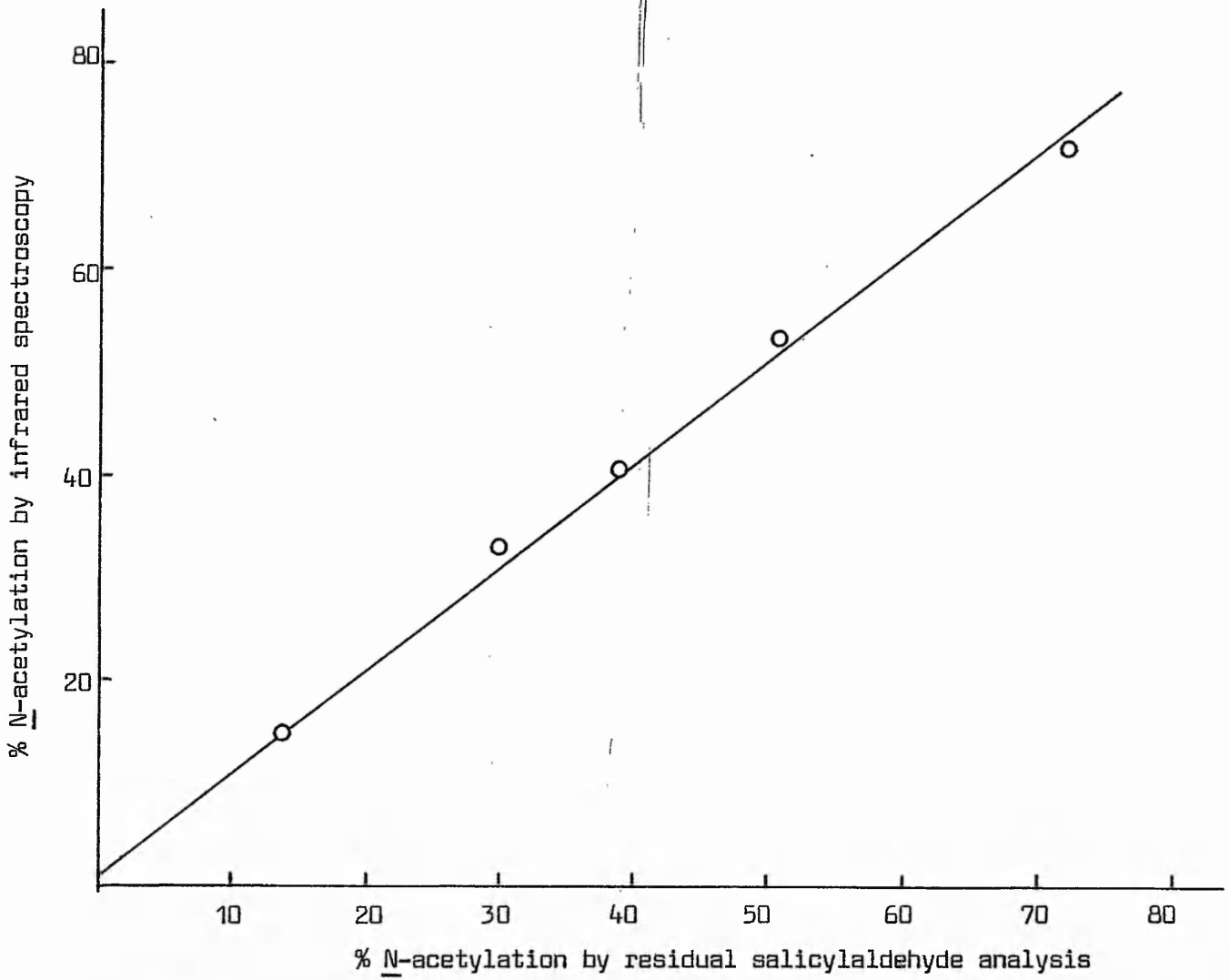


Figure 28 Plot of % N-acetylation by infrared spectroscopy versus % N-acetylation by residual salicylaldehyde analysis for chitosans of varying extents of N-acetylation.

an intercept of 1.6%, found by linear regression. For perfect agreement the slope should be unity and the intercept zero.

From the results in Table 21 the weight of salicylaldehyde reacted was found using the following expression:

$$\text{weight of reagent used} = \frac{M \times V}{\varepsilon \times 10^3} (A_1D_1 - A_2D_2) \text{ g}$$

where A_1 = absorbance of stock solution

A_2 = absorbance of residual solution

D_1 = dilution of stock solution

D_2 = dilution of residual solution

V = volume of solution used for steeping, cm^3

ε = extinction coefficient

M = molecular weight of salicylaldehyde

The salicylaldehyde technique has the added advantage that it can be applied to samples with relatively high degrees of N-acetylation, where many of the existing methods cannot be used due to the poor solubility of these highly N-acetylated products.

3.2.5.3 Homogeneous studies

The estimation of primary amines with salicylaldehyde by Milum[108] utilises the formation of the coloured Schiff's base to enable a spectroscopic analysis to be carried out. The determination is carried out in the presence of acetic acid, which is of interest since it is an ideal solvent for chitosan.

Initial studies of the reaction between chitosan and salicyl-

aldehyde in homogeneous solution were made using a high molecular weight chitosan. A 0.5% solution in 0.2% acetic acid was used. This was diluted 5 times with a solution containing a two-fold molar equivalent of salicylaldehyde in methanol. The reaction was seen to proceed by the gradual yellowing of the solution, the uv/visible spectrum of which showed a large absorption band at around 320 nm and a smaller one at 410 nm. The spectrum was run against a methanol standard with the band at 320 nm being attributed partially to the excess reagent and partially to the Schiff's base. The spectrum of N-salicylidene-D-glucosamine in water reveals bands at 315 nm and 395 nm. The higher wavelength bands can be attributed to the internal chelation between the nitrogen of the azomethine group and the hydrogen of the hydroxyl group in the Schiff's base form. For accurate spectroscopic study the close proximity of the two absorption bands requires that measurements are made against an equivalent salicylaldehyde standard.

The reaction with chitosan was followed as a function of time, using such a standard, by observation of the increase in the uv/visible absorption at 410 nm in 4 cm cells. The results given in Table 22 indicate that under the conditions used the reaction reaches a stage of completion after about 2.5 hours. The system after 16 hours was found to have undergone gelation, due to the insolubility of the product.

Attempts to maintain the system in an homogeneous state were investigated using chitin solvents such as 2-chloroethanol/hydrochloric acid[109] and trichloroacetic acid [110], which may tolerate the

Table 22

Formation of N-salicylidene chitosan as monitored by its uv/visible absorbance at 410 nm in 4 cm cells, using a solution of Kytex H in 80/20 (v/v) methanol/aqueous acid and salicylaldehyde.

Absorbance	Time/minutes	Absorbance	Time/minutes
0.21	10	1.0	90
0.40	20	1.04	105
0.55	30	1.1	120
0.62	40	1.13	130
0.80	60	1.3	162
0.91	74	1.3	16 hours

insoluble nature of the product. However using solutions of chitosan prepared in these solvents no subsequent reaction was observed on addition of the salicylaldehyde in methanol. It is assumed that the strength of the acids used causes rapid hydrolysis of any product.

The effect of acid hydrolysis by acetic acid, was therefore investigated, using the model compound D-glucosamine, formed from its more stable analogue D-glucosamine hydrochloride by the method of Breuer[111]. The uv/visible spectroscopic characteristics of two systems were investigated. One with D-glucosamine in a 20/80 (v/v) water/methanol solvent, with a 3.5 molar equivalence of salicylaldehyde. The second in an identical system except for the addition of acetic acid at a molar ratio of acid to amine of 2:1.

In the absence of acetic acid the position of λ max in the uv spectrum shifts downfield to 396 nm from that at 410 nm in the presence of acid. Also the relative rate and extent of reaction are reduced in the acetic acid system (see Table 23). The acetic acid has the effect of setting up an equilibrium between the forward reaction of Schiff's base formation and the reverse reaction of hydrolysis of the Schiff's base back to the starting components.

A similar trend was observed (Table 24) for the addition of excess acetic acid to a chitosan - salicylaldehyde system in that as the acid concentration is increased the absorbance intensity at 410 nm decreases. For this study a low molecular weight chitosan (Kytex L) was used to prevent gelation of the product due to the greater solubility of low molecular weight chitosans. The absorbance was recorded after 5 hours to allow the reaction to reach equilibrium.

Table 23

Formation of N-salicylidene-D-glucosamine as monitored by its uv/visible absorbance both in the presence (at 410 nm) and in the absence (at 396 nm) of acid, in 1 cm cells.

D-glucosamine solution concentration = 0.00107 mol/dm^{-3}

Ratio of salicylaldehyde:D-glucosamine = 7:2

Ratio of D-glucosamine:acetic acid = 1:2

Dilution of D-glucosamine solution = 5 times

Final solvent system = 80/20 (v/v) methanol/H_2O

Acid absent		Acid present	
Absorbance	Time/minutes	Absorbance	Time/minutes
0.77	12	0.052	11
0.86	15	0.069	15
0.99	23	0.109	25
1.1	30	0.126	30
1.18	38	0.235	65
1.25	46	0.260	75
1.32	60	0.299	90
1.36	70	0.327	105
1.43	126	0.361	130
1.43	273	0.393	150
		0.435	200
		0.459	240
		0.479	300
		0.508	390

Table 24

Effect of variations in acetic acid concentration on position of equilibrium in N-salicylidene chitosan; measurement of the equilibrium absorbance at 410 nm in 4 cm cells after 5 hours reaction.

Volume of chitosan solution (1 g dm^{-3}) /cm^3	Volume of 1% salicylaldehyde /cm^3	Volume of 1% acetic acid /cm^3	Total volume with methanol /cm^3	Abs.
10	3.3	1	50	0.227
10	"	2	"	0.168
10	"	3	"	0.146
10	"	4	"	0.132

Thus for a reproducible quantitative study, the conditions of acid concentration must be identical for the chitosan system and for any calibration using a model amine compound. Ideally the acid concentration should be at a minimum, and this will correspond to the amount required to dissolve the chitosan. In theory this would be based on a 1:1 free amine to acid ratio and may be achieved by the use of water soluble chitosan salts. Such salts are relatively easy to produce[112] using strong acid systems such as hydrobromic acid or hydrochloric acid, since chitosan in dilute solutions of these acids is readily reprecipitated, as the salt, by addition of concentrated acid to the system. Unfortunately when using the chitosan hydrobromide salt, no reaction was observed with salicylaldehyde. It seems that despite the mimimum possible acid concentration, the strength of the acid is still sufficient to completely reverse the Schiff's base formation. A weaker acid salt, such as chitosan acetate, would certainly prevent reversal, but such a product cannot readily be made in a solid form where all the free amine groups exist as the acetate salt. This is due to loss of acetic acid from the chitosan, and also conversion of the acetate to an amide[46], on drying.

Thus a compromise was reached involving the addition of acetic acid to chitosan at a ratio of acid : amine of 3:2, based upon the assumption that the material is completely de-N-acetylated. At any lower ratios of acid : amine the chitosan was not fully dissolved.

The effects of chitosan concentration and salicylaldehyde concentration were then studied.

a) Effect of variations in salicylaldehyde concentration;

A stock solution of 0.1% chitosan in acetic acid at an acid :

amine ratio of 3:2 was used for investigating the effect on the rate of reaction of variations in the initial salicylaldehyde concentration. A sample of Kytex L was used, previously characterised as being 16% N-acetylated. Based on this, molar ratios of chitosan free amine : salicylaldehyde of 1:3, 1:5, 1:10 and 1:20 were used and the rates of reaction at 25°C were followed by monitoring the uv/visible absorbance at 410 nm in 4 cm cells against an equivalent salicylaldehyde standard for each system. The conditions and results outlined in Tables 25 and 26 and Figure 29 indicate that the rate at which equilibrium is reached increases with increase in salicylaldehyde concentration, but that the position of that equilibrium is not greatly affected by the concentration.

b) Effect of variations in chitosan concentration;

The effect of varying the chitosan concentration on the rate of reaction was studied as described above (using constant conditions of salicylaldehyde concentration, based upon a 1:5 molar ratio of chitosan amines : salicylaldehyde for the highest chitosan concentration). Results are summarised in Table 27 and Figure 30. These show that the position of equilibrium is dependant on the chitosan concentration but the rate of establishment of the equilibrium is not under these conditions of excess salicylaldehyde.

3.2.5.4 Determination of the concentration of chitosan in solution using salicylaldehyde

The results given above show that provided the salicylaldehyde is present in excess, the position of equilibrium and hence the

Table 25

Effect of variation in the salicylaldehyde concentration on the formation of N-salicylidene chitosan (followed by uv/visible absorbance spectroscopy at 410 nm in 4 cm cells) at a constant chitosan concentration of 0.022%.

Ratio of amine:salicylaldehyde							
1:3		1:5		1:10		1:20	
Abs.	Time/m	Abs.	Time/m	Abs.	Time/m	Abs.	Time/m
0.013	10	0.024	10	0.125	10	0.1	5
0.047	20	0.184	30	0.195	15	0.214	10
0.084	30	0.22	35	0.275	20	0.315	15
0.119	40	0.288	45	0.425	30	0.407	20
0.150	50	0.412	60	0.72	45	0.668	30
0.182	60	0.54	75	0.938	60	0.92	40
0.271	90	0.81	105	0.065	90	1.030	50
0.361	120	0.96	135	1.085	120	1.07	60
0.576	180	1.036	165	1.087	180	1.078	70
0.787	240	1.064	195	1.083	210	1.083	80
0.936	300	1.073	225	1.080	240	1.081	90
1.020	360	1.078	255	1.079	275	1.079	120
1.075	470	1.087	290			1.069	180
1.087	450					1.076	240

Table 26

Reaction conditions for formation of N-salicylidene chitosan at varying salicylaldehyde concentrations.

Volume of 1% salicylaldehyde/cm^3	Volume of 0.1% chitosan/cm^3	Total volume with MeOH/cm^3	Chitosan:salicylidene ratio
3.3	10	50	1:5
6.5	"	"	1:10
13	"	"	1:20
1.96	"	"	1:3

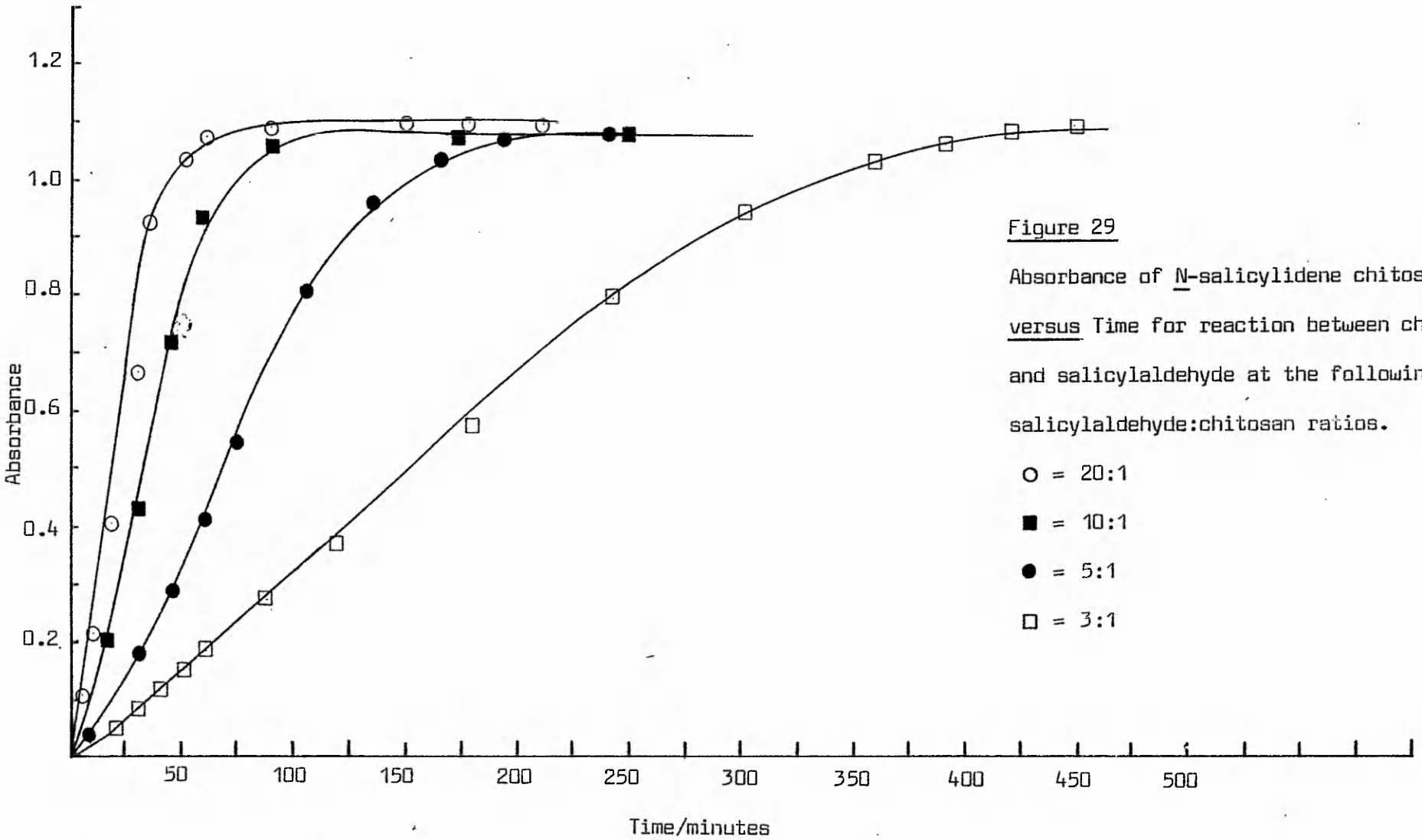


Figure 29

Absorbance of N-salicylidene chitosan versus Time for reaction between chitosan and salicylaldehyde at the following salicylaldehyde:chitosan ratios.

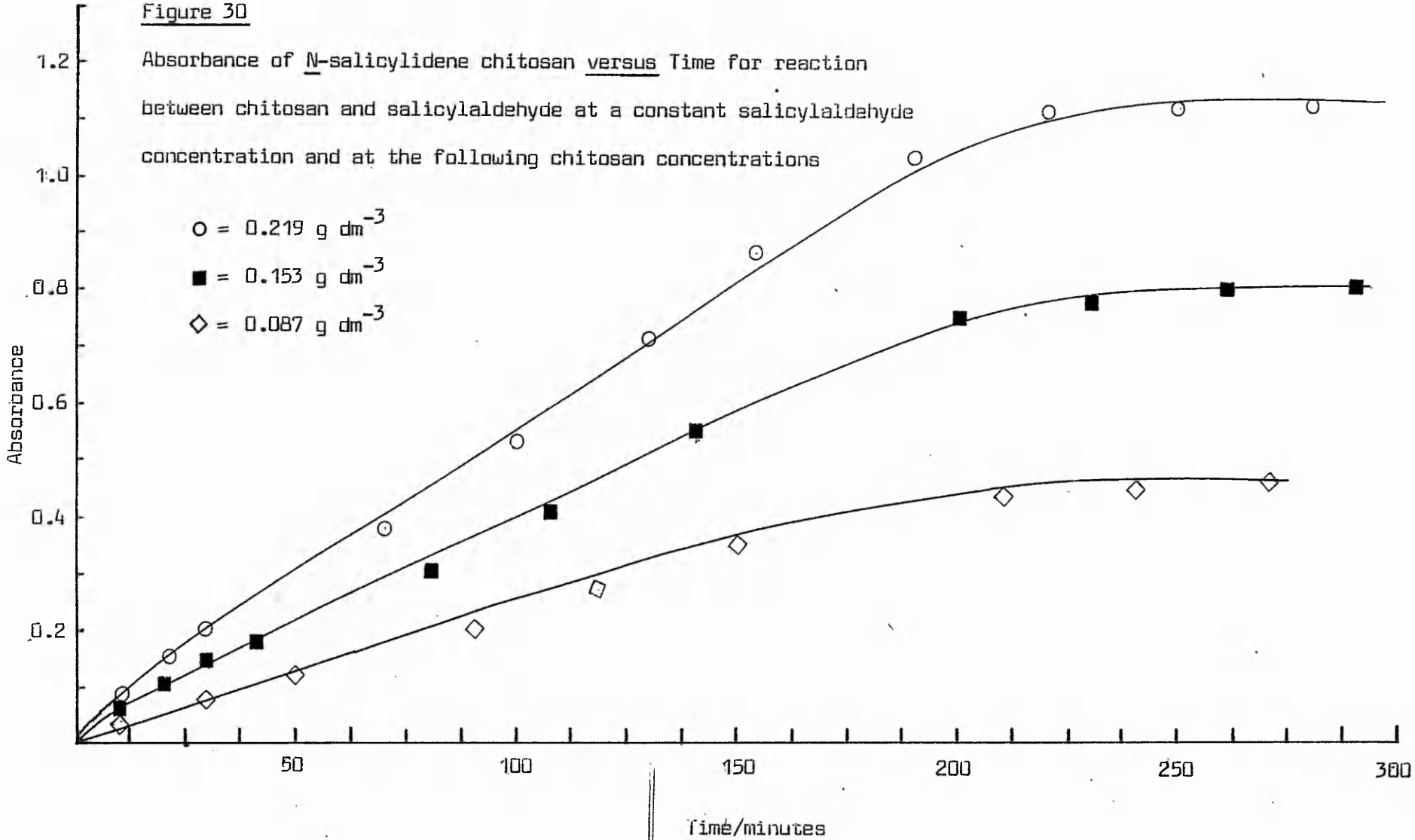
Table 27

Effect of variations in the chitosan concentration on the uv/visible absorbance of the N-salicylidene chitosan, at constant salicylaldehyde concentration (0.00536 mol/dm^{-3}), in 4 cm cells at 410 nm.

Chitosan concentration /g dm^{-3}					
0.0876		0.153		0.219	
Abs.	Time/m	Abs.	Time/m	Abs.	Time/m
0.04	10	0.062	10	0.083	10
0.063	20	0.104	20	0.145	20
0.084	30	0.143	30	0.200	30
0.102	40	0.176	40	0.388	70
0.120	50	0.398	80	0.525	100
0.193	90	0.410	110	0.715	130
0.262	120	0.555	140	1.028	190
0.356	150	0.755	200	1.116	220
0.440	210	0.780	230	1.115	250
0.452	240	0.800	260	1.115	280
0.450	270	0.805	290		
0.460	300				

Figure 30

Absorbance of N-salicylidene chitosan versus Time for reaction between chitosan and salicylaldehyde at a constant salicylaldehyde concentration and at the following chitosan concentrations



absorbance of the solution depends upon the concentration of the chitosan present. Hence using sufficient salicylaldehyde to ensure that the position of equilibrium is reached in a practical time it should be possible to construct an Absorbance versus Concentration plot that can be used for the determination of the concentration of chitosan in solution. Such a plot (Figure 31) was constructed using the reaction conditions outlined in Table 28. An excellent linear relationship was found between absorbance and chitosan concentration, from which it should be possible to determine the concentration of an unknown solution of chitosan. The calibration cannot be applied universally to any random chitosan solution unless the acid conditions of the solution are known to be identical to that used for the calibration plot. However it would be ideal for adsorption studies of chitosan, from solution, onto cellulose by means of residual analysis, as the initial chitosan solution can be prepared according to the required conditions.

3.2.6 Use of hydrobromic acid as an amine specific reagent

3.2.6.1 Introduction

The basic properties of the amine groups on chitosan have been extensively utilised for the determination of the extent of N-acetylation by alkaline titrimetric techniques. (These are discussed in Chapter 2). In this thesis a modification of the method of Hayes and Davies[34] is proposed to confirm the experimental agreement observed between the infrared and residual salicylaldehyde techniques (Figure 28). The method involves titration of water soluble chitosan salts with sodium hydroxide to give a direct estimation of the amine content.

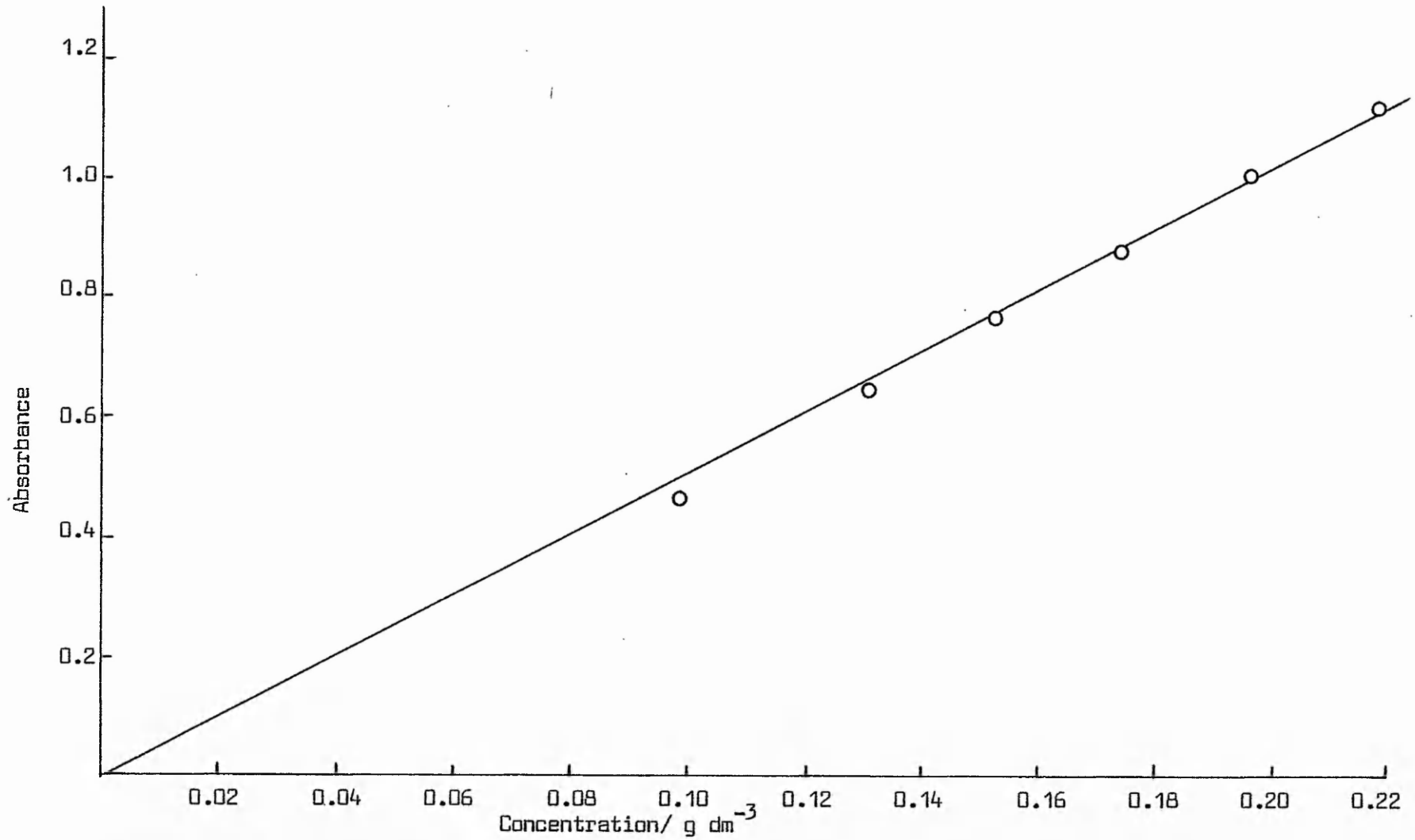


Figure 31 Equilibrium absorbance of N-salicylidene chitosan versus Chitosan concentration.

Table 28

Absorbance/concentration data for solution alalysis of chitosan by formation of N-salicylidene chitosan. Chitosan/acetic acid molar ratio of 2:3 and reaction time of 5 hours at 25°C.

Volume of 0.11% chitosan/cm^3	Volume of 1% salicylaldehyde/cm^3	Volume of water/cm^3	Total volume with MeOH/cm^3	Absorbance at 410 nm in 4 cm cell
10	3.3	0	50	1.117
9	"	1	"	1.002
8	"	2	"	0.873
7	"	3	"	0.765
6	"	4	"	0.648
4.5	"	5.5	"	0.465

The technique has been modified through the manufacture and use of the hydrobromide rather than the hydrochloride salts[34] of chitosan.

3.2.6.2 Production of chitosan hydrobromide salts

The use of chitosan hydrobromide salts for the titration was discounted by Hayes and Davies[34], due to the nature of the product which was found to discolour with time. A similar fate was observed for the hydroiodide salt. However production of the hydrobromide salt by precipitation from a dilute hydrobromic acid solution of chitosan, on addition of concentrated hydrobromic acid, gave a product that could be prevented from discolouring by extremely rigourous washing with methanol. In this way the hydrobromide salt of Kytex H and samples 14-18 were prepared. These are samples of varying degrees of N-acetylation produced initially for characterisation using the residual salicylaldehyde method (see Section 3.2.5.2). The samples prepared in this way could not be dried at elevated temperatures due to potential loss of the acid at such temperatures[46], and hence were treated initially by washing with methanol, which causes solvent exchange with water, and then with ether, which exchanges with the methanol to produce a low temperature drying system. They were then allowed to dry in a vacuum desiccator, over calcium chloride, and stored under these conditions until needed.

3.2.6.3 Titration of chitosan hydrobromide salts with alkali

The titrations were carried out using a standardised 0.1M sodium hydroxide solution and a microburette with phenolphthalein as indicator. The accurately weighed, water-soluble chitosan hydrobromide was dissolved in about 100 cm^3 of water. The solutions tended to turn

cloudy on the addition of a small excess of alkali and the chitosan reprecipitated on addition of a large excess of reagent.

From the value of the titre the number of moles and thus the weight of hydrobromide for each sample can be calculated. This is expressed as a ratio of total weight of sample to the weight of hydrobromide and is used to determine the extent of N-acetylation from a theoretical calibration plot of this ratio against the degree of N-acetylation (Figure 32). The results for the titrations are outlined in Table 29. Two titrations were carried out for each sample, with the average value for the ratio of sample weight to HBr weight being taken.

The values of the degree of N-acetylation obtained in this way, when compared against the values obtained by the infrared method (Table 20) again showed good agreement as indicated by the linearity of a plot of Degree of N-acetylation by infrared spectroscopy versus Degree of N-acetylation by HBr salt titration (Figure 33). The slope and intercept of this plot were found by linear regression to be 1.06 and -1.8 respectively which are reasonably close to the expected values, for complete agreement between the two techniques, of unity for the slope and zero for the intercept.

3.2.7 Studies with radioactive chitin

3.2.7.1 Introduction

The heterogeneous N-acetylation of chitosan can readily be achieved by the action of acetic anhydride in methanol[57]. In theory it should be possible, by the use of labelled acetic anhydride, to obtain a

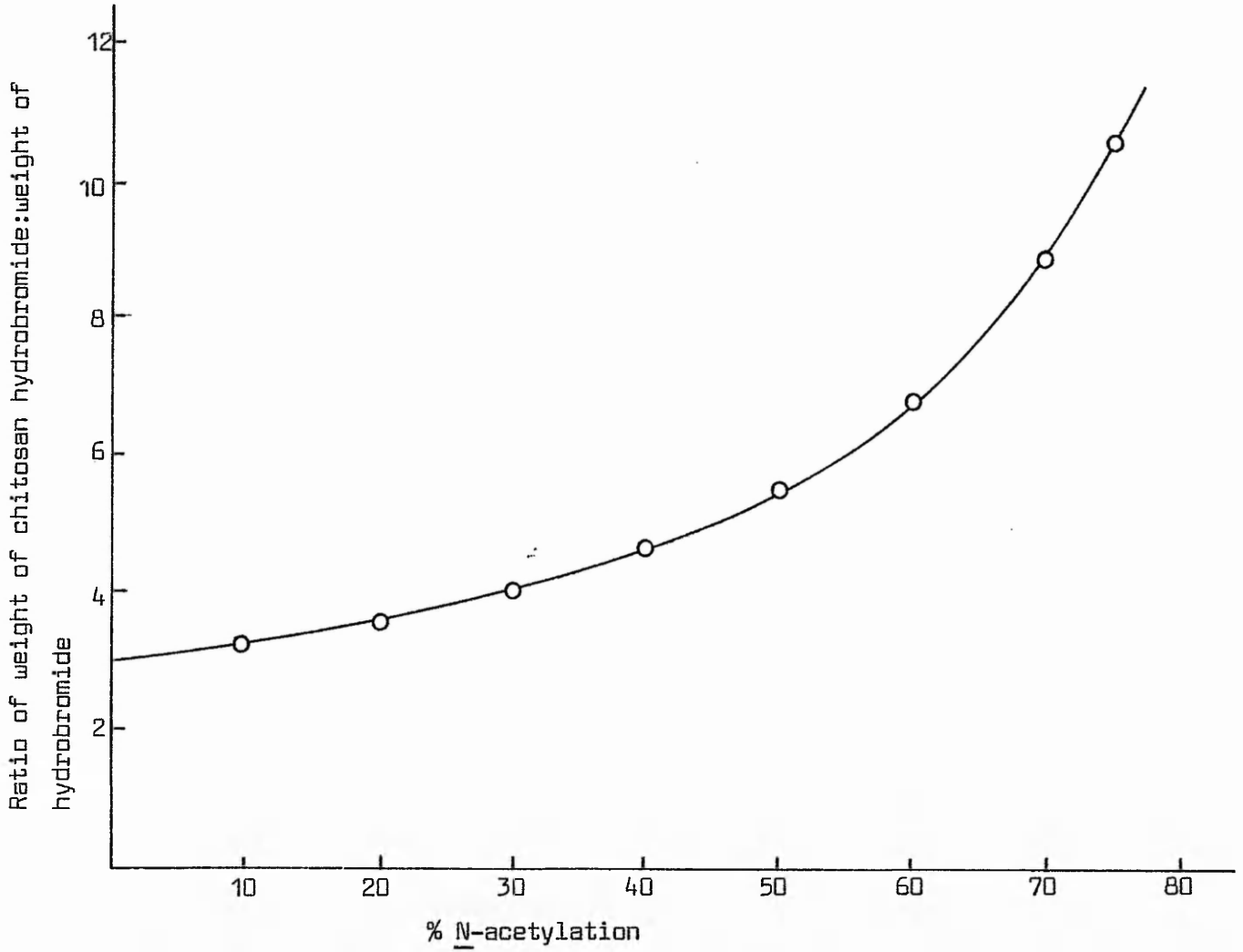


Figure 32 Theoretical calibration plot of Ratio of weight of chitosan hydrobromide: to weight of hydrobromide versus % N-acetylation.

Table 29

Determination of degree of N-acetylation of chitosan hydrobromide salts by titration against 0.1202 mol dm^{-3} NaOH.

Sample origin	Weight/g	Titre/cm^3	Weight of HBr/g	Sample weight /HBr weight	% N-acetyl groups
14	0.1864	2.25	0.0219	8.5	69
14	0.2432	2.95	0.0286	8.5	
15	0.2763	3.4	0.0332	6.8	60.5
15	0.1783	2.7	0.0263	6.8	
16	0.2059	3.75	0.0365	5.64	51
16	0.2546	4.7	0.0458	5.56	
17	0.1053	2.3	0.0220	4.7	41
17	0.1943	4.3	0.0409	4.75	
18	0.1336	3.2	0.0312	4.3	34.5
18	0.1455	3.45	0.0338	4.3	
Kytex H	0.1854	5.14	0.0499	3.7	22.5
Kytex H	0.1854	5.14	0.0499	3.7	

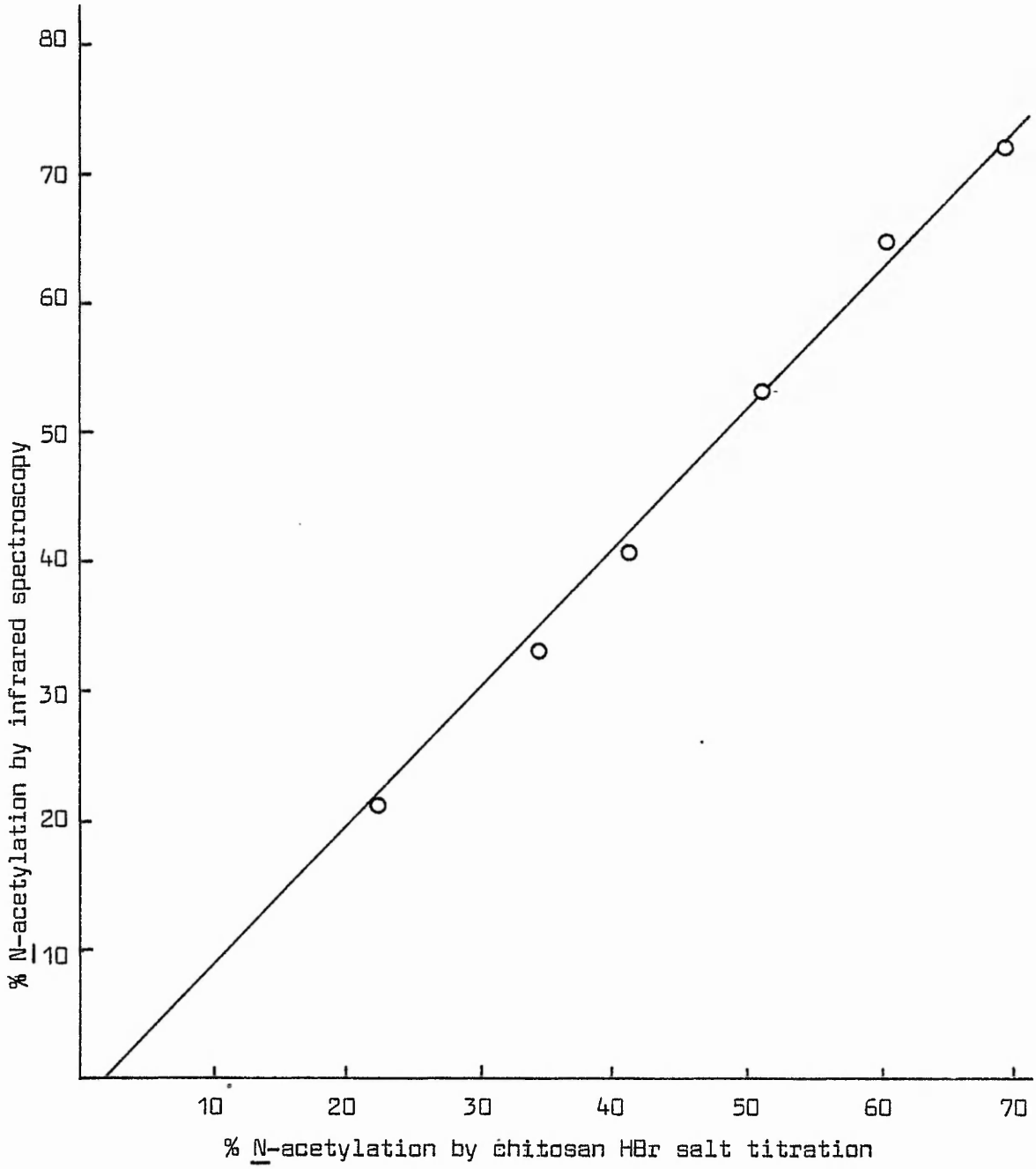


Figure 33 % N-acetylation by infrared spectroscopy versus % N-acetylation by chitosan HBr salt titration.

radioactive chitin where the level of the radioactivity will be dependant upon the free amine content of the original chitosan. ^{3}H labelled acetic anhydride has been used by Araki and Ito[103], for the N-acetylation of glycol chitin. This product was then enzymically deacetylated to liberate labelled acetic acid and the extent of deacetylation determined by extraction of the acetic acid from the aqueous phase into ethyl acetate followed by scintillation counting of the extracted phase.

3.2.7.2 ^{14}C labelled acetic anhydride

A sample of ^{14}C labelled acetic anhydride was used in which the labelled carbon atom is contained in only one of the acetyl groups of the molecule. The sample, when calibrated in 1972, contained 1×10^{3} μCi of radiation per 100 cm^{3}, with a sufficiently long half life to assume that this value would be constant. As direct use of this sample would be expensive as well as producing a product with an excessive radioactivity count, dilution with unlabelled acetic anhydride was necessary to give counts of the order of 10^{4} per minute. Thus when applied to chitosan it must be assumed that there is an equal probability of reaction of labelled and unlabelled acetic anhydride with the amine groups, and an equal probability of either of the two acetyl groups on the reagent being attached to the nitrogen of the chitosan. In order to obtain a value for the radioactive content in such a diluted system it is necessary to carry out a calibration.

3.2.7.3 Liquid scintillation counting

This involves the use of a substance which acts as a scintillator in that it emits a weak flash of light, of short duration, when struck

by an ionising particle. Measurement of this emitted light is the basis of scintillation counting. The liquid scintillator used was 2,5-diphenyloxazole (PPO) in toluene. For counting, a solution was prepared by dilution of the standard acetic anhydride solution, and counted in the scintillation fluid. Counting was repeated after the addition of excess unlabelled acetic anhydride to the system. The results in Table 30 show a drastic reduction in counting efficiency caused by the addition of the excess reagent and it is clear that acetic anhydride quenches the system. No counter is ever 100% efficient at detecting radioactive decays; they lose efficiency because samples contain materials which decrease the number of photons detected. This is known as quenching and in this instance is clearly occuring due to the presence of the acetic anhydride. Because of quenching the number of counts per minute (cpm) measured is less than the number of disintergrations per minute (dpm) which occur in the sample. Percentage counting efficiency is given as 100 x cpm/dpm. It is possible to correct for quenching by investigating the effect of any particular quenching agent on a series of samples of known efficiency.

3.2.7.4 Quench correction determination

For acetic anhydride the channels ratio method was used. The counting channel is split into two, a lower part and an upper part. The effect of quenching is to increase the net count rate in the lower and reduce it in the upper. The ratio of the upper to lower count rates is known as the channels ratio and is a measure of the quenching factor and is independant of the sample activity.

Table 30

Effect of acetic anhydride on scintillation counting of C^{14}-labelled acetic anhydride.

Stock solution of 1000 $\times 10^{-6}$ Ci/100 cm^3 diluted 2.7 times and this diluted a further 100 times.

Weight of diluted acetic anhydride/g	Weight of scintillation fluid/g	C^{14} counts/cpm (before addition)	Volume of acetic anhydride added/ cm^3	C^{14} counts/cpm (after addition)
1.0744	8.936	30,810	2	10,371
"	"	30,487	2	10,423
"	"	30,422	2	10,423

In practical terms this involves the preparation of a series of samples contianing known amounts of a standard, in this case ^{14}C-labelled hexadecane. The standard contains a precise and known amount of radioactivity usually expressed in terms of the dpm per g of material. A solution of the standard ^{14}C-labelled hexadecane was made up in the scintillation fluid and a series of samples prepared, each containing different volumes of added acetic anhydride. Counting was carried out at two different channel widths for each sample, a wide open channel and a narrow channel. The selection of the narrow channel depends on the quenching agent and for acetic anhydride the best results were obtained using a 50-100 channel width. For each sample the counting efficiency was calculated from the cpm in the wide open channel and knowledge of the dpm of the standard. Also the ratio of the counts in the narrow to wide channels were calculated (see Table 31). A plot of Counting efficiency versus Channels ratio gives a straight line (Figure 34) which can be used to calibrate a ^{14}C sample in the presence of acetic anhydride by measurement of the channels ratio under identical channel width conditions. The value obtained gives the counting efficiency from which the dpm of the sample can be found from the value of cpm obtained in the wide open channel. Thus for the diluted radioactive acetic anhydride solution, originally used for counting, the channels ratio at 50-100 to 50-1000 was determined (Table 32), and found to be 0.65. This corresponds to a percent efficiency from Figure 34 of 38%, and using the cpm of 30,803 from the wide open channel (50-1000) the dpm of the sample corresponds to 81,060, which is close to that of the theoretical value based on the manufacturers data. The initial dilution of the acetic anhydride standard of 2.7 times was designed for its reaction with chitosan, in order to produce

Table 31

Quench correction for acetic anhydride on C^{14} hexadecane.

Standard hexadecane = 1.117×10^{6} dpm/g

Stock solution = 0.4321 g in 250 cm^3

Volume of stock/cm^3	Volume of acetic anhydride/cm^3	dpm	50-1000 channel counts/cpm	50-100 channel counts/cpm	Channel ratio	% counting efficiency
10	0	19,306	14877	2574	0.173	77
"	0.05	"	14263	2905	0.204	74
"	0.1	"	13469	3580	0.266	68
"	0.2	"	12284	4001	0.326	64
"	0.3	"	11284	4266	0.378	58
"	0.5	"	9525	4589	0.482	49
"	0.7	"	7944	4589	0.578	41
"	1.0	"	6050	4050	0.670	31
"	1.5	"	3877	3092	0.798	20

Table 32

Channels ratio determination of quench for diluted C^{14}-labelled solution of acetic anhydride.

Diluted solution is 1/2700 as concentrated as the standard 1000×10^{-6} Ci/100 cm^3 stock acetic anhydride (1×10^{-6} Ci = 2.22×10^{-6} dpm).

Weight of diluted acetic anhydride/g	Theoretical dpm	Weight of scintiallation fluid/g	50-1000 channel count/cpm	50-100 channel count/cpm	Channel ratio
1.0744	80,960	8.9356	30,803	20,067	0.65

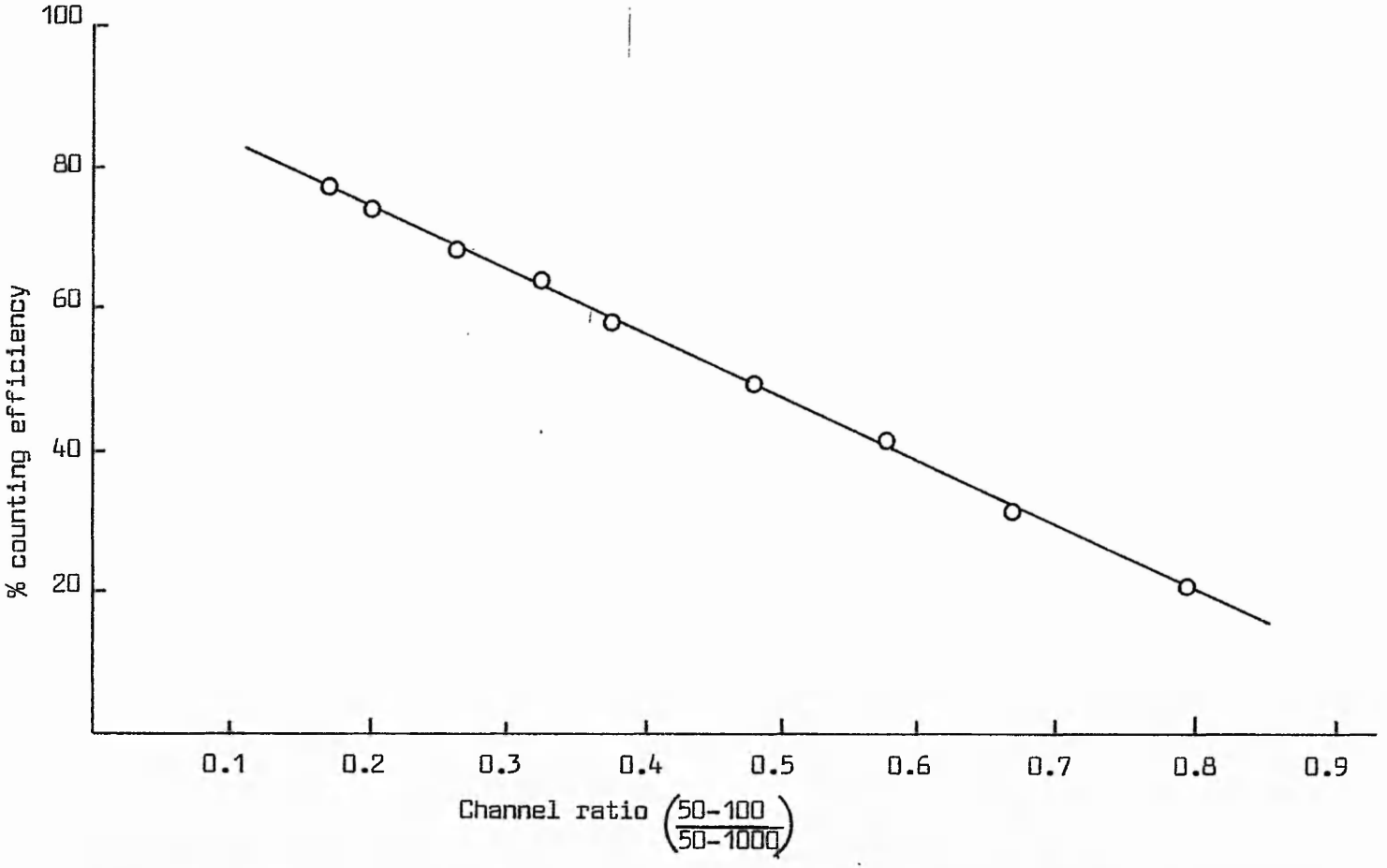


Figure 34 % counting efficiency versus the 50-100 to 50-1000 channels ratio for quenching caused by acetic anhydride on ^{14}C-hexadecane.

a product with a sufficiently high count rate, based on a sample weight of 40 mg of highly N-acetylated chitosan. For progressively lower degrees of N-acetylation the product count rate would increase using the same diluted solution. This is desirable since less counting errors are incurred at the higher count rates.

3.2.7.5 Application of ^{14}C-labelled acetic anhydride to chitosan

Difficulties were encountered in the application of labelled acetic anhydride to the N-acetylation of chitosan, due to the safety regulations preventing use of radioactive material on general purpose spectroscopic instruments. Thus it was not possible to carry out infrared determinations to see if N-acetylation had gone to completion or indeed if any O-acetylation has occured. Although the acetylation of the hydroxyl groups on chitosan is more difficult to achieve under the conditions used for N-acetylation, Moore and Roberts[57] have shown that some O-acetylation does occur after N-acetylation is complete. This can be demonstrated by the appearance of a band at 1740 cm^{-1} in the infrared spectrum. Any O-acetylation would provide extra labelled acetyl groups in addition to those of the amide groups and would render any results invalid.

Another more fundamental problem associated with the method is the insolublity of the product in all but concentrated acid. The toluene-based scintillation fluid cannot tolerate the addition of much aqueous media and as yet no system capable of dilution with aqueous acid has been developed that also maintains a reasonable counting efficiency. Thus although in theory the use of radio isotopes should provide a highly accurate method for analysis of amine

groups on chitosan, the practical difficulties encountered would require considerable further investigation. Such an investigation was not considered relevant to the main aim of the thesis, particularly as other techniques were simultaneously developed for characterising chitosan.

3.3 Adsorption of chitosan on paper

3.3.1 Investigation of the use of reflectance measurements for determining the concentration of chitosan adsorbed on paper

3.3.1.1 Introduction

If the effects of chitosan on the physical properties of paper are to be assessed it is essential that the chitosan content of the paper be accurately determined. The method previously used was based on a modification of the Kjedahl procedure[113]. Kjedahl techniques involve the formation of ammonia from any nitrogeneous materials, and subsequent titrimetric methods to determine the amount of ammonia liberated. However at the lower levels of chitosan addition to paper the nitrogen content is extremely small and thus the method may be prone to errors. For a 0.1% chitosan concentration on weight of fibre (o.w.f.) the nitrogen content of the treated paper would be 0.007%. Thus investigations were undertaken to provide a reliable technique for the estimation of chitosan on paper.

By the use of a colorimetric amine specific reagent it should be possible to react the chitosan present in paper with this reagent to obtain a coloured product, in which the intensity of the colour will be dependant on the chitosan content of the paper.

In the dyeing industry reflectance measurements on dyed samples have been used and related to the concentration of dye, so that the dye content of unknown samples can be determined from measurement of their reflectance under identical conditions. A similar method is proposed for the coloured paper, although it is restricted to bleached pulps which have a reasonably white appearance.

The amine-specific reagent chosen was salicylaldehyde since it fits all the requirements for the reflectance method when applied to chitosan on paper; the reaction between chitosan and the reagent goes to completion, its product, a chelated Schiff's base, absorbs in the visible region of the spectrum, and the paper is unaffected by the salicylaldehyde.

The reflectance characteristics of N-salicylidene chitosan when associated with paper were determined using a sample prepared by steeping a piece of filter paper in a solution of chitosan and pressing out the excess reagent. When dry the treated paper was reacted with a large excess of salicylaldehyde in methanol, then washed and soxhlet extracted with methanol to remove any unreacted salicylaldehyde. The reflectance of this sample measured against an untreated filter paper as standard, was recorded at various wavelengths on a single beam spectrophotometer. A plot of % Reflectance versus Wavelength (Figure 35) reveals that the wavelength of maximum absorption for the system is 408 nm.

In an attempt to rationalise the empirical relationship that exists between the reflectance and the colourant concentration an equation (14) has been devised.

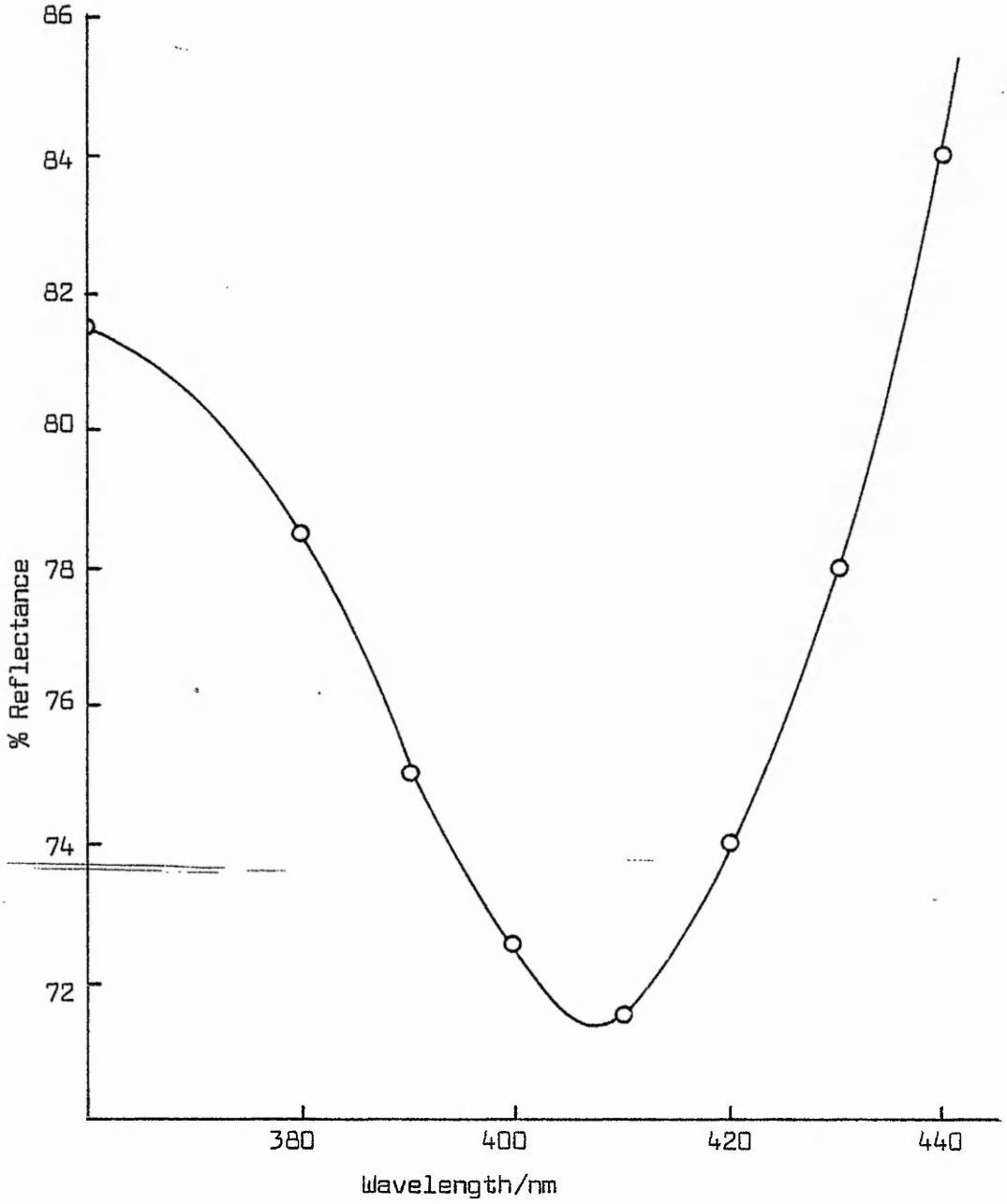


Figure 35 % reflectance versus Wavelength for N-salicylidene chitosan-paper.

$$Kc = F(R) \quad \ldots\ldots\ldots\ldots\ldots\ldots (14)$$

where K is a constant for a particular colourant, wavelength and substrate, and F(R) is some function of reflectance.

There are a number of ways in which the reflectance function can be determined, however the technique according to Kubelka and Munk[114] has widespread application. This relates the reflectance to the absorption and scattering coefficients of the ingredients, through equation (15)

$$F(R) = \frac{K}{S} = \frac{(1-R)^2}{2R} \quad \ldots\ldots\ldots\ldots (15)$$

where K = absorption coefficient

S = scattering coefficient

R = fractional reflectance

Beer's Law indicates that K is proportional to concentration, but S is independant of concentration. Combination of equations (14) and (15) lead to equation (16)

$$Kc = \frac{(1-R)^2}{2R} \quad \ldots\ldots\ldots\ldots\ldots\ldots (16)$$

From this the reflectance can be related to the colourant concentration. This simple equation is only applicable if the reflectance of the substrate is 100%. The substrate normally has an absorption coefficient which adds to that of the colourant. Therefore the fractional reflectance of the substrate must also be determined and leads to equation (17)

$$Kc = \frac{(1-R)^2}{2R} - \frac{(1-Rt)^2}{2Rt} \quad \ldots\ldots\ldots (17)$$

where Rt = fractional reflectance of substrate.

For high concentrations the reflectance function of the substrate is small compared to that of the colourant and can thus be ignored. It can however be completely removed by measurement of the reflectance of the colourant sample against the substrate rather than an arbitrary white standard.

The applicability of the Kubelka-Munk theory to any particular system can be assessed by means of a logarithmic expansion of equation (14) to give equation (18)

$$\log K + \log c = \log F(R) \quad \ldots\ldots\ldots\ldots (18)$$

Thus if the theory holds for a particular situation a plot of log F(R) versus log c should be linear with a slope of unity.

The constant K has to be initially evaluated by the construction of a calibration plot involving measurement of the reflectance of a series of samples of known concentration. Hence for application to the N-salicylidene chitosan-paper system the chitosan concentration must be determined initially by other means.

Two methods were used to determine the absolute chitosan content of paper. The first involves the deposition of a known quantity of chitosan onto preformed handsheets of paper. The second is a direct, novel and facile analytical technique for the determination of chitosan after it has been applied to the paper.

For this investigation a number of different pulps were chosen, the details of which are outlined in Table 33. They were formed into handsheets according to the TAPPI standard[115].

Table 33

Pulp	Description	Typical acid content
Stora 32	Bleached softwood kraft	140-350 milliequivalents/Kg
Stora 61	" hardwood Kraft	"
Port Hudson	" softwood kraft	"
Tyee	" softwood kraft	"
New Bern	" softwood kraft	"
Birch	" hardwood	-

3.3.1.2 Correlation of reflectance measurements with chitosan concentration determined by a gravimetric method

The amount of chitosan deposited on to a known weight of paper was found by determining the quantity of a solution of chitosan of known concentration, taken up during steeping of the paper in that solution. This was achieved by pressing out the excess solution with blotters and reweighing. The method relies on the assumption that the increase in weight is due solely to the presence of a solution of known concentration so that on evaporation of the solvent the weight of deposited chitosan can be calculated. Also to minimise the possibility of preferential adsorption of chitosan from solution onto the fibre, the time of steeping was kept very short, around 30 seconds. The solutions used were prepared in dilute acetic acid and the solvent evaporated off after steeping by heating at $50^{o}C$ in an oven. It is essential to evaporate the solvent rapidly to prevent migration of the solution which will cause a concentration gradient effect resulting in an uneven surface coverage.

When dry the paper samples were treated in an excess of salicylaldehyde (400 cm^3 of 1% salicylaldehyde in methanol per g of paper) for 16 hours, to ensure complete formation of the Schiff's base derivative. It is essential to thoroughly wash the samples with copious quantities of methanol, and soxhlet extract with methanol in the presence of a salicylaldehyde scavenger such as a solid primary amine, to remove any excess unreacted reagent that otherwise might interfere with the reflectance readings taken at 408 nm. These readings were carried out on a triple thickness of handsheet against an untreated standard prepared form the same pulp source. Measurements

were taken on both sides of the paper at four different positions per side by rotating the sample holder through 90° each time. The mean value of these were taken so that any uneven chitosan uptake could be averaged out. The results in terms of chitosan concentration expressed as a percentage on weight of fibre (o.w.f.), and the Kubelka-Munk reflectance function F(R) are given in Table 34. The numerous individual results from which this Table was constructed are given in Appendix II. For each of the pulps a plot of Log F(R) versus Log c was constructed (Figures 36 and 37) and from the intercept which is equal to log K, the constant K was determined. Also the slope of the line was calculated to give an indication of the applicability of the data to analysis by the Kubelka-Munk treatment. Details are summerised in Table 35. From these results it can be seen that the value of K varies from pulp to pulp as would be expected since it is dependant upon the nature of the substrate. The slope of the line for the Stora 61 and Stora 32 pulps were reasonably close to unity indicating that for these pulps the reflectance data fits the mathematical model of the Kubelka-Munk theory. However this was not the case for the other pulps, possibly due to the specific nature of the surface of the material, as shown by the excessive deviation of the slopes of the lines from unity. Nevertheless the reasonable linearity of the plots would still allow the use of the K values for the pulps under the conditions used for chitosan deposition and within the same chitosan concentration range.

Any possible errors in the evaluation of the chitosan concentration caused by preferential adsorption of chitosan from solution during deposition can be eliminated by the use of a direct analysis technique

Table 34

Chitosan concentration (measured by deposition)/reflectance data for N-salicylidene chitosan-paper samples.

Chitosan concentration /% o.w.f.	Log concentration	% average reflectance	F(R)	Log F(R)
		Stora 32		
0.209	-0.68	64.3	0.099	-1.004
0.197	-0.706	68.9	0.070	-1.154
0.42	-0.377	52.0	0.220	-0.657
0.438	-0.359	52.7	0.212	-0.673
1.26	0.100	33.8	0.652	-0.186
1.22	0.086	30.5	0.793	-0.101
0.394	-0.404	54.0	0.196	-0.708
0.514	-0.289	50.2	0.247	-0.607
0.586	-0.232	46.0	0.319	-0.496
0.403	-0.394	53.5	0.202	-0.695
0.558	-0.254	47.5	0.292	-0.534
0.545	-0.253	48.5	0.270	-0.568
0.24	-0.62	65.7	0.089	-1.05
0.227	-0.64	65.6	0.091	-1.04
0.74	-0.13	46.6	0.309	-0.51
0.816	-0.09	39.8	0.455	-0.342

Table 34 continued

Stora 61

Chitosan concentration /% o.w.f.	Log concentration	% average reflectance	F(R)	Log F(R)
0.24	-0.625	64.8	0.096	-1.018
0.215	-0.667	68.0	0.076	-1.120
0.540	-0.266	51.8	0.224	-0.650
0.580	-0.233	50.8	0.239	-0.622
0.96	-0.019	43.0	0.379	-0.421
0.936	-0.029	44.0	0.356	-0.448
1.270	0.104	38.0	0.506	-0.396
1.250	0.098	36.9	0.540	-0.267

Tyee

0.251	-0.600	73.3	0.049	-1.311
0.462	-0.336	57.3	0.160	-0.797
0.515	-0.289	67.9	0.109	-0.961
0.439	-0.358	69.3	0.068	-1.166
0.817	-0.088	51.9	0.223	-0.651
0.784	-0.106	54.4	0.191	-0.718
1.240	0.093	42.6	0.386	-0.413
1.580	0.199	37.6	0.518	-0.286

Table 34 continued

New Bern

Chitosan concentration /% o.w.f.	Log concentration	% average reflectance	F(R)	Log F(R)
0.211	-0.676	81.6	0.021	-1.684
0.208	-0.681	77.3	0.033	-1.475
0.412	-0.385	67.6	0.077	-1.11
0.390	-0.409	67.4	0.079	-1.102
0.729	-0.137	59.8	0.136	-0.868
0.716	-0.145	56.4	0.169	-0.773
1.040	0.017	46.5	0.308	-0.513
1.116	0.048	40.5	0.437	-0.359

Port Hudson

0.209	-0.680	78.6	0.029	-1.540
0.221	-0.656	78.6	0.029	-1.540
0.400	-0.389	67.7	0.077	-1.112
0.417	-0.380	67.6	0.077	-1.110
0.679	-0.168	55.9	0.173	-0.761
0.713	-0.196	53.1	0.202	-0.684
1.062	0.001	43.5	0.367	-0.435
0.880	-0.055	50.3	0.246	-0.609

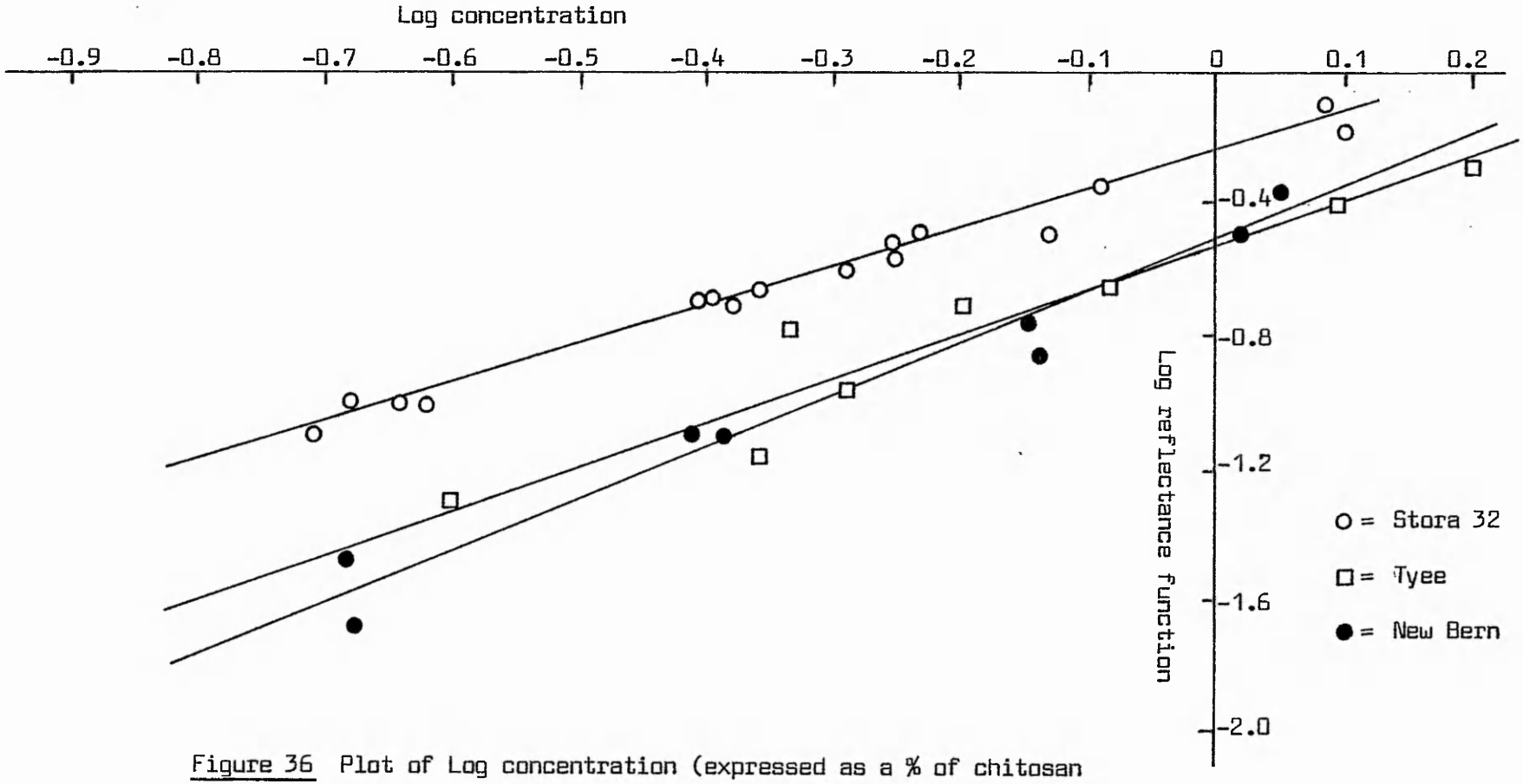


Figure 36 Plot of Log concentration (expressed as a % of chitosan on weight of fibre) versus Log of the Kubelka-Munk reflectance function.

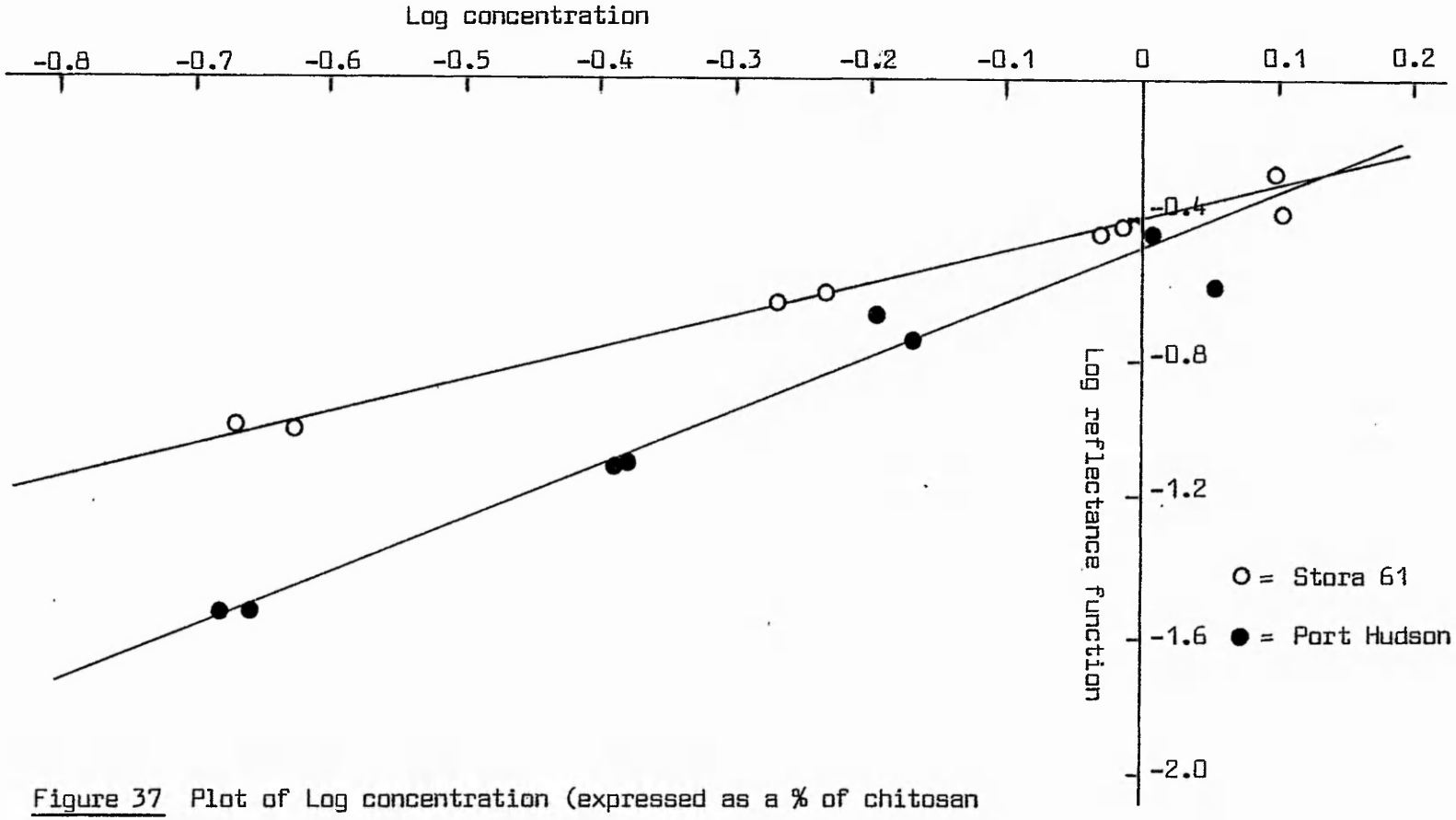


Figure 37 Plot of Log concentration (expressed as a % of chitosan on weight of fibre) versus Log of the Kubelka-Munk reflectance function.

Table 35

Regression analysis of results for plots of Log F(R) versus Log C for various pulps.

Pulp	Intercept	Slope	R^2	K
Stora 61	-0.392	1.042	0.995	0.406
Stora 32	-0.253	1.197	0.976	0.558
New Bern	-0.527	1.545	0.963	0.297
Port Hudson	-0.479	1.597	0.995	0.332
Tyee	-0.547	1.297	0.929	0.284

based on the determination of the chitosan content after deposition and drying.

3.3.1.3 Correlation of reflectance measurements with chitosan concentrations determined by a hydrolysis technique

The direct analysis of the chitosan content in paper is based on the formation of N-salicylidene chitosan and its hydrolysis to its parent constituents namely chitosan and salicylaldehyde. In the preparation of di-O-arylcarbamate-N-acetyl chitosan[94], the formation of N-salicylidene chitosan has been used to protect the amine group during O-arylcarbamate formation. This N-salicylidene product was then hydrolysed to allow N-acetylation to occur. Hydrolysis can readily be achieved using a strongly ionising acid such as hydrochloric acid or hydrobromic acid. This can be seen by the inability of the chitosan hydrobromide salt to undergo reaction with salicylaldehyde (see Section 3.2.5.3). ~~Hence if the~~ quantitative reaction of the free amine groups of chitosan, on paper, with salicylaldehyde can be achieved the yellow N-salicylidene chitosan produced can be readily hydrolysed to its parent components. The salicylaldehyde thus liberated can be spectroscopically analysed and the amount related to the initial chitosan content. These facts were established by treatment of a N-salicylidene chitosan film with a 80/20 (v/v) methanol/0.5N HBr mixed solvent for 16 hours at room temperature. The uv spectrum of the resulting solution showed absorption bands at 255 nm and 326 nm, which are identical to those found for salicylaldehyde in methanol.

There are three main factors that influence the choice of the hydrolysis medium:

a) There must be sufficient acid present to completely hydrolyse the Schiff's base. This is more rapidly achieved if the acid can also dissolve the chitosan. The presence of dissolved chitosan in the solution may interfere with the spectroscopic analysis of the salicylaldehyde hydrolysed off. Broussignac[12] has shown that a 3% chitosan solution has a uv absorbance intensity of about 0.5 at 255 nm in 1 cm cells, which is the wavelength of the highest intensity absorption band of salicylaldehyde. However the amount of salicylaldehyde required to give an "on scale" uv absorbance reading at this wavelength would correspond approximately to a chitosan concentration of 0.003%. Since this is 1000 times more dilute than the solution used by Broussignac the residual absorbance of the dissolved chitosan can be assumed to be negligible.

b) Since the initial stages of the hydrolysis, prior to dissolution of the chitosan, are carried out heterogeneously the reaction requires a considerable period of time for completion (16 hours). Thus the liberated salicylaldehyde must be stable for reasonably long periods.

c) The solvent must contain sufficient methanol to allow the hydrophobic salicylaldehyde to completely dissolve.

These factors were found to be completely satisfied by the use of a 80/20 (v/v) methanol/0.5N HBr solvent. The molar extinction coefficient for salicylaldehyde was determined in this solvent at 255 nm (Table 36) and found to be 10,205 which is in good agreement to that for salicylaldehyde in an 80/20 (v/v) methanol/1% acetic acid system (see Section 3.2.5.2).

Table 36

Uv absorbance data for determination of molar extinction coefficient of salicylaldehyde in 80/20 (v/v) methanol/0.5N HBr, at 255 nm in 1 cm cells.

Weight of salicylaldehyde/g	Volume of solution of salicylaldehyde /cm^3	Dilution factor	Absorbance	Extinction coefficient
0.2451	50	400	1.0241	10,205

Table 37

Uv spectroscopic analysis of salicylaldehyde, hydrolysed from N-salicylidene chitosan samples (from Kytex L).

Weight of chitosan/g	Volume of hydrolysis solution/cm^3	Dilution factor	Absorbance 255 nm 1 cm cells	Chitosan concentration /g dm^{-3}
0.0122	20	50	0.557	0.0122
0.0088	"	50	0.473	0.0088
0.0049	"	50	0.221	0.0049
0.0287	"	50	1.384	0.0287

For application of the hydrolysis technique to chitosan adsorbed on paper, calibration with an identical chitosan sample must be carried out so that the salicylaldedhyde absorbance intensity at 255 nm can be directly related to the chitosan concentration in the solution.

The calibration was carried out using a sample of Kytex L in the form of a thin film. Four pieces of dried film of different weights were used. They were initially treated with an excess of salicylaldehyde in methanol (1000 cm^3 of 1% salicylaldehyde per g of chitosan) for 48 hours. Removal of excess reagent after the formation of the N-salicylidene chitosan was achieved by careful washing with methanol and then by soxhlet extraction with methanol. The films were treated for 16 hours with the methanol/HBr solvent, after which the solutions were analysed by uv spectroscopy. The results are given in Table 37. From Beer's Law the absorbance of the solution is proportional to the concentration of salicylaldehyde. However since the salicylaldehyde concentration is proportional to the chitosan content it follows that a direct plot of Absorbance versus Chitosan concentration can be constructed to obtain an extinction coefficient expressed in g dm^{-3} (Figure 38). This was found from the slope to be 48.4 and can be applied to any system with a chitosan having a similar degree of N-acetylation.

Since the technique relies on the quantitative reaction between the amine groups of chitosan and salicylaldehyde, as well as the quantitative hydrolysis of the product, it will also give a value for the degree of N-acetylation of the Kytex L used. This can be achieved by calculating the ratio of chitosan used to the amount of

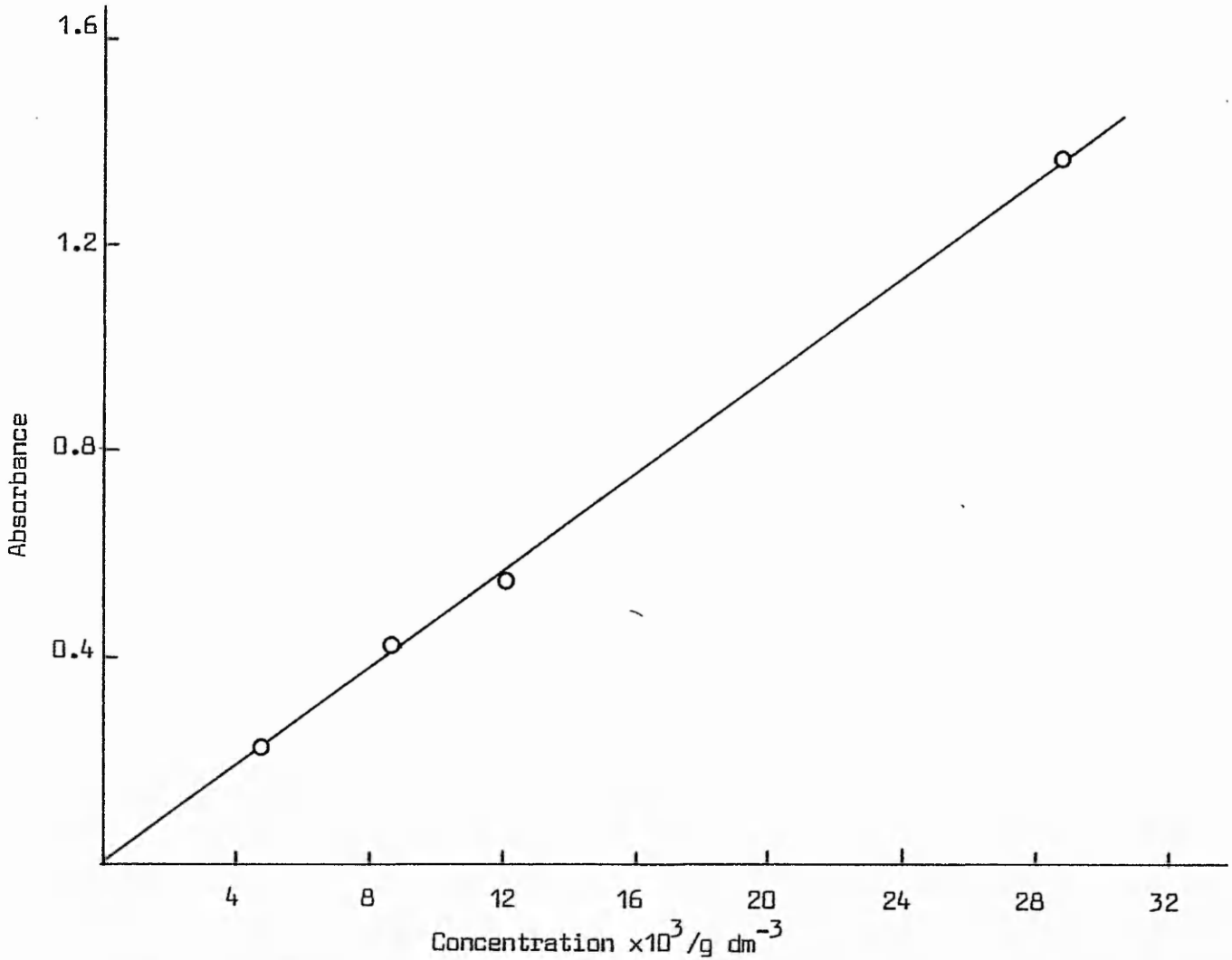


Figure 38 Absorbance of salicylaldehyde hydrolysed from N-salicylidene chitosan versus Chitosan concentration.

salicylaldehyde hydrolysed off from the N-salicylidene chitosan and by using this ratio with the calibration curve for the residual chitosan analysis technique (see Section 3.2.5.2, Figure 27). Using the data in Table 37, the ratio of chitosan:salicylaldehyde was found to be 1.73, which corresponds to a degree of N-acetylation of 17%, from the calibration plot. This fits reasonably well with the values of 16% and 14% found by infrared spectroscopy and residual salicylaldehyde analysis methods respectively. The good agreement also confirms that both the reaction with salicylaldehyde and the subsequent hydrolysis of the Schiff's base are occuring completely quantitatively.

For use of the hydrolysis method with chitosan treated papers a modification to the deposition technique for chitosan application is required. It is not practical to weigh the paper before steeping in a chitosan solution, since further wet treatments will be required during which the paper will tend to lose fines. After steeping and pressing out the excess chitosan solution the handsheets were allowed to dry in air, rather than in an oven, to prevent the formation of an amide from the acetate[46] which would alter the free amine content of the material. Drying was carried out with the sheet fixed in a horizontal position so that any solution migration would occur in the plane of the paper thickness. In this way any uneveness in the nature of the surface, with respect to measurement of its reflectance, can be averaged out over both sides of the sheet. After drying the sheet was immersed in methanolic ammonia to desalt the chitosan and thus obtain it in the free amine form. Reaction with salicylaldehyde and subsequent aftertreatment with methanol was carried out in an

identical manner to that used for treatment of the chitosan for the calibration. At this stage, prior to hydrolysis, part of the paper sample was used for a moisture determination so that the dry weight of the material could be determined. Because chitosan concentrations on paper are usually given in terms of a percentage on weight of dry fibre, in the final analysis a correction factor is used to adjust the weight of the N-salicylidene chitosan-paper system to that for the paper alone. This adjustment involves the subtraction from the dry weight of the N-salicylidene chitosan-paper system the weight of the N-salicylidene chitosan which is 1.513 times (based on a degree of N-acetylation of 17%) the weight of the chitosan present in the paper. The difference between the dry weight of N-salicylidene-paper and that of the paper alone is usually very small, and is of little significance except at the higher levels of chitosan content. Hydrolysis of the N-salicylidene chitosan-paper system was readily achieved using the acidic methanol solvent for 16 hours, as indicated by the loss of the yellow tint in the paper after this time. Initial experiments were carried out using Kytex L on Stora 61 handsheets, at four different levels of chitosan impregnation. This was achieved by adjusting the concentration of the solution of chitosan used for steeping. A sufficient quantity of each sample was prepared so that prior to hydrolysis the reflectance of the sheet could also be recorded. This was carried out in a similar manner to that previously described (see Section 3.3.1.2) except that a magnesium carbonate block was used as a standard against which the untreated pulp was also calibrated to take into account the reflectance of the substrate. The results for the hydrolysis analysis and the reflectance data are outlined in Tables 38. The chitosan content of the samples

Table 38

Chitosan content analysis, by hydrolysis of N-salicylidene chitosan-paper, for Stora 61 handsheets; with associated reflectance data at 410 nm against a magnesium carbonate standard.

Dry weight of N-salicylidene chitosan-paper/g	Dry weight of paper/g	Volume of hydrolysis solvent/cm^3	Dilution factor	Absorbance at 255 nm in 1 cm cells	Chitosan content/g	Concentration of chitosan/% o.w.f	Log conc.
0.2266	0.2238	20	10	0.450	1.86×10^{-3}	0.831	-0.80
0.2167	0.2156	20	5	0.340	7.02×10^{-4}	0.326	-0.487
0.2224	0.2204	20	10	0.320	1.32×10^{-3}	0.600	-0.222
0.2704	0.2393	20	10	0.580	2.40×10^{-3}	1.002	0.001

Reflectance data for the above samples

Concentration of chitosan/% o.w.f	% average reflectance of sample	% average reflectance of substrate	Reflectance function F(R)	Log F(R)
0.831	36.5	86.5	0.542	-0.266
0.326	50.6	"	0.231	-0.636
0.600	41.0	"	0.412	-0.385
1.002	31.4	"	0.737	-0.132

were calculated using equation (19).

$$\% \text{ chitosan content} = 100(A \times D \times V/1000\varepsilon) \,/\, [W-(A \times V \times F \times D/1000\varepsilon)] \quad ..(19)$$

where A = absorbance of hydrolysis solution at 255 nm in 1 cm cells

D = dilution factor

V = volume of hydrolysis solution in cm^3

ε = extinction coefficient (48.4 for Kytex L)

W = dry weight of N-salicylidene chitosan-paper

F = ratio of N-salicylidene chitosan to chitosan = 1.513 for the 17% N-acetylated Kytex L

For ease of calculation a simple computer program was written in Basic (see Appendix III), that also calculates the Kubelka-Munk reflectance function for each sample from its reflectance measurement.

For Stora 61 a regression analysis of the plot of Log F(R) versus Log c (Figure 39) gave a slope of 0.995, which is a good fit for the Kubelka-Munk treatment, although the value for K, the antilog of the intercept, was found to be 0.69 which is considerably higher than that found using the gravimetric analysis technique (0.406). However the value of K would be expected to be lower for the gravimetric method, where the solvent was evaporated by heating, thus probably converting a proportion of the salt linkages to the amide[46] which will not undergo Schiff's base formation. To test this hypothesis, further reflectance - concentration plots were constructed for other pulps, using the hydrolysis technique as described. The three pulps New Bern, Port Hudson and Tyee were chosen and the results are given in Table 39. The regression analysis details for the respective plots of Log F(R) versus Log c (Figure 39) are summarised

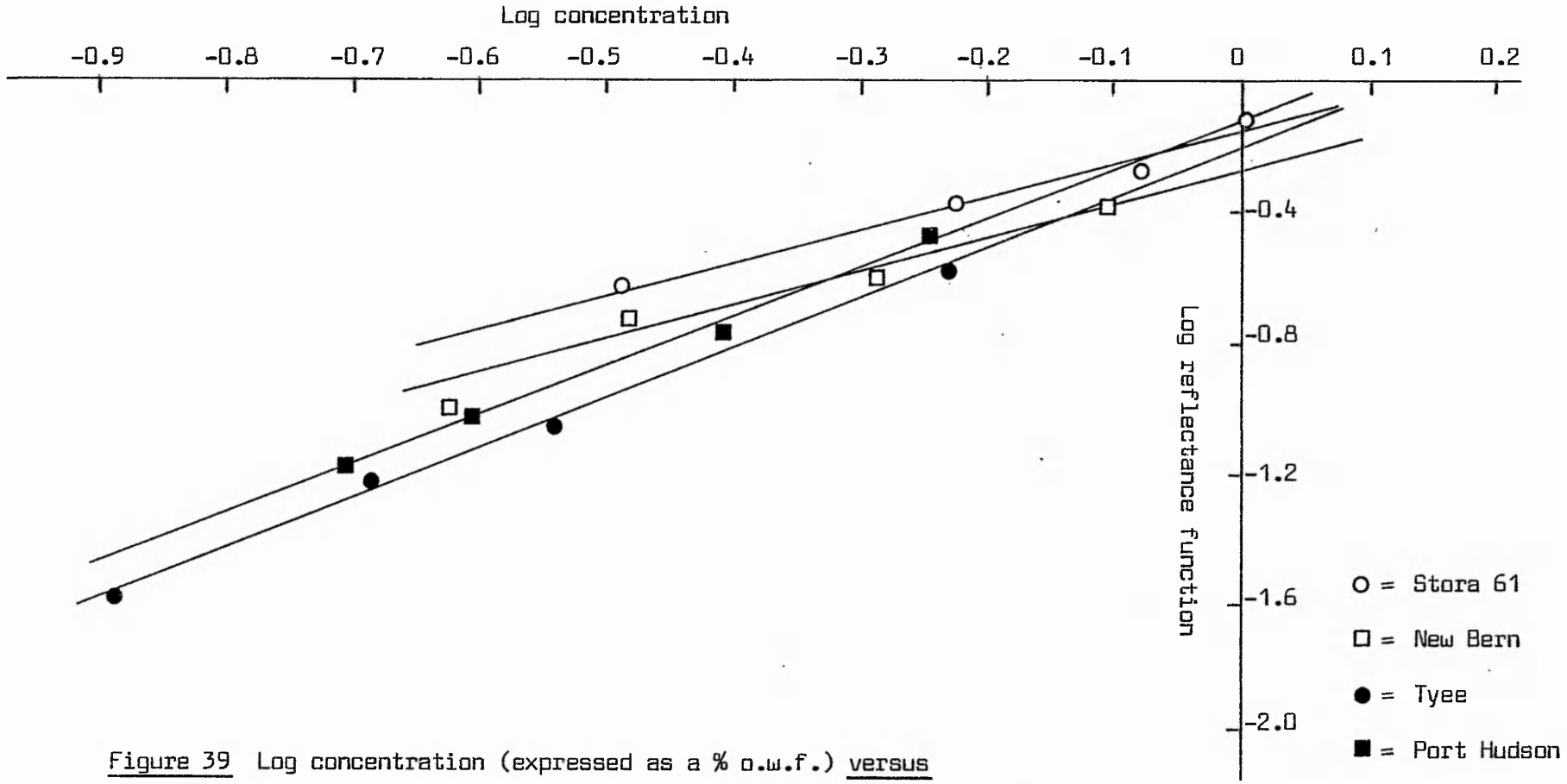


Figure 39 Log concentration (expressed as a % o.w.f.) versus Log Kubelka-Munk reflectance function for various N-salicylidene papers.

Table 39

Chitosan content analysis data with associated reflectance data for various pulps.

Pulp type	Analysis data: Dry weight of N-salicylidene chitosan-paper/g	Dry weight of paper/g	Volume of hydrolysis solvent/cm^3	Absorbance at 255 nm in 1 cm cells	Chitosan content $x10^4$/g	Conc. of chitosan /% o.w.f.	Log conc.	Dilution factor
New Bern	0.1652	0.1644	20	0.263	5.43	0.330	-0.481	5
"	0.1524	0.1518	"	0.175	3.62	0.238	-0.623	5
"	0.1369	0.1353	"	0.254	10.5	0.776	-0.110	10
"	0.1421	0.1410	"	0.177	7.31	0.519	-0.285	10
Tyee	0.1671	0.1663	"	0.116	4.79	0.288	-0.540	10
"	0.1420	0.1407	"	0.201	8.3	0.590	-0.229	10
"	0.1354	0.1352	"	0.085	1.76	0.130	-0.886	5
"	0.1450	0.1446	"	0.145	3.00	0.207	-0.684	5
Port Hudson	0.1520	0.1515	"	0.145	3.00	0.198	-0.704	5
"	0.1493	0.1487	"	0.179	3.70	0.249	-0.604	5
"	0.1558	0.1549	"	0.147	6.07	0.392	-0.407	10
"	0.1608	0.1593	"	0.230	9.50	0.597	-0.244	10

Table 39 continued

Pulp type	Concentration of chitosan/% o.w.f.	Reflectance data: % average reflectance of sample	% average reflectance of substrate	Reflectance function F(R)	Log F(R)
New Bern	0.330	54.9	94.1	0.184	-0.736
"	0.238	64.4	"	0.096	-1.015
"	0.776	41.7	"	0.406	-0.392
"	0.519	49.9	"	0.250	-0.603
Tyee	0.288	66.2	100.9	0.086	-1.064
"	0.590	49.6	"	0.256	-0.592
"	0.130	79.9	"	0.025	-1.597
"	0.207	70.9	"	0.060	-1.224
Port Hudson	0.198	70.0	99.6	0.064	-1.192
"	0.249	65.3	"	0.092	-1.035
"	0.392	56.3	"	0.170	-0.771
"	0.597	44.9	"	0.338	-0.471

in Table 40. [For this method the reflectance of the substrate was also taken into account, with F(R) being taken as the numerical value of the right hand side of equation (17), (see Section 3.3.1.1)].

The K values for these pulps follow the trend observed for Stora 61 in that they are greater than those found using the gravimetric method. However the slopes of the Log F(R) versus Log c plots, indicate that the relationship between reflectance and concentration does not exactly correspond to the requirements of the Kubelka-Munk theory. Thus in their present form the reflectance-concentration data can only be used as a calibration for similar systems at the same concentration range.

A second more modern approach to the measurement of reflectance was investigated, involving the use of the Internal Colour Systems Micromatch Spectrophotometer. This instrument has the ability to measure the reflectance at 16 different wavelengths from 400 nm in 20 nm increments. For any sample it will give an average value for the colour strength over the 16 different wavelengths. The colour strength is the ratio of the absorption to scattering coefficients for the sample against that for the substrate. Measurements were made on the N-salicylidene chitosan-paper systems for the New Bern, Tyee and Port Hudson pulps. Each sample was measured on both sides of the sheet at four different positions. The results are given in Table 41. In theory a plot of Colour strength versus Concentration should give a straight line with an intercept of 1, which corresponds to the point of zero concentration where the sample and substrate are identical. Plots of Colour strength versus Concentration (Figure 40) show a reasonable convergence to the intercept at a value of 1 for the colour strength, but again they were not ideal.

Table 40

Regression analysis data for plots of Log F(R) versus Log conc. for various pulps.

Pulp type	Slope	Intercept	Square of correlation coefficient	K = antilog of intercept
Stora 61	0.995	-0.1587	0.989	0.69
Tyee	1.54	-0.2396	0.995	0.58
New Bern	1.145	-0.256	0.963	0.554
Port Hudson	1.53	-0.114	0.995	0.77

Table 41

Colour strength/concentration data for N-salicylidene chitosan-paper systems.

Pulp type	Colour strength	Concentration of chitosan /% o.w.f.	Pulp type	Colour strength	Concentration of chitosan /% o.w.f.
Port Hudson	3.9	0.597	Port Hudson	2.81	0.392
"	2.22	0.249	"	1.54	0.198
New Bern	4.46	0.519	New Bern	6.11	0.776
"	2.56	0.238	"	3.61	0.330
Tyee	2.27	0.288	Tyee	3.82	0.590
"	1.58	0.13	"	1.98	0.207

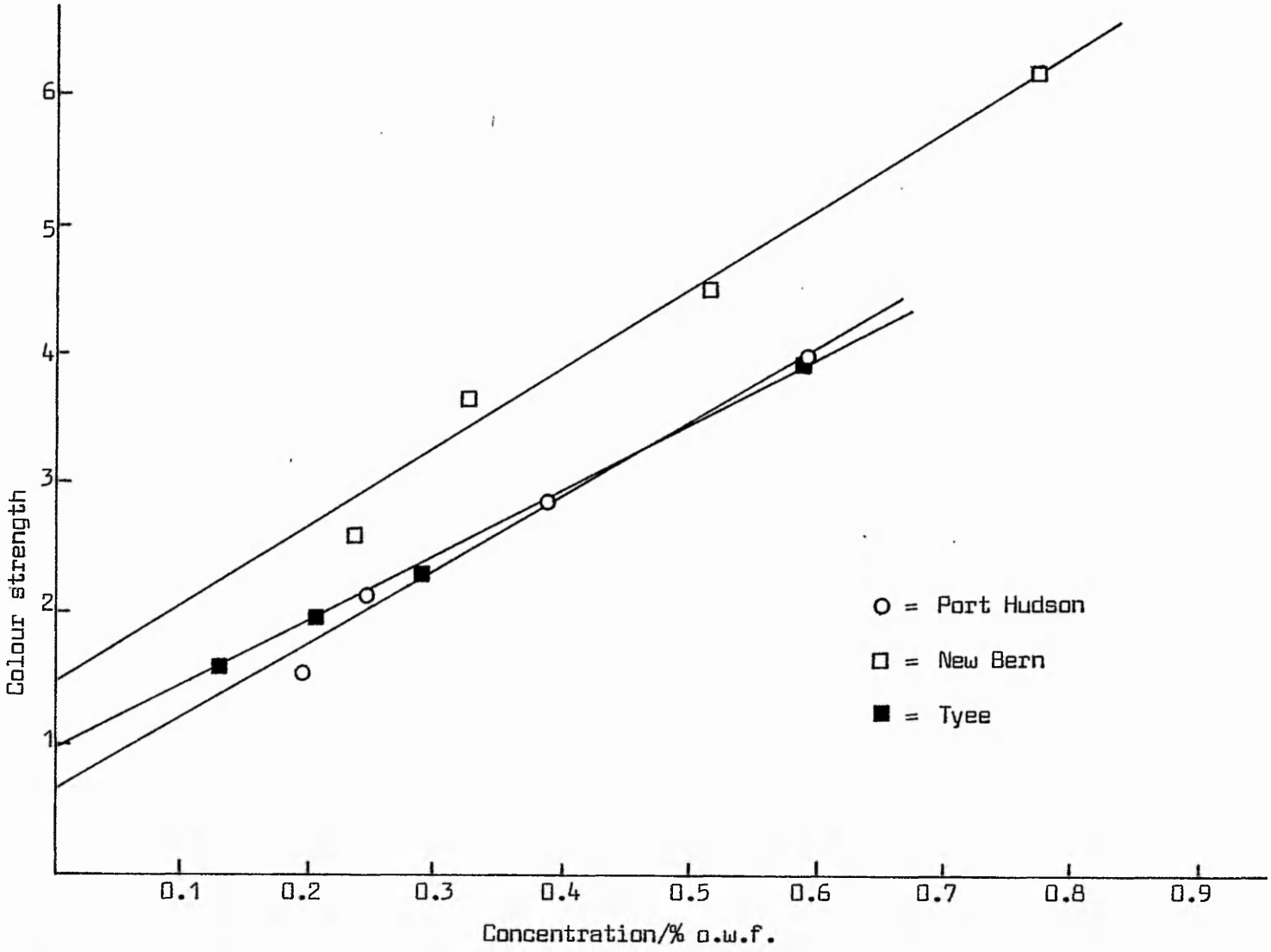


Figure 40 Plot of Colour strength versus Concentration of chitosan on weight of fibre for various N-salicylidene chitosan-papers.

3.3.1.4 Comparison of the Kubelka-Munk and the Atherton relationships

Since it is clear that the reflectance data does not exactly fit the Kubelka-Munk expression, another relationship between reflectance and the adsorption and scattering coefficients was investigated. This has been developed by Atherton[116] and is expressed in equation (20)

$$F(R) = \frac{K}{S} = \left(\frac{1}{R} - \frac{1}{Rs}\right) \quad \text{.....(20)}$$

where K = adsorption coefficient ,

S = scattering coefficient

R = fractional reflectance of sample

Rs = fractional reflectance of substrate

In this case the reflectance function is determined in a different way to that for the Kubelka-Munk analyses, and is derived on the basis of modification of the Kubelka-Munk theory to take into account the practical realities of spectrophotometric measurement techniques. The data in Tables 38 and 39 were reassessed accordingly and given in Table 42. Ideally the logarimetric plot of F(R) versus Concentration should still have a slope of 1 (Figure 41). The regression analysis of these results (Table 43) shows an improvement over the Kubelka-Munk treatment, in the proximity of the slopes for the plots to the desired value of unity.

It is clear that any reflectance function used must be representative of the actual relationship between the absorption coefficient, scattering coefficient and reflectance. Thus if the chitosan content on paper is to be assessed using the reflectance of a coloured

Table 42

Reflectance/concentration data for N-salicylidene chitosan-papers using the Atherton treatment.

Pulp type	Concentration of chitosan/% o.w.f.	Log conc.	Reflectance function F(R)	Log F(R)
Stora 61	0.831	-0.080	1.58	0.200
"	0.326	-0.487	0.82	-0.086
"	0.600	-0.222	1.28	0.108
"	1.002	0.001	2.03	0.307
Tyee	0.238	-0.540	0.519	-0.284
"	0.590	-0.229	1.03	0.011
"	0.130	-0.886	0.26	-0.584
"	0.207	-0.684	0.42	-0.377
New Bern	0.330	-0.481	0.76	-0.12
"	0.238	-0.623	0.49	-0.31
"	0.776	-0.110	1.33	0.126
"	0.519	-0.285	0.94	-0.026
Port Hudson	0.198	-0.704	0.43	-0.372
"	0.249	-0.604	0.53	-0.278
"	0.392	-0.407	0.77	-0.112
"	0.598	-0.244	1.22	0.087

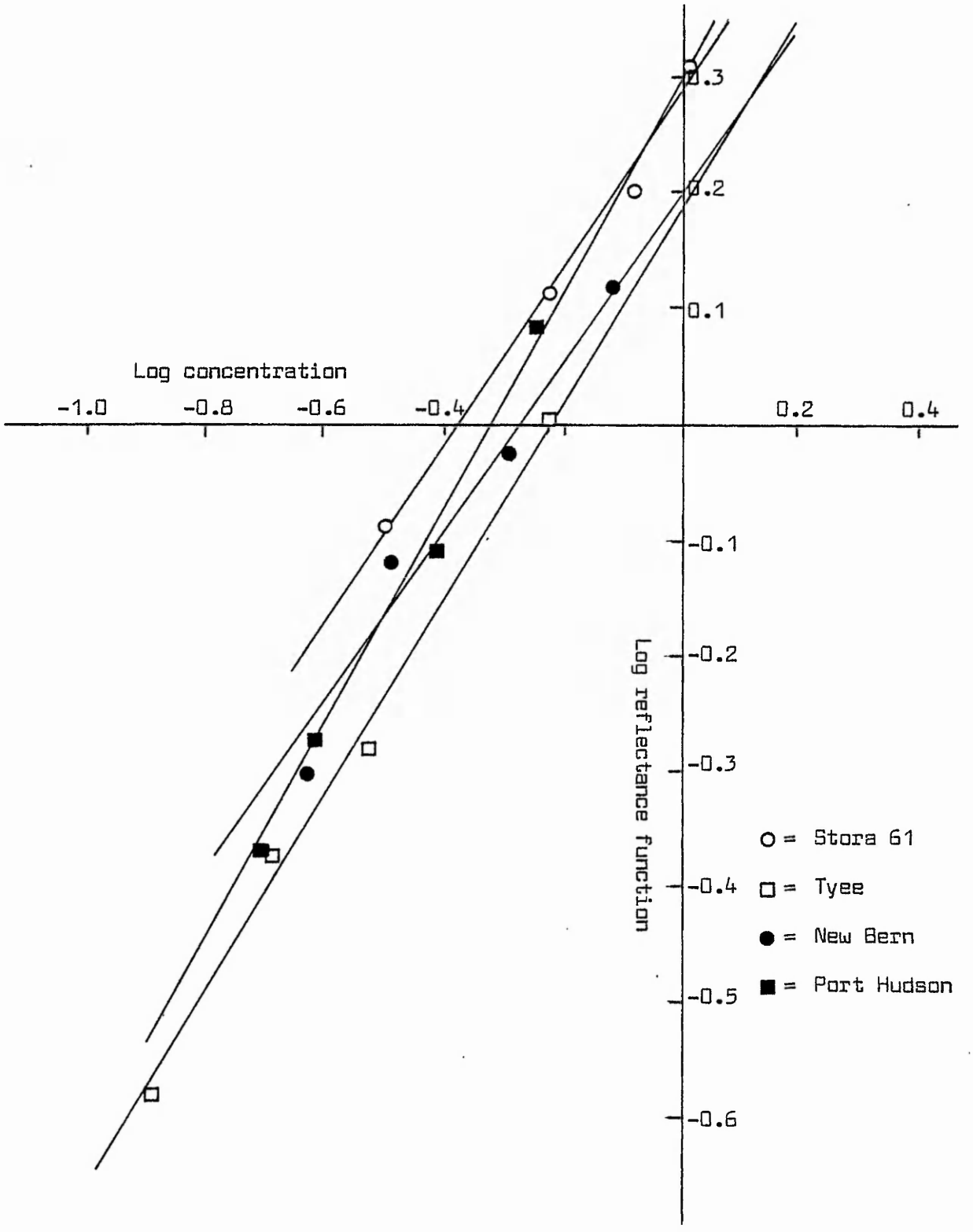


Figure 41 Log concentration versus Log Atherton reflectance function for various N-salicylidene chitosan-papers.

Table 43

Regression analysis data for plots of Log F(R) versus Log conc. for various pulps, using the Atherton treatment.

Pulp type	Slope	Intercept	Square of correlation coefficient	K = antilog of intercept
Stora 61	0.77	0.284	0.987	1.9
Tyee	0.89	0.213	0.996	1.63
Port Hudson	0.98	0.311	0.993	2.05
New Bern	0.80	0.22	0.967	1.66

derivative then a more suitable reflectance function must be found. There exist a number of alternative approaches[117-119] to the determination of reflectance functions, and these may warrant further investigation at a later stage.

3.3.2 Effects of adsorbed chitosan on the strength of paper

Due to a lack of time only a brief investigation into the properties of chitosan as an additive for strength improvements of paper was undertaken. Chitosan addition was carried out by adsorption from solution at the disintergration stage of handsheet formation[115]. Four levels of addition to a Stora 32 stock were applied at 1%, 2%, 3% and 4% based on the dry weight of the pulp using a 1% chitosan solution in acetic acid. The chitosan content was determined using the N-salicylidene chitosan hydrolysis technique (see Section 3.3.1.3 and Table 44). The formed handsheets were then conditioned[120] and their physical properties tested[121]. The results outlined in Table 45 show that at the low retention levels obtained, little improvement in the physical properties is observed. Under the conditions used the adsorption process is relatively inefficient, especially at the higher initial chitosan addition levels, where the majority of the chitosan in solution was not adsorbed. The fact that the adsorption is poor is not surprising when considering the conditions used for the production of the handsheets[115]. The initial disintergration is performed at a liquor ratio of approximately 70:1 with an overall chitosan solution concentration of only 0.15 g dm^{-3}, for the 1% addition level. High liquor ratios and low chitosan concentrations have been found to be detrimental to the amount of adsorption taking place (see Sections 3.4.7.1 and 3.4.7.3). Also after the initial

Table 44

N-salicylidene-paper hydrolysis data for chitosan content.

Pulp	Dry weight of paper/g	Volume of hydrolysis solution/cm^3	Dilution factor	Absorbance in 1 cm cells at 255 nm	Conc./%o.w.f.
Stora 32	0.2011	20	1	0.44	0.09
"	0.2234	"	1	0.702	0.13
"	0.1986	"	1	0.673	0.14
"	0.1992	"	1	0.772	0.16
UBK	0.2042	40	1	0.469	0.19

Table 45

Physical test properties of treated pulps.

Sample	Chitosan addition level/%	Chitosan conc./% o.w.f.	Burst index /Kpa m^2g^{-1}	Tear index /mN^2g^{-1}	Breaking length /Km	Bulk /cm^3g^{-1}
Stora 32	0	0	2.73	15	3.71	1.57
"	1	0.09	3.17	16	3.64	1.33
"	2	0.13	3.57	16.6	3.79	1.54
"	3	0.14	3.34	16	3.83	1.55
"	4	0.16	3.66	15.9	4.06	1.51
UBK	0	0	3.8	16.6	5.3	1.67
"	1	0.19	5.94	14.9	6.58	1.57

disintergration, which took approximately 30 minutes, the stock was diluted a further 5 times, before being used for hand sheet production. Hence the adsorption of chitosan cannot have attained equilibrium in such a short time (compare with the 48 hours required as shown in Section 3.4.6), and the dilution may cause desorption of the polymer. Thus in order to improve the retention of chitosan in an adsorption process, the conditions for the adsorption must be optimised with respect to the factors that favourably influence the uptake as reported in Section 3.4.

Nevertheless favourable increases in the physical properties of handsheets produced using an unbleached kraft pulp (UBK) and a 1% chitosan addition level were observed (Table 45). For this system the concentration of the chitosan on the fibre was found to be 0.19%. This was considerably higher than the value for the bleached kraft pulp Stora 32, at the same addition level. Although the bleaching process may increase the carboxyl content of the pulp by oxidation, it is likely that the improved chitosan uptake is due to the presence of lignin in the unbleached pulp which will contain anionic sulphonic acid groups, thus increasing the surface charge of the pulp, and hence the uptake (see Section 3.4.7.8). Similar trends were observed by Allan et al.[53] using an unbleached sulphite pulp.

For the UBK pulp there is a 24% increase in the Breaking Length, and a 56% increase in the Burst Index over the untreated pulp (Table 45). These few results indicate that there are considerable possibilities for the use of chitosan as a wet and dry strength improvement additive but that further work is required to optimise the variables involved in the adsorption.

3.4 Studies on the adsorption of chitosan onto cellulose substrates

3.4.1 Introduction

The use of equilibrium adsorption techniques for the application of chitosan onto paper has been studied by Allan et al.[53,56], with respect to the resulting physical properties of the paper. As an applicative method Allan seems to have dismissed its use due to the detrimental effects on the physical properties caused at higher chitosan retention levels. However up to now no study into the factors influencing the equilibrium adsorption have been carried out. In this thesis the adsorption of chitosan onto cellulose has been investigated, with respect to the nature of the chitosan as well as the substrate, so that a clearer picture of the forces involved during adsorption can be obtained.

3.4.2 Preparation of chitosan solutions for adsorption studies

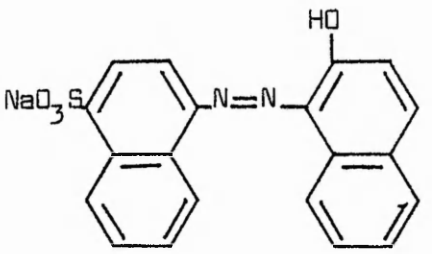
The chitosan solutions used for adsorption studies were in the concentration range between 0.25 and 1 g dm^{-3}. They were made up by the addition of aqueous acetic acid to the chitosan sample at an approximate molar ratio of acetic acid:free amine of 3:2. The final pH of the solutions was adjusted to 4 by the addition of more acetic acid. The solutions were not buffered because of the potential effects of added electrolyte on the uptake of the polyelectrolyte. For similar reasons the ionic strength of the solutions were not maintained at a constant level because this would require the addition of an excess of electrolyte.

3.4.3 Analysis of the concentration of chitosan in solution by metachromic titration

Little previous study into the adsorption of cationic polymers on cellulose substrates has been carried out [122]. Horn and Melzer[123] have investigated the influence of molecular weight and charge density on the adsorption of polyethyleneimines and cationic polyacrylamides on cellulose, using methods of residual polymer solution analysis after adsorption to evaluate the uptake. For most work on polymer adsorption, residual analysis is the technique used to determine the amount of adsorption. For chitosan this requires a procedure whereby the concentration of polymer in solution can be accurately determined. Originally a method involving the homogeneous quantitative reaction between chitosan and salicylaldehyde was proposed as outlined in Section 3.2.5.3. However another technique developed in this laboratory[124] has been found to be considerably more accurate and sensitive. It is based on the metachromic effects polyelectrolytes in solution exert on dyes of the opposite charge. Metachromacy is the shift in the main uv/visible absorption band of the dye to another wavelength, caused by the presence of the polyelectrolyte.

The effect was originally observed by Erhlich[125] and has been extensively used with anionic polymeric systems using cationic (basis) dyes. Until the work by Roberts and Potts[124] no study of the use of anionic dyes with cationic polyelectrolytes had been carried out. They found that the dye molecules interacted quantitatively with the amine groups of chitosan, and thus could be used to analyse chitosan in the solution and also to determine the extent of N-acetylation of any soluble chitosan sample. The acid (anionic)

dye chosen for the solution analysis was C.I. Acid Red 88 (XIV)



(XIV) C.I. Acid Red 88

For quantitative work this dye is required in a pure form. Most commercial dyes contain large quantities of salt as well as impurities as a result of the byproducts of their manufacture. The commercial C.I. Acid Red 88 was purified according to the method of Nursten and Williams[126] which involves treatment of a solution of the dye using ion exchange resins. The dye in the pure solid state was kept as the sodium salt due to its thermal instability in its free acid form. The metachromic shift in the position of the absorption band for C.I. Acid Red 88 in a 0.6% acetic acid solution, on addition of a chitosan solution involved a hypsochromic shift in λ max from 505 nm to 450 nm. This shift of 55 nm is clearly visible due to the change of colour from red to yellow. Unfortunately if a solution of the dye is titrated with a chitosan solution the colour change is gradual and there is no really sharp end point. Thus for quantitative analysis the absorbance values at 505 nm of a series of dye solutions, at constant dye concentration and containing varying amounts of chitosan were determined. The absorbance intensity at 505 nm decreases with increasing chitosan content, due to the shift to 450 nm, until a point was reached where the absorbance remained constant. Addition of excess chitosan beyond this point has no further effect on the absorbance

because all the dye anions have been used up. Thus if the absorbance is plotted (Figure 42) against the volume of chitosan added, two distinct lines will be observed, one where the absorbance decreases with addition of chitosan and a second where it remains constant. The intersection of these two lines corresponds to the exact amount of chitosan required to interact completely with the dye. The data for the construction of this plot is outlined in Table 46. Thus by using a chitosan of known free amine content the amount adsorbed by the cellulosic substrate can be determined by a metachromic analysis of the solution before and after adsorption. The effect can be seen in Figure 42 where a greater volume of the solution is required (after adsorption) to interact with the same volume of the dye. Details of this adsorption analysis are given in Table 47. The difference between the volumes of the two solutions required to interact with all the dye can be converted into a weight of chitosan which is equivalent to the amount of polymer adsorbed by the pulp. This amount can be calculated using the following equation;

amount of chitosan adsorbed in grams $= [C_2-(C_1V_1D_2/D_1V_2)]V_3/1000$...(21)

where C_1 = concentration of chitosan solution in g dm^{-3} required to calibrate the dye

C_2 = concentration of chitosan solution in g dm^{-3} used for adsorption

D_1 = dilution factor for calibration solution

D_2 = dilution factor for residual solution after adsorption

V_1 = metachromic end point for calibration solution in cm^3

V_2 = metachromic end point for residual solution in cm^3

V_3 = volume of original solution used for adsorption in cm^3

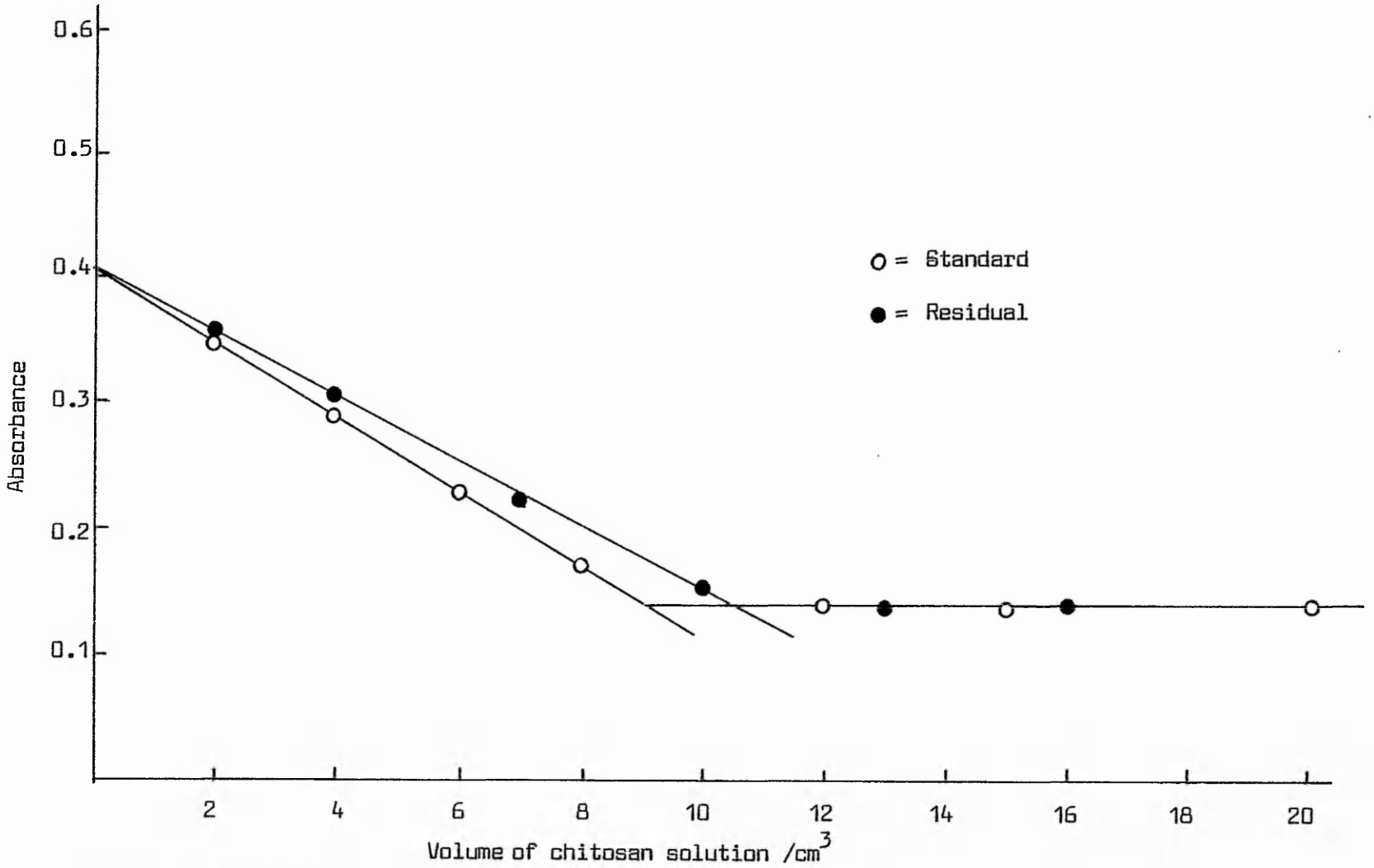


Figure 42 Plot of Absorbance of C.I. Acid Red 88 versus Volume of chitosan for a standard chitosan solution and a residual chitosan solution.

Table 46

Solution analysis data for chitosan standard by metachromic titration.

Volume of 0.398 g dm^{-3} soln. of C.I. Acid Red 88/cm^3	Conc. of chitosan soln./g dm^{-3}	Dilution factor	Volume of chitosan /cm^3	Total volume /cm^3	Absorbance at 505 nm in 1 cm cells
5	0.962	10	20	250	0.134
5	"	10	15	"	0.133
5	"	10	12	"	0.134
5	"	10	8	"	0.17
5	"	10	6	"	0.23
5	"	10	4	"	0.29
5	"	10	2	"	0.346

Table 47

Metachromic analysis of residual chitosan in solution after adsorption.

Volume of 0.398 g dm^{-3} soln. of C.I. Acid Red 88/cm^3	Conc. of chitosan soln./g dm^{-3}	Dilution factor	Volume of chitosan /cm^3	Total volume /cm^3	Absorbance at 505 nm in 1 cm cells
5	0.962	10	16	250	0.134
5	"	"	13	"	0.133
5	"	"	10	"	0.152
5	"	"	7	"	0.224
5	"	"	4	"	0.306
5	"	"	2	"	0.357

The calibration and residual analysis must be carried out on the same volume of the same dye solution. If the solution used for adsorption is the same as that used for the calibration then $C_2 = C_1$.

From the knowledge of the weight of the pulp the uptake can be expressed as a percentage on the weight of fibre. The results in Tables 46 and 47 have been treated according to equation (21), as shown in Table 48). For the lower levels of chitosan uptake the differences between the chitosan content of the original and residual solutions will be smaller and thus for accuracy the intercept is worked out from a regression analysis of the two lines involved in the metachromic plot of Absorbance versus Volume of chitosan solution. Any system where the correlation coefficient R^2 for the regression was less than 0.99 was repeated. During the adsorption analysis of chitosan on cellulose a considerable number of spectroscopic metachromic titrations were carried out. For convenience these are tabulated in the style of Table 48 and are contained in Appendix IV.

3.4.4 Preparation of cellulose substrates for adsorption studies

3.4.4.1 Preparation of celluloses containing carboxyl groups through periodate/chlorous acid oxidation

The standard substrate for pulp preparations was a sample of purified cotton in a feathery physical form. This purified cotton has a negligible carboxyl content and since it seemed likely that the cationic chitosan may interact with anionic sites[56] on the pulp it was considered desirable to modify the cotton to produce such sites. Modification of cotton to provide carboxyl groups requires some form of oxidation. Although most oxidising agents are unspecific in their action upon cellulose, periodic acid and its salts are exceptions in

Table 48

Metachromic analysis data for determination of the chitosan adsorbed on cellulose.

Symbols are explained in the script.

C_2/g dm^{-3}	C_1/g dm^{-1}	D_1	D_2	V_1/cm^3	V_2/cm^3	V_3/cm^3	Weight of pulp/g	Chitosan adsorbed/g	Chitosan content/% o.w.f.
0.962	0.962	10	10	9.29	10.66	40	0.9447	4.94×10^{-3}	0.52

that they specifically oxidise[127-130] vis-glycol groupings of the anhydro-D-glucose units to pairs of aldehyde groups. In the absence of light it is possible, by the use of periodates, to convert the C(2) and C(3) hydroxyls on cellulose to their respective aldehydes without simultaneous occurrence of side reactions. The extent of the reaction can be controlled by varying conditions of periodate concentration, ratio of cellulose to solution and reaction time.

A second oxidation procedure is required to convert the dialdehyde group, produced by periodate oxidation, into carboxyl groups. This can be achieved by the use of chlorous acid and was first studied by Harris et al.[131]. The periodate oxidised cellulose was treated with sodium chlorite dissolved in acetic acid, the concentration of sodium chlorite and the amount of acetic acid depending on the initial dialdehyde content of the cellulose.

Using the purified cotton as starting material a series of oxidised pulps were obtained according to the reaction conditions outlined in Table 49 and were labelled A to F. These oxidised celluloses were later converted to the free acid form for analysis (see Section 3.4.5.2).

3.4.4.2 Preparation of celluloses containing sulphonic acid groups through reaction with a reactive dye

At the higher carboxyl contents, oxidative modification of cellulose will tend to give a product that is increasingly hydrophilic in nature with possible concomitant effects on the surface charge of the pulp. Thus a second method of fibre modification was used, involving the application of a reactive dye containing sulphonic

Table 49

Oxidation conditions for purified cotton samples.

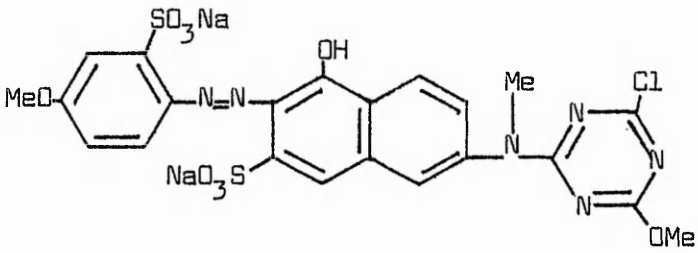
Sample	Weight of pulp/g	Volume of 0.01M $NaIO_4/cm^3$	Reaction time/h	Volume of 0.2M sodium chlorite in 1M acetic acid/cm^3
A	10	500	24	500
B	30	1500	48	1500
C	20	1000	8	1000
D	50	1500	12	2500
E	20	500	48	1000
F	20	2000	10	1000

Table 50

Conditions for dyeing purified cotton with C.I. Reactive Red 13.

Sample	Weight of pulp/g	Weight of dye/g	Liquor ratio	Weight of salt/g	Prefix dyeing time/h	Weight of Na_2CO_3/g	Fixation time at $80^oC/h$
R1	10	0.1	40:1	32	72	10	5
R5	10	0.5	"	32	72	10	5
R10	10	1.0	"	32	72	10	5

acid groups as the anionic site source. The reactive dye chosen was C.I. Reactive Red 13 (XV).



(XV)

C.I. Reactive Red 13.

Three reactive dyeings were carried out at levels of 1%, 5% and 10% based on the weight of the cotton used. The dye was initially applied in the presence of salt and then treated with sodium carbonate to bring about reaction with the fibre. Careful washing after fixation was required so that any unreacted dye could be removed. The samples were labelled R1, R5 and R10 (see Table 50).

3.4.5 Analysis of chemically modified celluloses

3.4.5.1 Introduction

The anionic content in both the oxidised pulps and the reactive dyed pulps must be determined if their effect on the adsorption of chitosan is to be quantified.

3.4.5.2 Determination of carboxylic acid content

The carboxyl content is best determined by means of alkalimetric techniques, where the counter ions on the carboxyl groups are initially exchanged for hydrogen ions which are then neutralised by the addition of excess alkali. Back-titration of the residual alkali affords an estimation of the carboxyl content. The neutralisation is carried out

in the presence of salt to suppress the Donnan effect (see Section 2.2.2.1).

Samples that contain appreciable quantities of carbonyl groups tend to give incorrect results due to interaction of the alkali with these groups. Alkaline degradation of oxidised celluloses has been extensively studied. It is suggested that the ability of a carbonyl group in oxidised cellulose, to exist in its enol form makes the cellulose susceptible to alkaline hydrolysis by the machanism proposed by Evans et al.[132], where the double bond involved with the enol group weakens the glycosidic linkage.

In the technique used, chlorous acid oxidation has been found by Davidson and Nevell[133] to be only 90% efficient and thus the final product will contain a reasonable number of carbonyl groups that would interfere with the alkali titration. Hence prior to carboxyl estimation the pulps were treated for several days with a large excess of sodium hydroxide to negate the effects of the carbonyl groups. The suggested products[134] of the resulting alkaline degradation would be C_4-saccharinic acids, lactic acids and glycollic acids. These were assumed to be removed during the subsequent washing of the pulp prior to the addition of hydrochloric acid to convert the carboxyl groups into their free acid form. In this form the pulp was carefully washed to remove any excess acid, before being analysed by a modification of the method of Samuelson and Wennerblom[135]. This involves steeping the sample in an excess of a sodium hydroxide - sodium chloride solution for sufficient time to allow equilibrium to be attained (48 hours). The remaining alkali is then titrated against a standard hydrochloric acid to a phenolphthalein end point. At this stage a known volume of the sample is removed and titrated against

more of the acid using a mixed indicator of methyl red and bromo-cresol green. This titration is continued until the change in colour to red is stable for a few seconds. The whole solution is then boiled for two minutes to remove dissolved atmospheric carbon dioxide and finally titrated to a neutral grey end point. The second titration provides a correction for the dissolved carbon dioxide that at the low concentrations of reagent required (0.01M) for the titrations, would adversely effect the results. The original alkali salt solution is also titrated under identical conditions so that the amount used in the neutralisation can be evaluated using equation (22)

carboxyl content in milliequivalents/kg

$$N \times 10^5 [a-(b + P \times C/Q)]/[G(100-f)] \quad .....(22)$$

where G = moist weight of pulp

f = moisture content of pulp

a = volume of standard hydrochloric acid consumed by X cm^3 of alkaline salt solution in cm^3

b = volume of standard hydrochloric acid consumed by the sample using phenolphthalein in cm^3

C = volume of standard hydrochloric acid consumed by part of the sample using the mixed indicator in cm^3

P = X + b cm^3

Q = volume of solution titrated using the mixed indicator in cm^3

N = normality of the acid.

The details for each pulp are given in Table 51. In general the pulps were stored at low temperatures (0^oC) to prevent any autohydrolysis by the hydrogen ions derived from the carboxyl groups.

Table 51

Carboxyl group determinations of pulps. Explanations of symbols is given in the script (Section 3.3.2.5).

Sample	N	a/cm^3	x/cm^3	b/cm^3	P/cm^3	Q/cm^3	c/cm^3	G/g	f/%	Carboxyl content /milliequiv.kg^{-1}
A	0.00959	43.2	40	21	61	20	0.6	0.4374	5.76	471
B	"	43.5	40	19.85	59.85	20	0.8	0.2081	5.31	1026
C	"	21.8	20	14.1	34.1	20	1.1	0.3593	5.3	162
D	"	21.8	20	17.75	37.75	20	1.2	0.3927	3.99	454
E	"	43.5	40	26.9	66.9	20	0.6	0.3338	6.50	445
F	"	43.5	40	30	70.0	20	0.8	0.2360	7.55	466
Purified cotton	"	43.5	40	41	81	20	0.65	0.2568	4.63	0

3.4.5.3 Determination of the sulphonic acid group content

In order to determine the number of acid groups in the reactive dyed pulp an analysis was developed which involves the dissolution of the cellulose samples using 70% sulphuric acid and the spectroscopic determination of the dye in that solution. For this spectroanalysis an extinction coefficient for the dye in the sulphuric acid solvent system was required. For this purpose the commercial C.I. Reactive Red 13 was purified by dissolution in methanol after which the solution was filtered to remove the insoluble sodium chloride and the dye recovered by evaporation on a steam bath. During this process it is possible that the dye may react with the methanol to replace the chlorine with a methoxyl group. As the chromophore is not likely to be affected and the molecular weight will remain almost the same i.e. replacement of a chlorine atom at 35.5 with a methoxyl group at 31, in a total molecular weight of over 600, this should have little effect. The dye was then dissolved in water and passed through a cationic exchange resin (H^+ form) to remove the sodium ions contained in any remaining salt, and also those on the sulphonic acid groups of the dye. This leaves the dye in its free acid form and the chloride from the salt as hydrochloric acid. Low temperature evaporation of the resulting solution will leave the dye in a pure from, suitable for spectroscopic assessment of its extinction coefficient. The uv/visible spectrum of the dye in both water and 70% sulphuric acid were run and revealed a shift in the position of λmax from 505 nm in water to 573 nm in sulphuric acid. The absorbance intensity of the solution was stable over a period of 16 hours, and the extinction coefficient was found from the data in Table 52 to be 29,960 at 573 nm.

Table 52

Extinction coefficient data for C.I. Reactive Red 13 in 70% sulphuric acid at 573 nm.

Weight of dye/g	Mol. weight of dye	Volume of acid/cm^3	Dilution factor	Absorbance in 1 cm cells	Extinction coefficient
0.0506	610	50	50	0.994	29,960

Table 53

Sulphonic acid group determination in milliequivalents kg^{-1} for C.I. Reactive Red dyed pulps.

Sample	Dry weight of pulp/g	Volume of acid/cm^3	Dilution factor	Absorbance at 573 nm in 1 cm cells	Sulphonic acid content/milli-equiv. kg^{-1}
R1	0.0985	25	1	0.86	14.6
R5	0.1036	25	1	1.138	18.3
R10	0.1047	25	5	0.428	34.1

Dissolution of the reactive dyed cellulose in 70% sulphuric acid was complete in 2 hours, using 250 cm^3 of acid per gram of pulp. Using the extinction coefficient of 29,960 the dye content in milli-equivalents kg^{-1}, for each pulp was found and converted into acid group content by doubling its value, since each dye molecule contains two sulphonic acid groups. Results are shown in Table 53. Comparison of the sulphonic acid content of the pulps with the carboxyl content of the oxidised pulps shows that a considerably larger number of anionic groups can be introduced into the fibre by periodate oxidation than through reactive dyeing. The highest sulphonic acid content of 34.1 milliequivalents kg^{-1} is less than the lowest carboxyl content of 45.4 milliequivalents kg^{-1} and much less than the highest carboxyl content of 1026 milliequivalents kg^{-1}. Thus any direct comparison between the two systems, of the chitosan adsorption as a function of acid group content, cannot easily be studied. However the strong acid nature of the sulphonic acid groups may help to negate the effect of their low content when compared to the weakly acidic carboxyl groupings.

3.4.6 Rate of attainment of equilibrium in the adsorption of chitosan on cellulose

The rate of adsorption of polymers from solution is dependant on factors such as the nature of the polymer adsorbate, the nature of the adsorbent and the rate of stirring the solution. In the adsorption experiments on chitosan the system was not stirred so that agitation effects would remain constant, though minimal, throughout.

The physical nature of the pulps used with respect to their surface area and porosity were assumed to be constant so that any

variations in the adsorbent were of a chemical nature. The assumption may not be true at the higher carboxyl contents where the swelling, in water, caused by the hydrophilic carboxyl groups will effect the surface area of the pulp.

The amount of chitosan adsorbed by the various pulps as a function of time was investigated at a chitosan level of about 1 g dm^{-3}, a liquor ratio of 30:1 and a temperature of 20°C. The results are shown in Figure 43 (full results are tabulated in Appendix IV), from which it can be seen that the adsorption of chitosan is an equilibrium process, the time to equilibrium being independant of the carboxyl content of the pulp. For all the pulp samples studied the equilibrium adsorption was reached within 48 hours. The relatively long time required to reach equilibrium is most probably due to the high molecular weight of the chitosan used (Kytex L), around 380,000. Heller[136] found that for the adsorption of poly(ethyleneglycols) on charcoal the time required for attainment of equilibrium increased greatly with increasing molecular weight of the poly(ethyleneglycol). Kindler and Swanson[122] obtained similar results for the adsorption of poly (ethyleneimine) on cellulose from solution. The time taken to reach equilibrium for poly(ethyleneimine) on cellulose was found by Horn and Melzer[123] to occur in minutes. This can be explained by the low molecular weights of the samples used i.e. less than 20,000. For higher molecular weights the time to establish equilibrium would be greater and Yuzhenka and Maleyeu[137] have suggested that the time required for complete adsorption is proportional to the square of the Limiting Viscosity Number of the solution. When investigating the effects of other variables on the adsorption of chitosan onto cellulose, sufficient time must be allowed for the system to reach equilibrium.

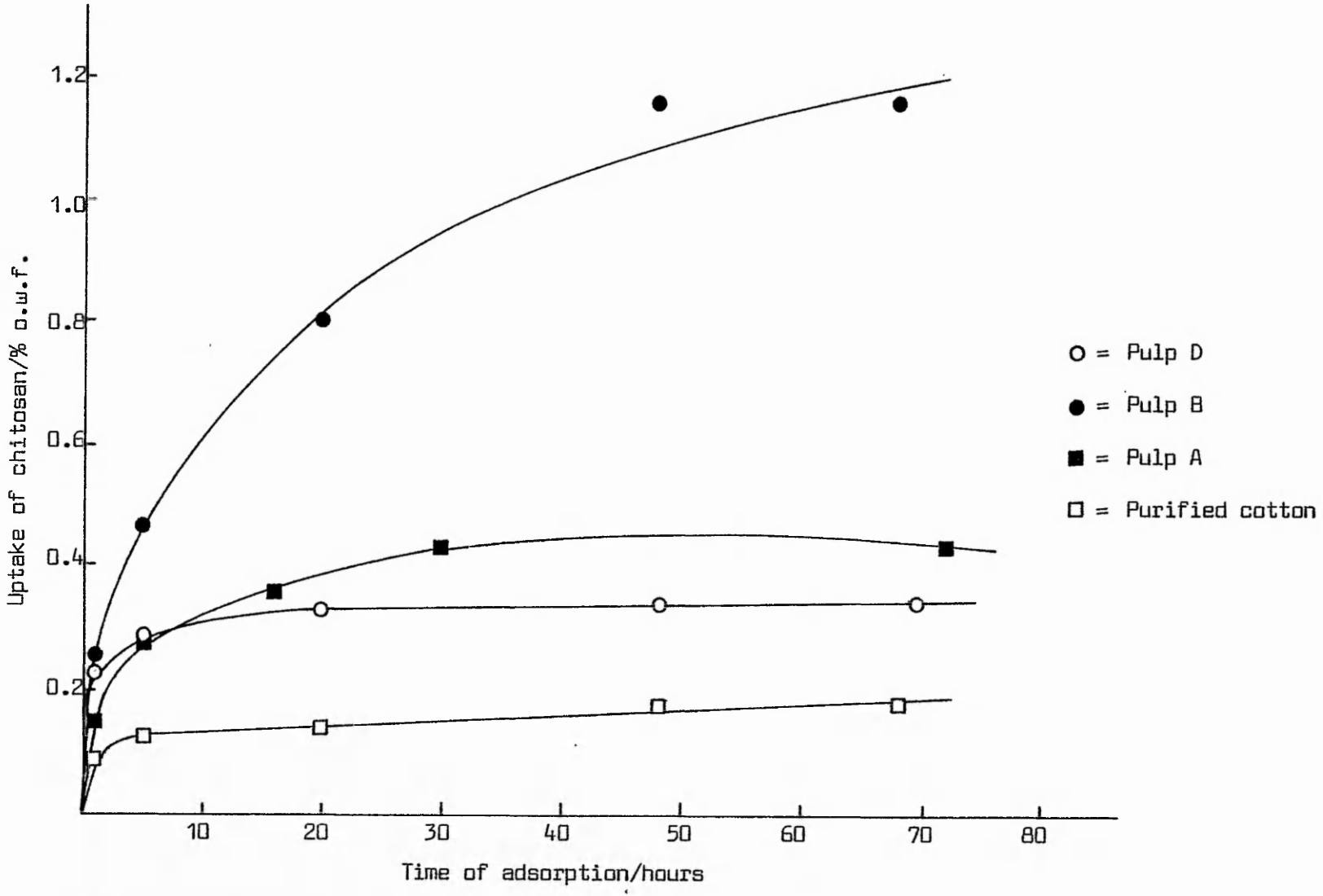


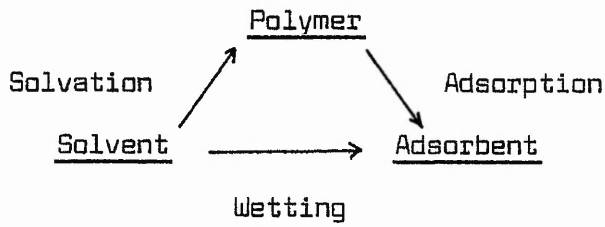
Figure 43 Uptake versus Time of adsorption for various pulps.

From the adsorption plots this may be assumed to have occured after 48 hours for the particular molecular weight of chitosan used.

3.4.7 Effects of selected variables on the equilibrium adsorption of chitosan on cellulose

3.4.7.1 Liquor ratio

The extent of polymer adsorption from solution is determined by the balance of three interactions: polymer/solvent, polymer/adsorbent and solvent/adsorbent. The relationship between these interactions is illustrated below.



Thus any modification in the surface nature of the adsorbent, any alteration in polymer structure or change to a different solvent may effect at least two of the above interactions.

When polymer segments approach the surface of an adsorbent the solvent molecules must be desorbed before the segments can be adsorbed. The competition between the polymer molecules and the solvent for the surface can be the predominant factor in the adsorption process[138].

The equilibrium adsorption of chitosan onto cellulose as a function of the liquor ratio of the adsorption solution at a constant chitosan concentration of about 1 g dm^{-3} and at $20^{o}C$ was examined. Pulp E was chosen for these experiments. It was initially chopped into a finer form using a blender in order to increase the surface area and hence

the adsorption. The results were plotted as Uptake at equilibrium versus Liquor ratio (Figure 44). The initial increase in the uptake up to a liquor ratio of 30:1 can be explained in terms of the accessibility of the pulp to the solution. At these liquor ratios there is insufficient solution to completely cover all of the fibre, unless it is considerably compressed. Once complete coverage has been achieved without compression, at a liquor ratio of 30:1, the uptake then decreases sharply with increasing liquor ratio before levelling off. If the polymer/adsorbent interaction is predominant then one would expect the uptake to increase before levelling off at the point of fibre saturation. The fact that it decreases suggests that the solvent has a considerable effect on the adsorption process. The decrease may be explained in terms of the inability of the solvent at the lower liquor ratios to wet out the fibre thus allowing for a more efficient polymer adsorption process, since there is less solvent to desorb from the fibre. Howard and McConnell[139] found that the adsorption of poly(ethyleneoxide) onto charcoal is governed by the affinity of the solvent molecule for the charcoal. The less the adsorption of the solvent the greater the amount of adsorption of the polymer from the solvent in the liquid phase. If the affinity of solvent for adsorbent is extremely strong the solvent molecules may be preferentially adsorbed forming a thick solvation layer resulting in an increase in the concentration of the polymer in the bulk of the solution. This is known as negative adsorption and has been observed for the adsorption of poly(isobutene)[140] from carbon tetrachloride and benzene.

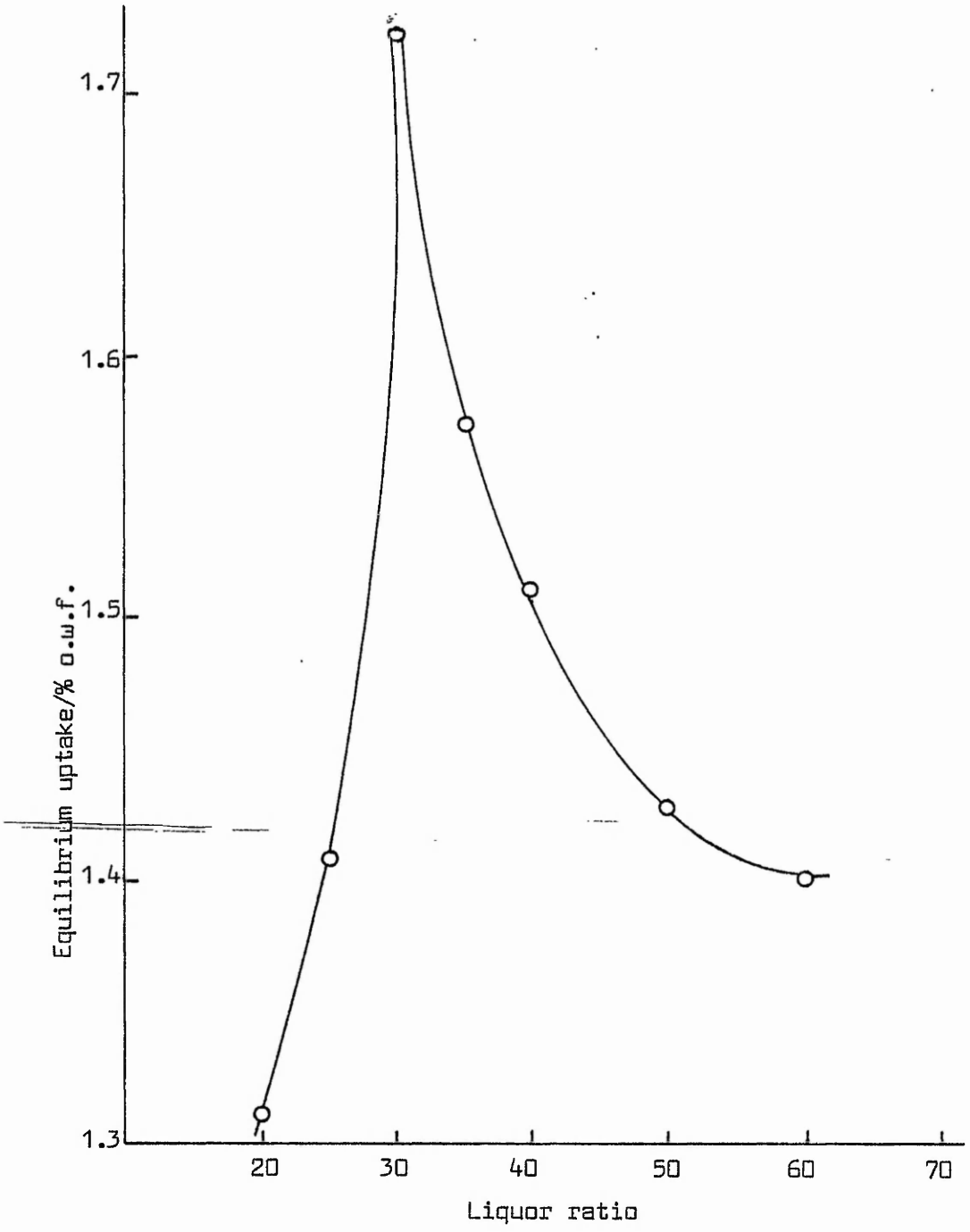


Figure 44 Equilibrium uptake versus Liquor ratio.

3.4.7.2 Temperature

The effect of temperature on the adsorption of polymers has been extensively studied, but a universal rule applicable to all systems has not, as yet, been found. Both increases[141] and decreases[142] in the amount of adsorption with increasing temperature have been reported. In some cases[143] both maxima and minima of adsorption have been observed at various temperatures. The effect of temperature on the equilibrium adsorption was studied using pulp D at 20°C, 30°C and 40°C, and constant conditions of liqor ratio (30:1) and chitosan concentration (1 g dm^{-3}). The plots of Uptake *versus* Time for each temperature (Figure 45) show that there was no difference in the equilibrium adsorption at 20°C and 30°C although at 40°C there was an increase of about 25% in the uptake. It is difficult to rationalise these results unless more is known about the effects of temperature on polymer solubility and on the desorption of the solvent, as well as the enthalpy changes that accompany the adsorption process. Often the solubility of the polymer molecules increases with temperature and thus adsorption is probably occuring from almost two different solvents, a poor (low temperature) and a good (high temperature) solvent.

3.4.7.3 Concentration of chitosan

The adsorption of chitosan at three concentration levels (1.0, 0.5 and 0.25 g dm^{-3}) at a liquor ratio of 30:1 and a temperature of 20°C was investigated using pulp A and a plot of Uptake *versus* Time for each concentration was obtained (Figure 46). The amount of chitosan adsorbed at equilibrium increases with increase in the

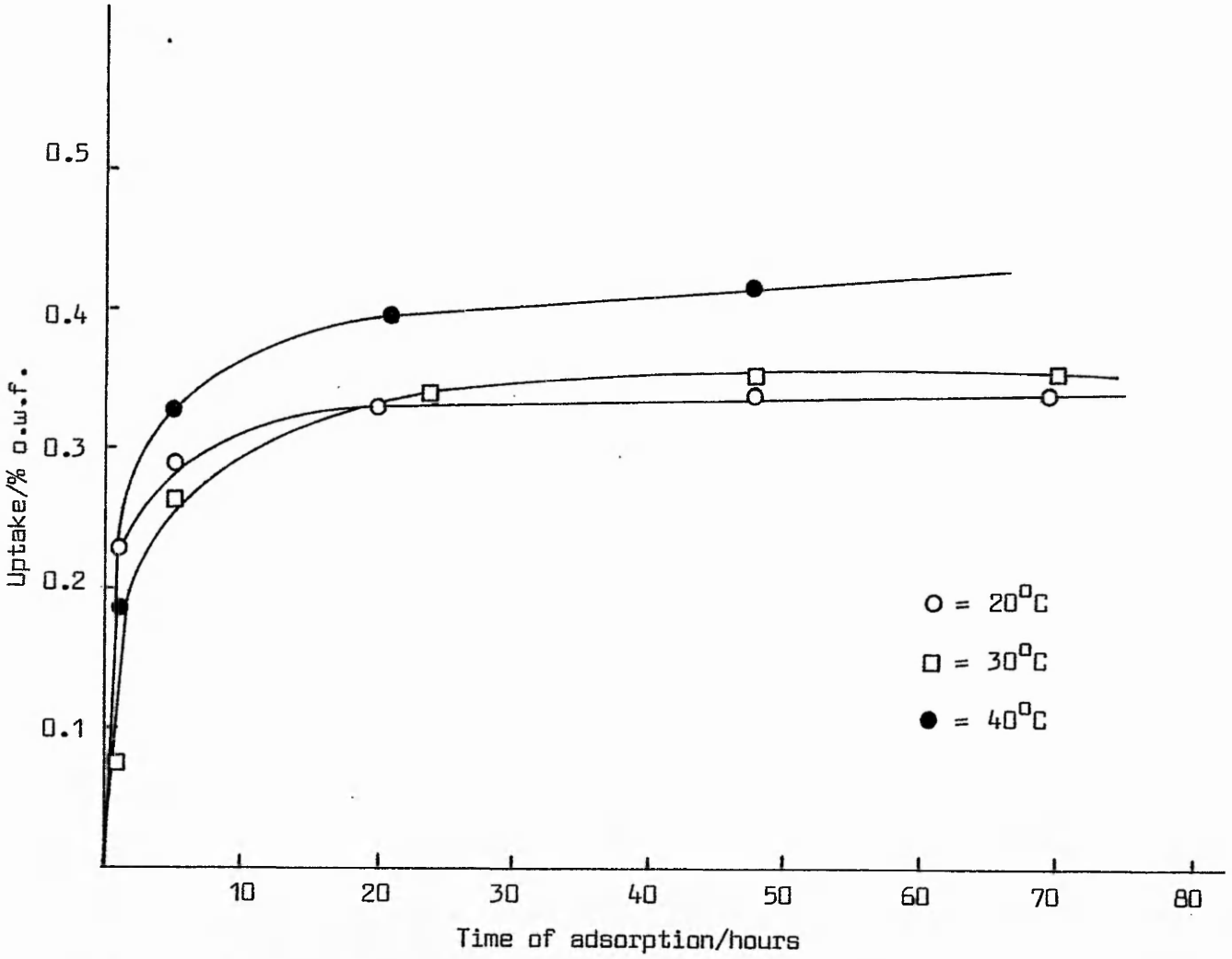


Figure 45 Uptake versus Adsorption time at 3 different temperatures for pulp D.

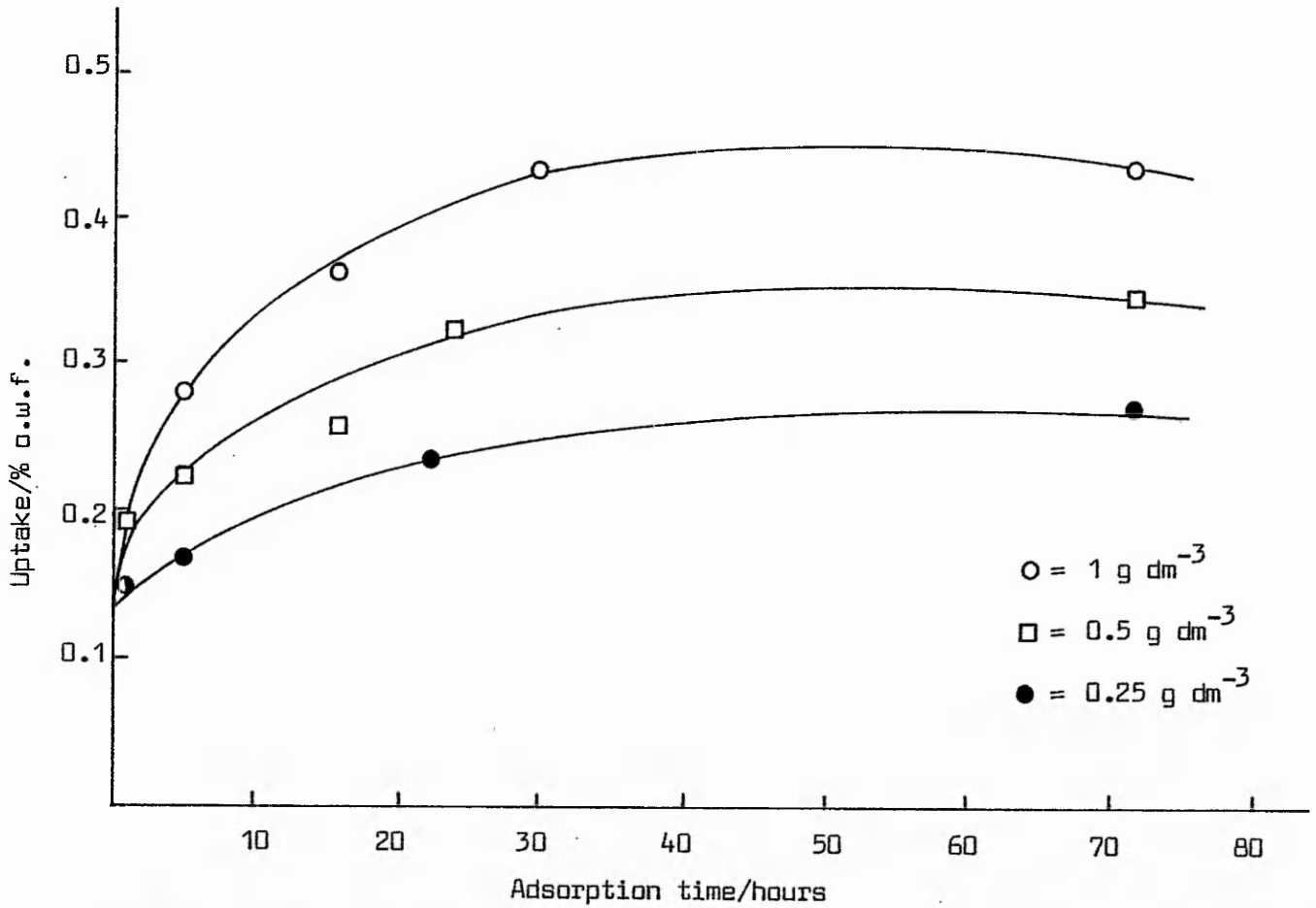


Figure 46 Uptake versus Adsorption time for adsorption onto pulp A at 3 different chitosan concentrations.

chitosan concentration. This can be explained in terms of the effect of chitosan on the position of equilibrium. As the concentration of chitosan is increased the equilibrium is shifted in favour of the adsorption process. The equilibrium adsorption for this particular system is not linearly dependant upon the chitosan concentration, and infact begins to level off at the higher concentrations. This could correspond to either a total shift of the equilibrium position to the adsorption side or to the surface of the fibre becoming completely saturated.

3.4.7.4 Added electrolyte

The effect of varying the concentration of added electrolyte on the equilibrium adsorption of chitosan was studied using pulp C and the usual conditions of liquor ratio (30:1) and temperature ($20^{o}C$). The chitosan concentration used was 0.5 g dm^{-3} prepared by the two fold dilution of a stock 1 g dm^{-3} solution with appropriate quantities of a 0.2M sodium chloride solution. Salt is known to have an effect on the metachromacy[144] of polyelectrolytes with dyes, tending to reverse the metachromic shift. Therefore the presence of salt in the adsorption solutions may have some effect on the accuracy of the metachromic titrations, but the salt concentrations used in the experiments were extremely low compared to those required to reverse the metachromic shift and thus their effect can be assumed to be negligible.

The equilibrium uptake, when plotted against salt concentrations from 0 to 1.0M (Figure 47) shows an initial rapid increase in the uptake to a plateau followed by a gradual decline as the salt concentration increases.

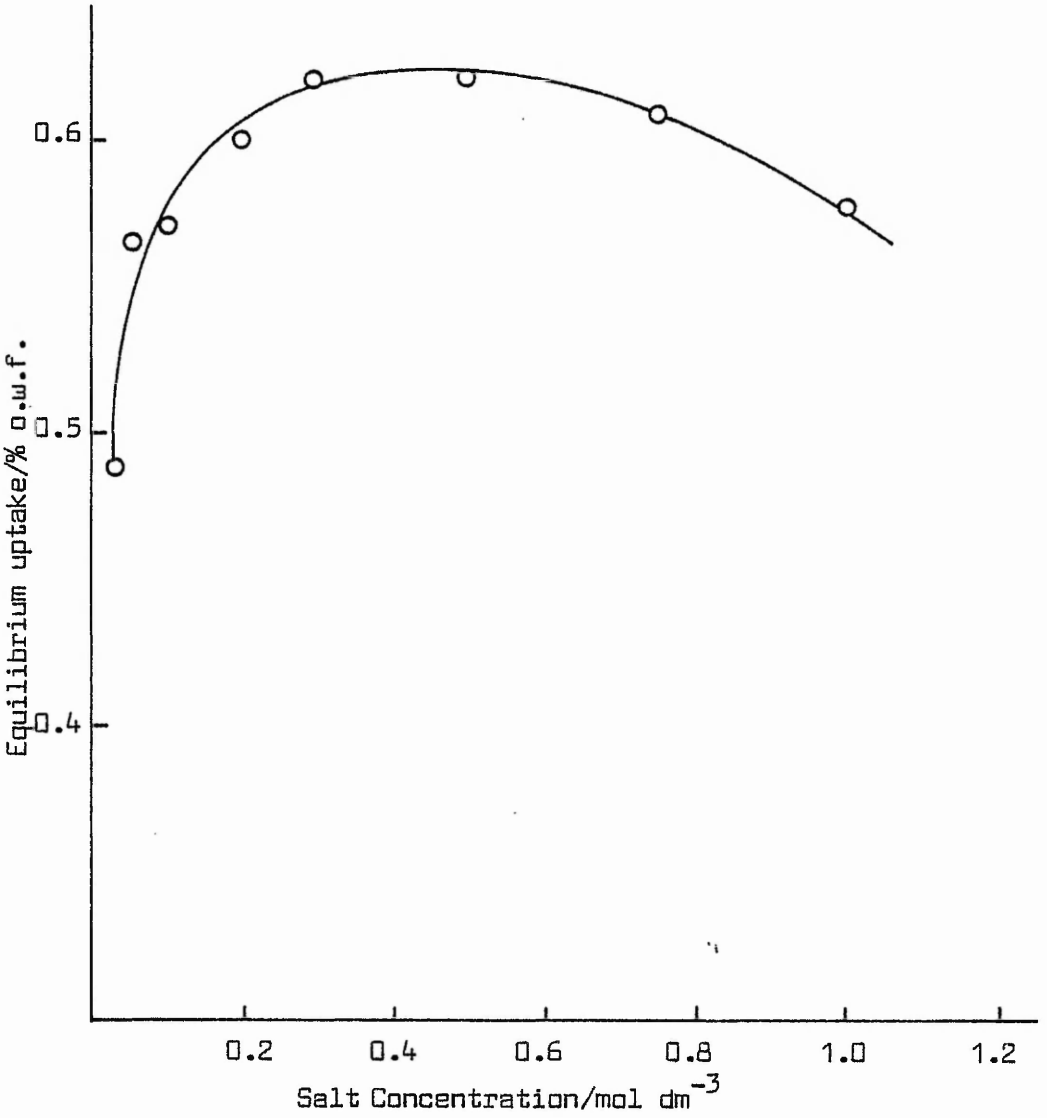


Figure 47 Equilibrium uptake versus Salt concentration for chitosan acetate on pulp C.

The shape of the graph suggests that two opposing mechanisms are operating, one causing an increase in adsorption, the other a decrease at the higher salt concentrations. If the attraction of the polymer by the pulp is assumed to be related to the surface charge of the fibre then the decrease in the uptake can be explained in terms of suppression of the charge by the positive ions of the added electrolyte. These added positive charges will tend to surround the fibre surface thus reducing the effect of the anionic sites on the fibre. This effect is analogous to the flocculation of hydrophobic colloids where the added electrolyte reduces their surface charge and thus overcomes the repulsive forces between negatively charged colloidal particles, allowing them to agglomerate.

For the initial increase in the adsorption three possible explanations can be suggested;

a) The added electrolyte causes the polymer to salt out of solution, in a similar manner to the salting out of dyestuffs. This would reduce the final solution concentration and give an adsorption value higher than expected. This theory was tested by carrying out an equilibrium adsorption measurement under identical conditions except that sodium thiocyanate at a 0.1M concentration was the added electrolyte used, instead of sodium chloride. Sodium thiocyanate[145] has a salting in effect and therefore should reverse the uptake trend. However when using sodium thiocyanate the uptake increased from 0.37% o.w.f. without salt, and 0.57% o.w.f. with 0.1M sodium chloride, to 0.76% o.w.f. with the thiocyanate. Thus it is unlikely that the sodium chloride causes any "salting out" effects.

b) The adsorption study was carried out using aqueous solutions

of chitosan acetate and the addition of salt to such a system might bring about an ion exchange between the acetate counter ion on chitosan and the chloride ion from the salt, leaving the chitosan in the form of its hydrochloride. Hayes and Davies[34] have produced chitosan hydrochloride by addition of sodium chloride to a solution of chitosan in acetic acid and thus an ion exchange process of this type is perfectly feasible.

The effect, if any, of the counter ion on the adsorption of the chitosan, must be related to its ability to aid the attraction between the surface anionic sites and the cationic chitosan. Chitosan in solution may be assumed to act as a liquid anion exchanger, in a similar way to tris(6-methylheptyl)amines[146] in aqueous solution. In ion exchange sytems for ions of equal charge it has been found that large ions are more strongly held than smaller ones[146]. Thus when comparing the bulky acetate ion with the smaller chloride ion, the chloride will be less firmly held by the chitosan and should exchange more readily with the anionic sites on the fibre and thus allow for an increased uptake.

The possible effects of the chloride counter ion was tested using a solution of chitosan hydrochloride which was calibrated against a standard chitosan acetate solution so that its concentration could be determined in terms of its chitosan rather than chitosan hydrochloride content. The hydrochloride salt was produced by precipitation from a chitosan solution in dilute hydrochloric acid by the addition of concentrated hydrochloric acid. Since the hydrochloride salt already has a chloride counter ion and assuming the above mechanism to operate, it would be in its most advantageous form for

adsorption and tha addition of salt should therefore not cause an increase in adsorption. The uptake at equilibrium was investigated under similar conditions to those used for the chitosan acetate. The plot of Equilibrium uptake versus Salt concentration (Figure 48) was however similar in shape to that of Figure 47. The value of 0.43% at zero concentration was greater than that of the acetate (0.37%) at the same concentration. Thus the chloride counter ion has some effect on the adsorption but in the presence of added electrolyte the nature of the counter ion is not the dominant factor.

c) It is likely that the initial increase in the uptake is a consequence of the polyelectrolyte nature of the chitosan (see Section 3.1.3.1). In the absence of salt the polymer coil is highly expanded but as salt is added the size of the random coil decreases resulting in an increase in the saturation level for a given surface area of cellulose. Also the smaller the polymer coil the more likely it is to penetrate the pores of the system, increasing the available surface area for bonding. This effect of salt is however not infinite and reaches a stage where addition of excess electrolyte has little effect on the size of the polymer coil and thus contributes only to the reduction in the surface charge of the fibre. At this point the uptake levels off and begins to decrease on further addition of salt.

3.4.7.5 Degree of N-acetylation of the chitosan

Four different extents of N-acetylation were investigated, the samples being produced by homogeneous[101] N-acetylation in dilute acetic acid/methanol using acetic anhydride (samples 19-22). The equilibrium adsorption of a 1 g dm^{-3} solution of each sample on

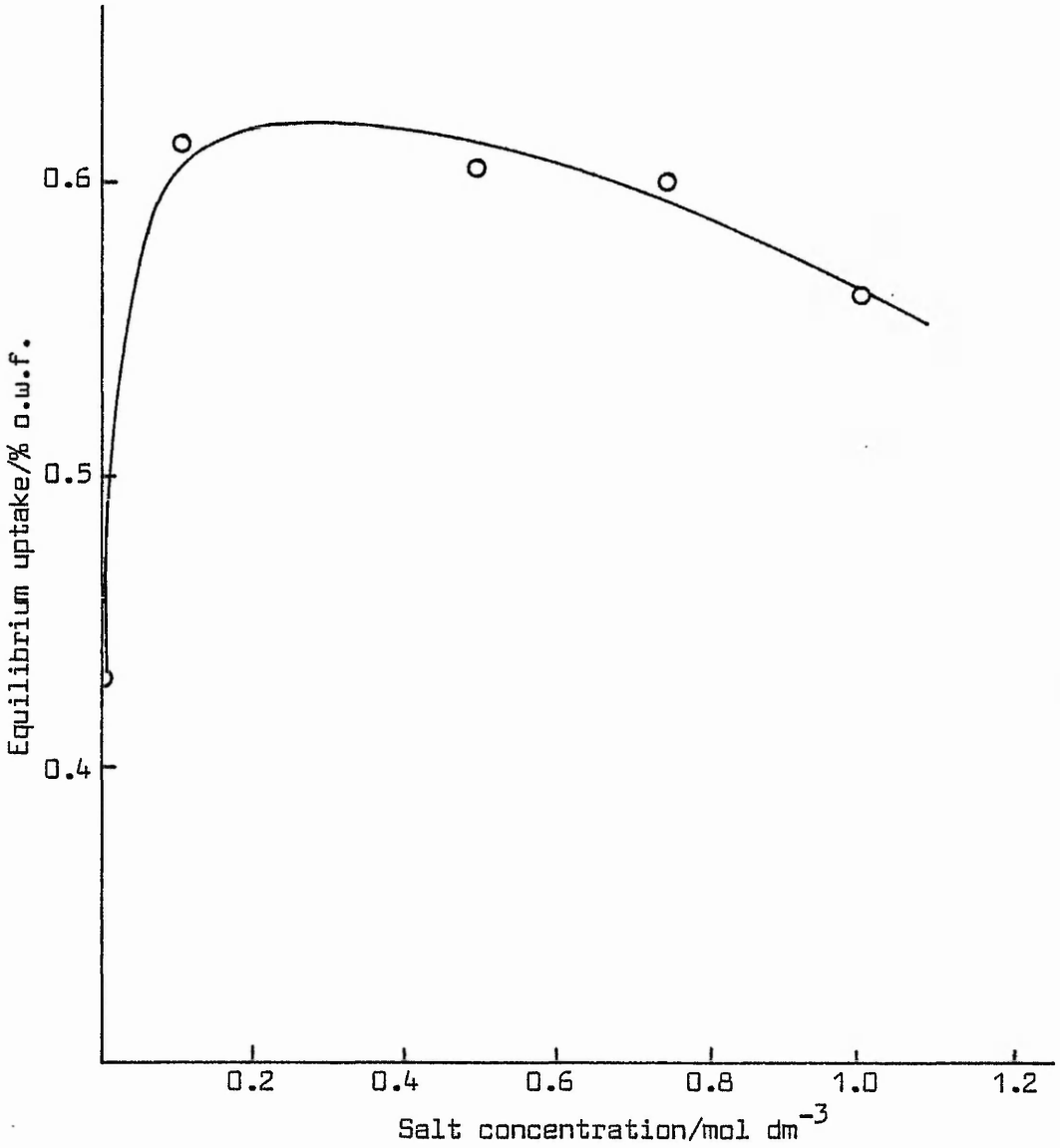


Figure 48 Equilibrium uptake versus Salt concentration for chitosan hydrochloride adsorption on pulp C.

pulp C at a liquor ratio of 30:1 and at a temperature of 20°C was measured and plotted against the free amine content of the sample (Figure 49). The free amine content was calculated on the basis of the metachromic analysis[124] of the standard adsorption solution, since the dye interacts quantitatively with the amine groups. The concentration of dye in the titrimetric solution was determined spectroscopically in the absence of any chitosan using an extinction coefficient of 20,350[126]. From the metachromic analysis the amount of chitosan required to interact with a given volume of this standard dye solution was found and expressed as a ratio of the weight of chitosan to the moles of dye which can be converted into a degree of N-acetylation from a theoretical calibration plot (Figure 50). The results for the free amine analysis are shown in Table 54.

Figure 49 shows that the uptake at equilibrium decreases linearly with increase in the free amine content of the chitosan. This trend can again be explained as a consequence of the polyelectrolyte nature of chitosan in solution. It is the ability of the free amine groups on chitosan to be protonated that gives the polymer its polyelectrolyte properties (see Section 3.1.3.1). Thus as the free amine content is decreased these properties will also decline, resulting in a reduction in the size of the polymer coil and concomitant increases in the adsorption, as outlined for size reduction in Section 3.4.7.4.

3.4.7.6 Molecular weight of chitosan

The effect of molecular weight of an adsorbate on its adsorption has been extensively studied for a number of systems. However care must be taken in interpreting these studies since the molecular

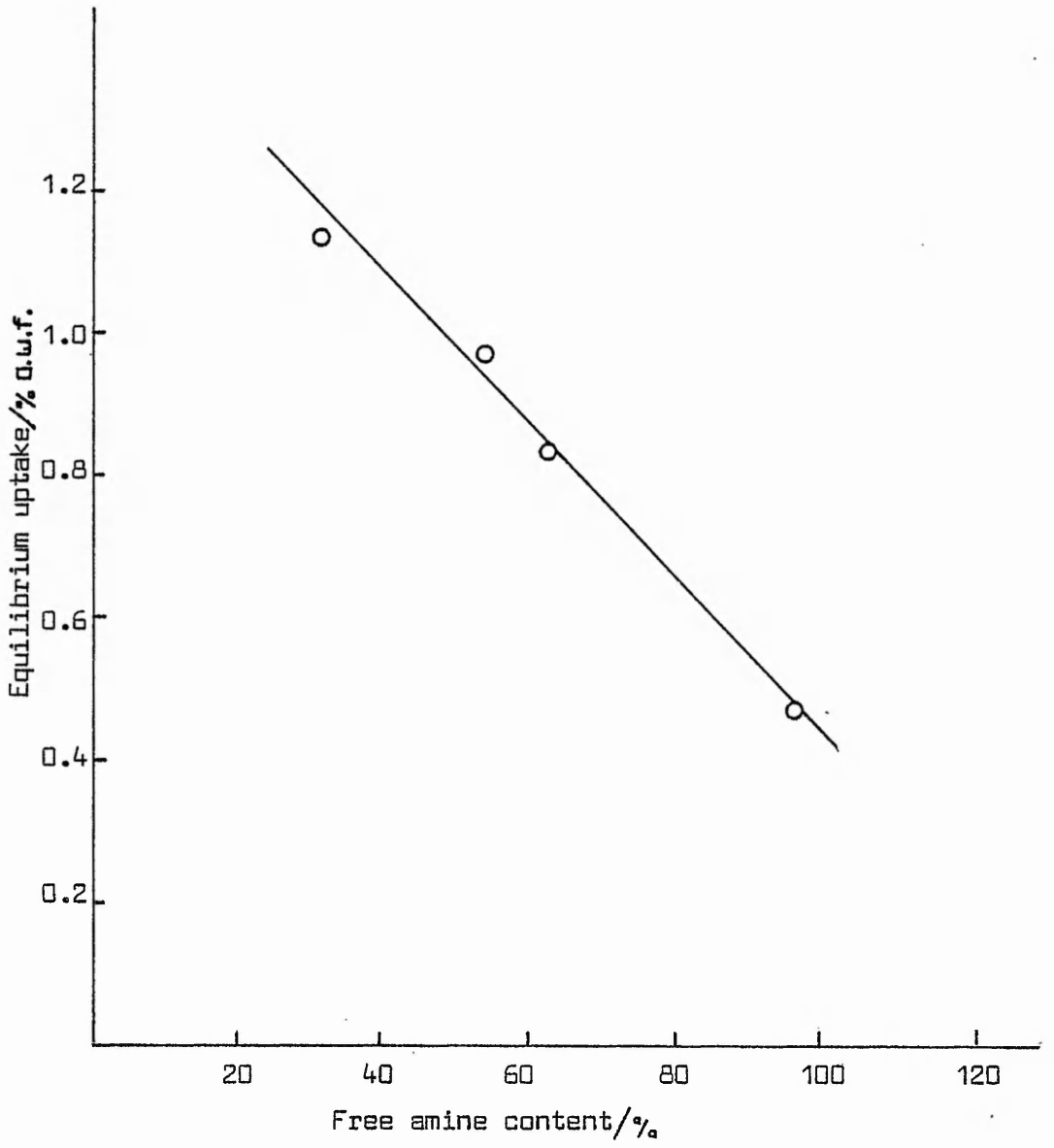


Figure 49 Equilibrium uptake versus Free amine content of the chitosan for adsorption onto pulp C.

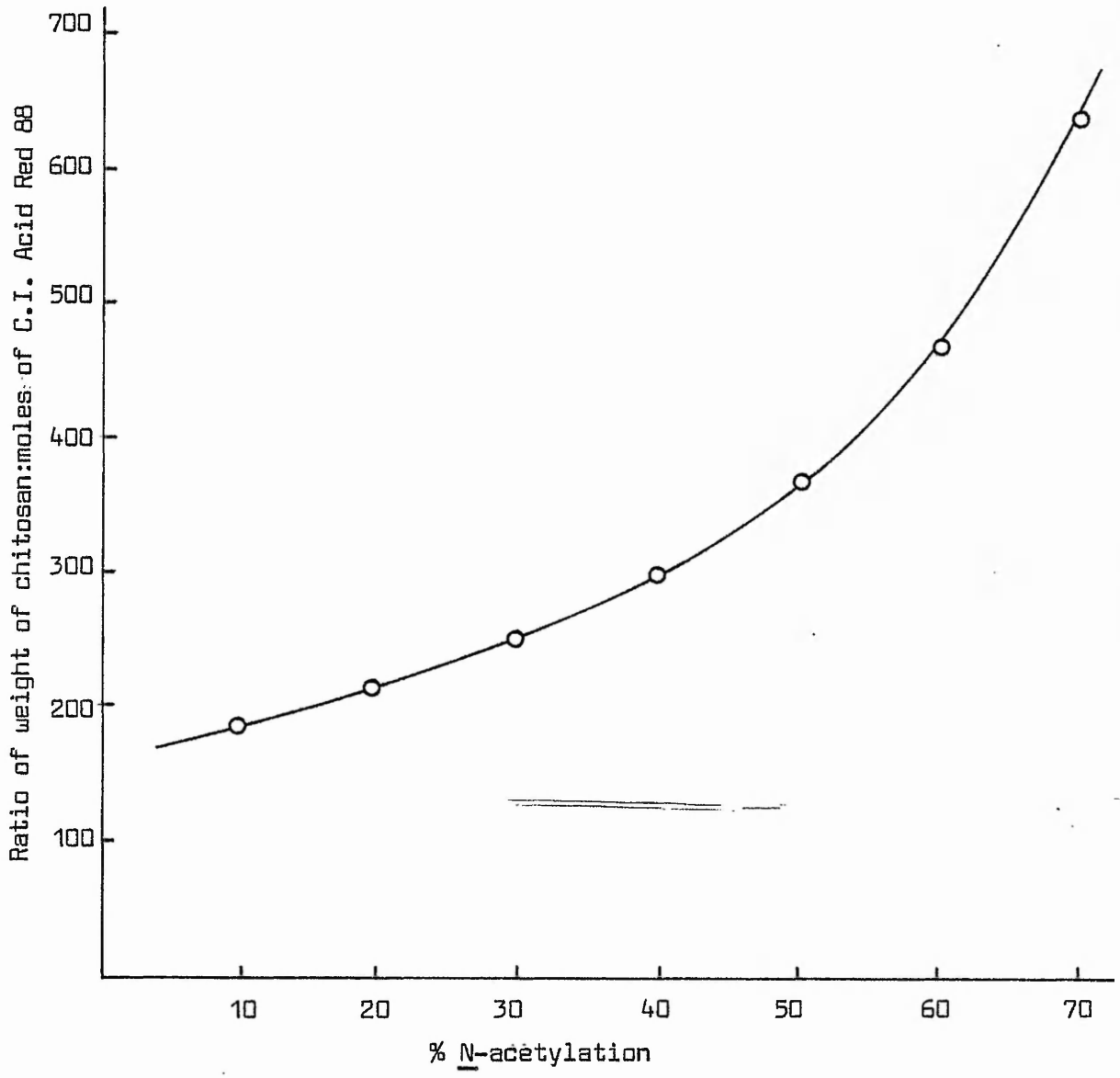


Figure 50 Theoretical calibration plot of Ratio of weight of chitosan:moles of C.I. Acid Red 88 versus % N-acetylation of that chitosan.

Table 54

Metachromic analysis for determination of degree of N-acetylation of chitosan.

Spectroscopic analysis

Sample	Volume of dye/cm^3	Dilution factor	Absorbance at 505 nm in 1 cm cells	Moles of dye $\times 10^6$
19	5	20	0.484	2.38
20	5	20	0.480	2.36
21	5	20	0.478	2.34
22	5	20	0.477	2.34

Metachromic analysis on same volume of dye

Sample	Concentration of chitosan standard /g dm^{-3}	Dilution factor	Volume of diluted standard required for dye/cm^3	Weight of diluted standard required for dye/g	Ratio of chitosan:dye /g mol^{-1}	% N-acetyl groups
19	0.9524	20.06	8.4	3.988×10^{-4}	167	4
20	0.9959	17.41	11.61	6.64×10^{-4}	281	38
21	0.9885	19.65	15.84	7.969×10^{-4}	337	46.5
22	0.9865	10.02	12.69	12.43×10^{-4}	530	64

weight effect is also a function of the porosity of the adsorbent. With a porous adsorbent high molecular weight polymer fractions may be effectively prevented from being adsorbed, except on the external surface of the substrate, as they cannot physically enter the pores of the system.

For non-porous systems Perkel and Ullman[142] concluded that the adsorption is either independant of molecular weight or increases with increasing molecular weight. An empirical relationship between adsorption and molecular weight was found.

$$As = KM^{a} \quad \ldots\ldots\ldots\ldots\ldots\ldots (23)$$

where As is the saturation level of adsorption, M is the molecular weight and K and a are constants (these are not the same as the viscometric constants K and a) The value of a is a function of the conformation of the adsorbate polymer molecules at the solution/ adsorbent interface. If a is zero then adsorption is independant of molecular weight and all the polymer segments would lie in the plane of the adsorbent. If a is 1 then adsorption would be directly proportional to the molecular weight. In this case all the polymer molecules are attached by a single segment. Values of a between 0 and 1 correspond to different conformational models e.g. for a value between 0 and 0.1 the polymer molecule is assumed to be adsorbed as a set of continuous spheres. This corresponds to the adsorbed polymer retaining its random coil configuration after attachment to the surface.

The equilibrium uptake of chitosan of different molecular weights was carried out on pulp C at a liquor ratio of 30:1 and at $20^{o}C$.

Since the attainment of equilibrium is known to be dependant on the molecular weight[136] the system was allowed to equilibrate for 7 days before analysis because a high molecular weight chitosan (Kytex H) was used at a concentration of about 1 g dm^{-3}. After each adsorption experiment the solution was degraded to a lower molecular weight by heating, in a sealed system to prevent solvent evaporation, at 70°C for 48 hours. The molecular weight was determined after each degradation by a single concentration viscometric analysis. For this a two-fold dilution of the chitosan stock was carried out using a solution containing an appropriate quantity of sodium chloride and acetic acid so that the final salt and acid concentration of the diluted system were the same as those used for the determination of the viscometeric constants for chitosan (see Section 3.1.3). From the plots (Figure 13), for this determination, of Viscosity number versus Concentration, the viscosity numbers at the concentration of the diluted adsorption solution (approximately 0.5 g dm^{-3} or 5×10^{-4} g cm^{-3}) were obtained. These were plotted (Figure 51) against the viscosity average molecular weights calculated from the modified Staudinger equation. This graph was used as a calibration so that the molecular weight of the degraded chitosans could be found from a single concentration viscosity number determination. The results are given in Table 55.

A plot of Equilibrium uptake versus Molecular weight (Figure 52) shows an initial decrease in the adsorption, as the molecular weight increases, after which the adsorption becomes independant of the molecular weight.

Although anomalous cases that do not conform to the Perkel-Ullman model[142] have been reported[147,148] where low molecular weight

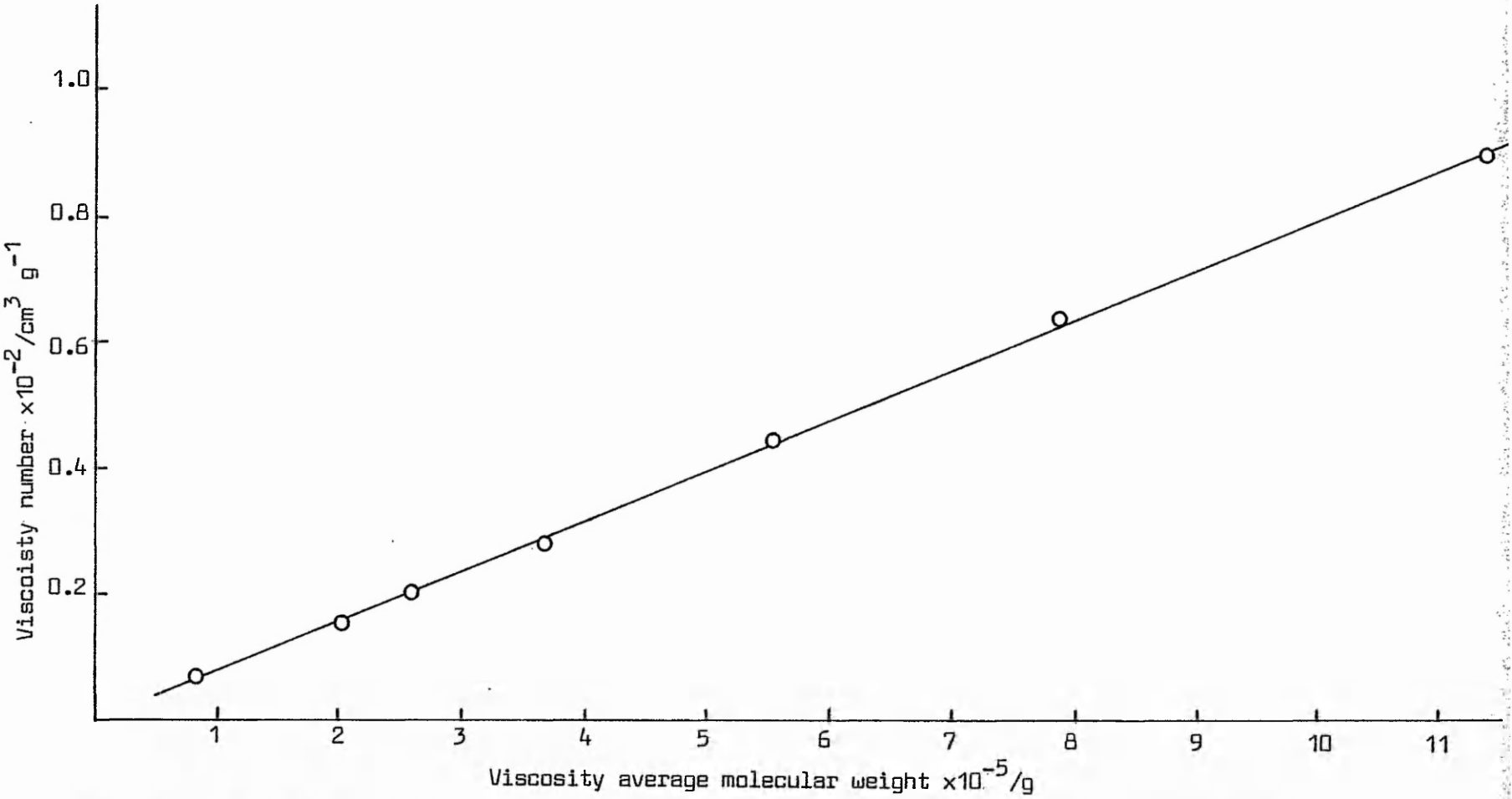


Figure 51 Viscosity number of chitosan phenylosazone samples 7 to 13, at a concentration of 0.45 g dm^{-3} versus Viscosity average molecular weights of these samples in 0.1M acetic acid/0.2M sodium chloride.

Table 55

$\bar{M}v$ from single point viscometric determinations at a polymer concentration of 0.45 g dm^{-3} in 0.1M acetic acid/0.2M sodium chloride.

Sample	Viscosity number	Molecular weight
H1	1580	1,975,000
H2	517	647,900
H3	428	536,000
H4	200	251,400

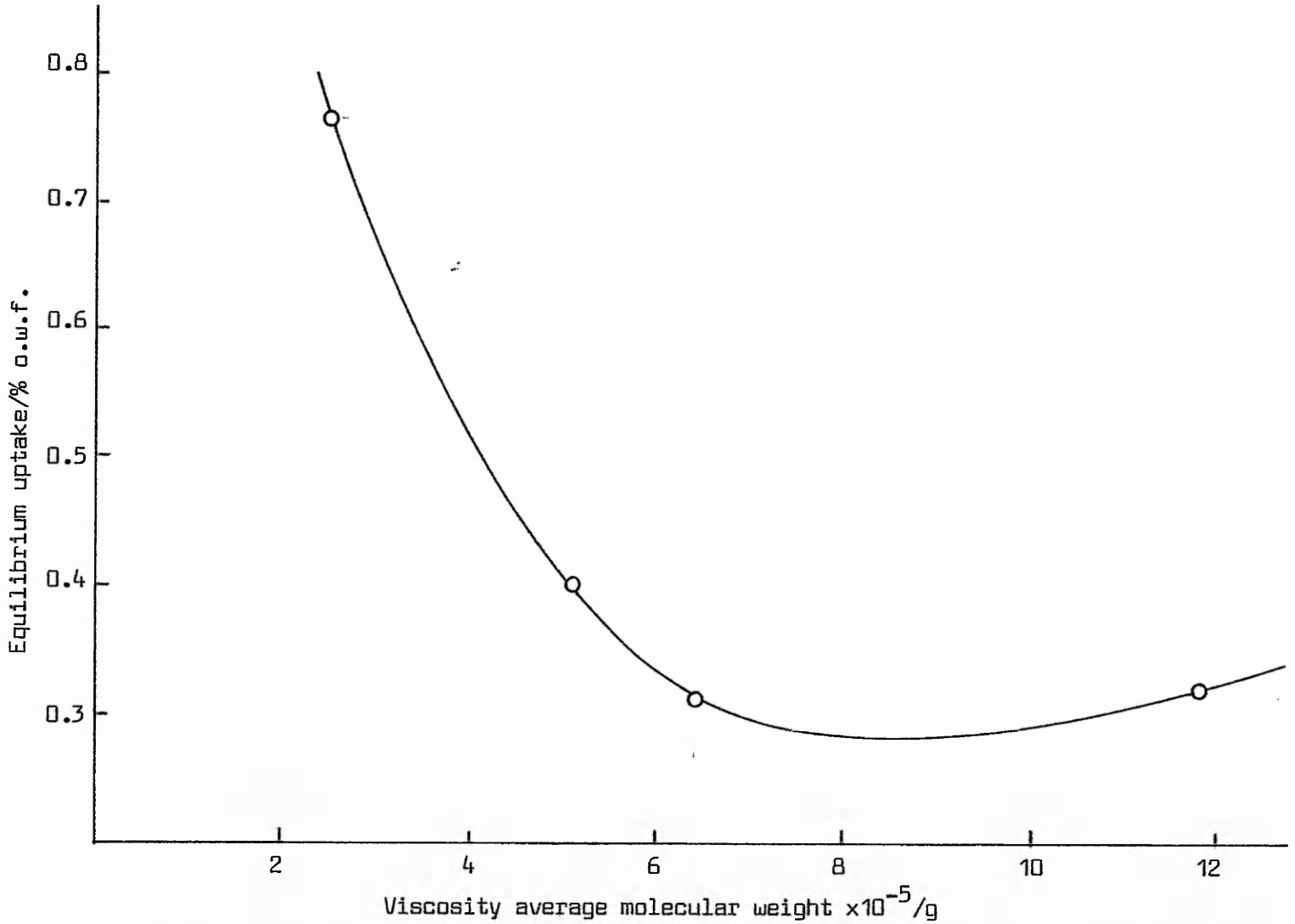


Figure 52 Equilibrium uptake versus Viscosity average molecular weight of chitosan samples for adsorption onto pulp C.

polymers are adsorbed preferentially on non-porous systems, it may be possible to explain the adsorption of chitosan in terms of their model with suitable allowance being given to the porous nature of cellulose as an absorbent. The initial decline in the adsorption with increasing molecular weight, is caused by the effect of increased polymer size on the accessibility of the molecules to the pores of the cellulose. Flory[149] has shown that the volume occupied by a polymer solution is proportional to its molecular weight raised to the power of 3/2, and thus as the molecular weight increases fewer polymer molecules can penetrate the pores of the cellulose resulting in a reduction in the available surface area for adsorption. At and beyond the point where the adsorption becomes independant of the molecular weight it may be assumed that the chitosan molecules are so large that the majority of them are prevented from entering the pores. Thus for the higher molecular weights the adsorption is effectively occuring on a non-porous system. Here the independance of adsorption on molecular weight would suggest that the polymer is adsorbed flat onto the surface as a in equation (23) would be zero.

3.4.7.7 Physical nature of the cellulose substrate

Two important features of the adsorbent are its porosity and its surface area. The area of surface available to solute molecules depends on the size of the pores and of the polymer molecules. Thus by increasing the available surface area the adsorption of chitosan should increase, assuming constant molecular weight. This was investigated by determining the equilibrium adsorption from a 1 g dm^{-3} solution at a liquor ratio of 30:1, for various pulps before and after mechanical treatment of the fibre. This treatment consisted

of chopping the pulps in a high speed rotary blender. Direct comparison between each pulp shows the expected increase in the equilibrium uptake after mechanical processing of the fibre (see Table 56). For most of the samples there was nearly a 90% increase. Thus for any study involving a direct comparison of adsorption on different pulps, the available surface area of each must be identical. During the oxidation reactions on the purified cotton to increase the carboxyl content, mechanical processing was avoided so that the surface area of all the pulps could be assumed to be the same. However for these oxidised pulps there is a tendancy for those with a high carboxyl content to swell in water, and thus increase their surface area.

3.4.7.8 Carboxylic acid group content of the cellulose substrate

The adsorptions at equilibrium for six pulps of different carboxyl content were determined using a chitosan concentration of 1 g dm^{-3} and a liquor ratio of 30:1 at 20°C. A plot of Equilibrium adsorption versus Carboxyl content (Figure 53) indicates that the uptake is dependant, at least partially, on the carboxyl content. The fact that at a zero carboxyl content there was still some adsorption suggests that the forces causing the chitosan to adhere to the cellulose are not solely ionic in nature. An ionic mechanism would explain the increase in carboxyl content due to the greater number of available anionic sites for the cationic chitosan. However it may be better to consider the system in terms of the surface charge of the fibre. This charge is likely to increase with increasing carboxyl content. However the hydrophilic nature of the pulp also increases markedly at the higher carboxyl contents. Hence in the presence of water the pulp will tend to swell causing an increase in the surface area but

Table 56

Equilibrium adsorption uptake of chitosan before and after mechanical treatment. (Liquor ratio 30:1, temperature 20°C, adsorption time 48 hours; chitosan concentration 0.95 g dm^{-3}).

Pulp	Carboxyl content milliequiv. kg^{-1}	Equilibrium uptake before/% o.w.f.	Equilibrium uptake after/% o.w.f.
B	1026	1.15	2.03
C	162	0.47	0.8
D	454	0.34	0.52
E	445	0.91	1.72
F	466	0.79	1.32

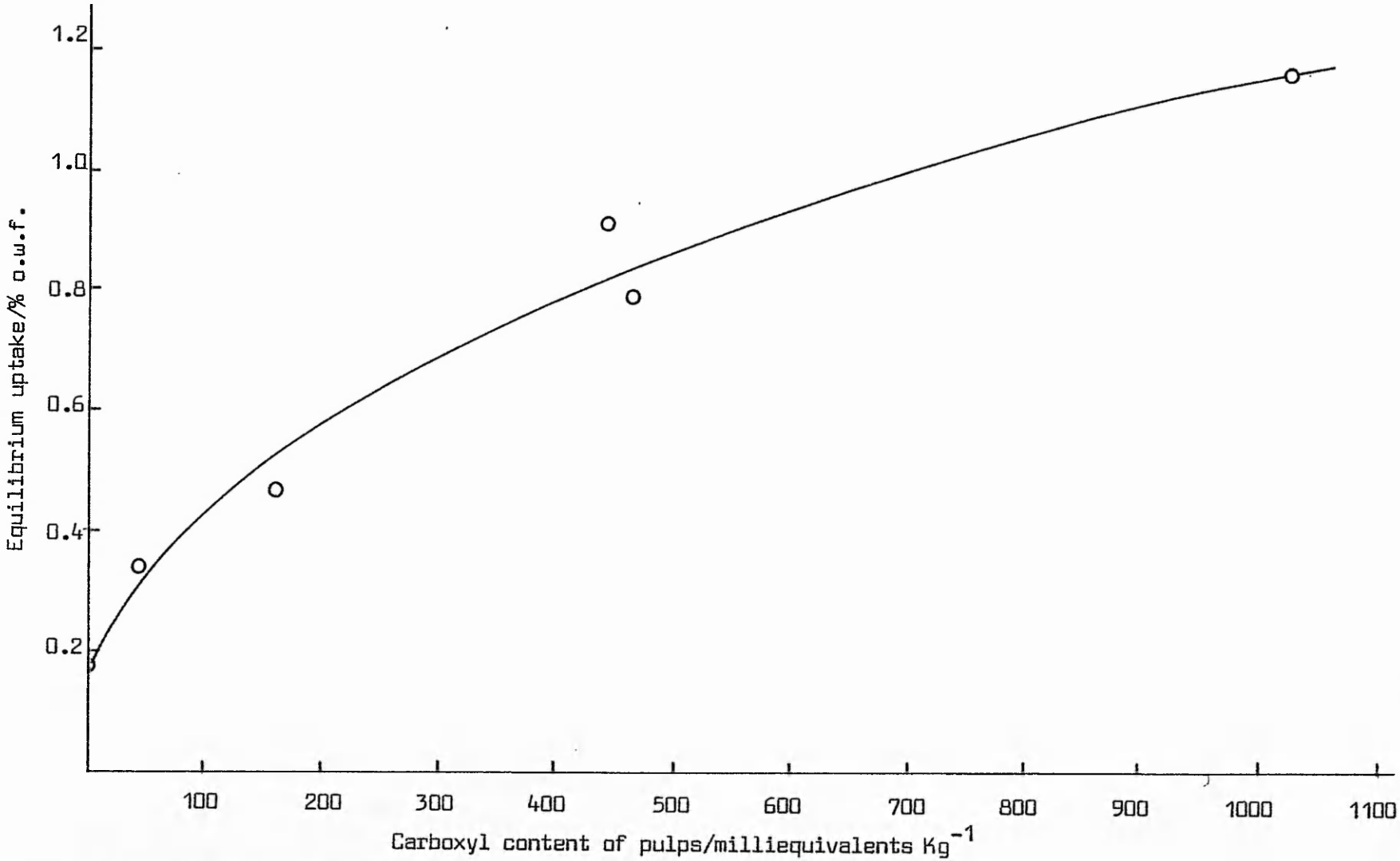


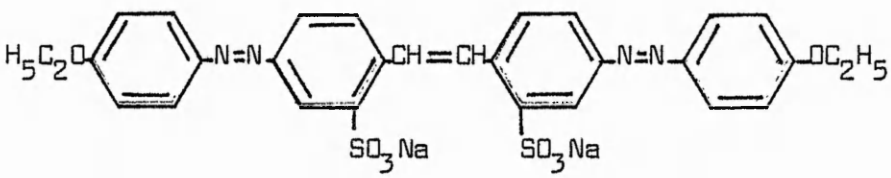
Figure 53 Plot of Equilibrium uptake versus Carboxyl content of pulps.

effectively reducing the charge per unit surface area. Although an increase in surface area may improve the adsorption (see Section 3.4.7.7), the reduction in the effective surface charge appears to be dominant since the equilibrium uptake tends to level off at the higher carboxyl contents.

Another factor that may contribute to the levelling off of the adsorption is the increased solvent/cellulose interaction as a direct consequence of the increasing hydrophilic nature of the fibre thus decreasing the ability of the chitosan to desorb the solvent from the cellulose surface prior to its own adsorption.

3.4.7.9 Variation in the nature of the anionic groups on the cellulose substrate

A series of equilibrium adsorption experiments were carried out, using a chitosan concentration of about 1 g dm^{-3} and the usual conditions of liquor ratio and temperature, on the three reactive dyed pulps (R1, R5 and R10), and also on samples of purified cotton and direct dyed purified cotton. The direct dye used was C.I. Direct Yellow 12 (XVI)



(XVI)

C.I. Direct Yellow 12.

For these last two adsorption measurements a salt concentration of 0.5M was used, because direct dyes are not particularly wash-fast, and thus salt is required to keep them adsorbed on the fibre. The adsorption results are outlined in Table 57.

Although the sulphonic acid group content of the reactive dyed pulps are low when compared to those for the carboxyl group content of the oxidised celluloses, the equilibrium uptake for these dyed systems were very low. They were infact lower than the uptake value (0.173%, see Figure 53) for the purified cotton (from which they were made) under identical adsorption conditions. Also as the concentration of the dye molecules on the fibre is increased the uptake tends to decrease slightly.

These results may be explained in terms of the forces other than ionic that assist the adsorption process e.g. van der Waals forces and hydrophobic bonding[150,151]. Van der Waals forces of attraction are greater the greater the contact area between the two bonding systems e.g. planar systems show an increased interaction in comparison with non-planar systems[152]. Thus for the oxidised celluloses the chitosan can approach relatively closely to the fibre surface and hence increase the contribution of the van der Waals forces. However for the reactive dyed pulps the large dye molecule protrudes from the polymer chain surface because of the constraints imposed by the covalent bond between dye and polymer and thus disrupts the planarity of that surface, reducing the potential contact area. The disruption increases as the dye concentration increases, as shown by the slight decrease in the uptake with increase in dye content.

The driving force of the hydrophobic bond is the increase in the entropy caused by the disruption of the ordered structure of water

Table 57

Equilibrium uptake of chitosan on various substrates. (Liquor ratio 30:1, temperature 20°C, time 48 hours, chitosan concentration 0.95 g dm^{-3}).

Sample	Salt concentration /mol dm^{-3}	Uptake/% o.w.f.	Acid content /milliequiv. kg^{-1}
Purified cotton	0	0.173	0
R1	0	0.11	146
R5	0	0.086	183
R10	0	0.08	341
Purified cotton	0.5	0.376	0
Direct dyed cotton	0.5	0.397	-

that exists around a hydrophobic surface, when two such surfaces combine to expel that water. In general, factors that reduce the van der Waals forces of attraction will also reduce the hydrophobic contact.

Further evidence to support the theory of the existence of other bonding forces comes from the values for the equilibrium uptake for the direct dyed pulp compared to the undyed purified cotton. There is a slight increase in the uptake of chitosan with the dyed pulp over that with the cotton. In this case there is little disruption of the fibre surface by the dye because direct dyes are planar and are known to interact with cellulose via van der Waals forces, and thus lie in the plane of the fibre surface. Hence the chitosan can still approach fairly closely to the fibre surface and maximise the van der Waals forces between itself and the surface or the dye molecules. The increase in adsorption can be attributed to the slight increase in the surface charge of the pulp caused by the sulphonic acid groups on the direct dye.

3.4.8 Extent of ionic interaction in the adsorption of chitosan on cellulose

In order to determine the extent of ionic bonding between the chitosan and any anionic pulp sites a sample of blended pulp C was dyed with an alkaline solution (pH8) of Methylene Blue to bring about ion exchange between the carboxyl hydrogen ions and the cationic dye ions. After careful washing to remove excess dye the pulp was used for an equilibrium adsorption experiment, using a 1 g dm^{-3} chitosan solution under the usual conditions of liquor ratio (30:1) and temperature ($20^{o}C$). After adsorption, but prior to metachromic analysis

to determine the residual chitosan concentration, a portion of the solution was removed for spectroscopic analysis to determine the concentration of Methylene Blue ions that had been displaced by ionic interaction between the protonated amine groups of chitosan and the anionic carboxyl group. For this analysis an extinction coefficient for Methylene Blue was determined in a 0.6% solution of acetic acid, by preparing two identical solutions of Methylene Blue, one in ethanol and the other in 0.6% acetic acid. The extinction coefficient in ethanol is known to be 89,125 at 655 nm and using this the value of 72,708 was obtained for the molar extinction coefficient in the 0.6% acetic acid at 664 nm.

After steeping, the absorbance of the solution at 664 nm was found to be 0.096 in 1 cm cells. This is equivalent to a Methylene Blue concentration of 1.32×10^{-6}M and 1.98×10^{-8}mol in the 15 cm^3 of the solution used for adsorption. The metachromic analysis (see Appendix IV) revealed that the pulp had adsorbed 3.25×10^{-3} g of chitosan leading to an uptake of 0.643% o.w.f. From the fact that the chitosan used (Kytex L) has a degree of N-acetylation of 16%, the 3.25×10^{-3} g adsorbed would contain 1.63×10^{-5} moles of amine groups. Hence the ratio of amine to carboxyl was found to be $1.63 \times 10^{-5} / 1.98 \times 10^{-8}$ = 822. Hence approximately only 1 amine group in every 800 is actually ionically bound to the fibre. Thus unless other forces (see Section 3.4.7.9) contribute it would appear that the chitosan is attached at a very small number of points on the fibre surface. This would correspond to the case in the Perkel-Ullman model where the adsorbed polymer retains its random coil configuration after adsorption. In this configuration the adsorbed layer would have a considerable thickness, approximately the diameter of the coil.

However the results from Section 3.4.7.9 suggest that other forces are operating in the adsorption process. It therefore seems likely that the anionic sites on the pulp serve to attract the cationic chitosan molecules to the vicinity of the fibre surface where the adsorption process is then enhanced by van der Waals and hydrophobic interactions, with little ionic exchange occuring. The idea of the chitosan being distributed by planar interactions over the surface is consistent with the model proposed for the adsorption based on the molecular weight experiments (see Section 3.4.7.6).

CHAPTER 4

CONCLUSIONS

1) The reducing end groups in chitosan can form an arylosazone derivative by homogeneous reaction with an arylhydrazine in aqueous acetic acid. Arylosazone formation occurs both when the reducing end group is a 2-amino-2-deoxy-D-glucose residue and when it is a 2-acetamido-2-deoxy-D-glucose residue.

2) Number average molecular weights of chitosan samples, in which all the end groups were initially in the reducing form can be determined from absorbance measurements on solutions of their phenylosazone derivatives.

3) The viscometric constants a and K in the modified Staudinger equation can be determined for chitosan in 0.1M acetic acid/0.2M sodium chloride and in 0.1M acetic acid/0.02M sodium chloride using a technique that utilises the properties of the molecular weight distribution resulting from random degradation of the polymer chains, in this case from acid hydrolysis of the polymer in solution. The values obtained for the viscometeric constants differ from those obtained previously but are similar to those reported for related β-(1→4)- linked ionic polysaccharides. The modified Staudinger equation for chitosan in 0.1M acetic acid/0.2M sodium chloride can be expressed as:

$$[\eta] = 1.81 \times 10^{-3} \bar{M}_v^{0.93}$$

and in 0.1M acetic acid/0.02M sodium chloride as:

$$[\eta] = 3.04 \times 10^{-5} \bar{M}_v^{1.26}$$

where $[\eta]$ is expressed in $cm^3\ g^{-1}$.

4) Heterogeneous reaction of chitosan with arylhydrazines in methanol at room temperature leads to the formation of arylhydrazone derivatives of chitosan which undergo formazan formation with diazotised aniline in the absence of base.

5) The structure of the arylosazone derivatives of chitosan involves internal chelation between the nitrogen of the C(1) arylhydrazone group and the imino hydrogen of the C(2) arylhydrazone group. The electrons of the C(2) arylhydrazone group are highly delocalised, whereas those of the C(1) group are not.

6) Both homogeneous and heterogeneous reaction between chitosan and the amine specific reagent 2,4-dinitrofluorobenzene yields a product in which the amine groups have only partially reacted. Gelation occurs in the homogeneous reaction due to the desolubilising effect of the N-(2,4-DNP)- residues. Incomplete reaction is assumed to be caused by steric effects associated with the bulky N-(2,4-DNP)-groups.

7) The destruction of the N-(2,4-dinitrophenyl)-D-glucosamine chromophore system in aqueous media at temperatures greater than approximately 50°C is a consequence of the carbonyl group, formed during mutarotation of the product in amphiprotic solvents, such as water. The effect of the carbonyl group is specific due to its position relative to that of the chromophore and operates probably by chemical reduction of the nitro group on the benzene ring.

8) The degree of N-acetylation of a series of chitosan samples of varying extents of N-acetylation found by both residual salicylaldehyde analysis and HBr titrimetric techniques were in good agree-

ment with the previously published infrared method based on the absorbance ratio A1665/A3450.

10) Homogeneous reaction between chitosan and salicylaldehyde in 80/20 (v/v) methanol/1% acetic acid is an equilibrium process. The position of equilibrium is sensitive to both the acid and the salicylaldehyde concentrations. However at salicylaldehyde concentrations in excess of 3:1, salicylaldehyde:chitosan amine groups, the equilibrium is shifted completely toward the product (Schiff's base) side. At constant acid conditions and in the presence of excess salicylaldehyde the position of equilibrium, found by measuring the uv/visible absorbance intensity at 410 nm, was quantitatively dependant upon the chitosan concentration.

11) The amount of salicylaldehyde, as determined by uv spectroscopic analysis, hydrolysed from N-salicylidene chitosan by the action of a 80/20 (v/v) methanol/0.5N HBr solvent is quanititatively dependant upon the amine content of the chitosan, and can be used to characterise the free amine group content of a sample of chitosan or to determine the concentration of chitosan, of known free amine group content, adsorbed on a substrate.

12) The reflectance spectra of N-salicylidene chitosan-paper systems can be related empirically to the amount of chitosan adsorbed on the paper through a reflectance function. The Atherton function appears to give a closer theoretical agreement with the results than does the Kubelka-Munk reflectance function, although neither gives perfect agreement.

13) The adsorption of chitosan from solution onto cellulose is an equilibrium process.

14) The equilibrium uptake is dependant on the surface charge of the pulp, in that factors that increase the surface charge, such as the presence of carboxyl groups will also increase the uptake. The effect of the carboxyl group is reduced at the higher carboxyl contents due to the hydrophilic nature of these groups, which in the presence of water swells the fibre and effectively reduces the surface charge per unit area.

15) The equilibrium uptake is dependant upon the physical nature of the pulp with respect to the available surface area for adsorption. This is also a function of the porosity of the cellulose and in general an increase in the surface area results in an increase in adsorption. The effect of increase in the surface area caused by swelling due to hydrophilic carboxyl groups is offset by the adverse effect this swelling has on the suface charge/unit area.

16) The adsorption process is dependant not only on polymer/adsorbent interactions but also on polymer/solvent and solvent/adsorbent interactions as shown by the effects on the equilibrium uptake of polymer concentration and solution liquor ratio.

17) Factors that decrease the size of the polyelectrolyte random coil tend to increase the equilibrium uptake e.g. addition of small amounts of added electrolyte or decrease in the free amine content. In the case of addition of large amounts of added electrolyte the effect on the polyelectrolyte random coil size reaches a maximum and the excess electrolyte will then cause a reduction in the adsorption

by charge neutralisation of the surface anionic groups on the fibre.

18) The effect of the molecular weight of the chitosan on the adsorption process is controlled by the relationship between the molecular size of the polymer and the pore size of the pulp.

19) During the adsorption process very little ionic exchange occurs between the protonated amine groups on the chitosan and the free acid groups on the fibre.

20) Disruption of the planarity of the fibre surface causes a reduction in the equilibrium uptake, even in the presence of an increased anionic character.

21) A mechanism is proposed for the adsorption of chitosan onto cellulose. The cationic polymer is initially attracted to the anionic surface of the fibre where it becomes attached to the fibre predominantly through van der Waals forces of attraction and hydrophobic interactions, rather than by ionic exchange. The greater the surface charge of the fibre the greater the initial attraction and thus the greater the uptake. Reduction in size of the polymer random coil improves its ability to penetrate the pores of the system thus increasing the available surface area for adsorption.

In the vicinity of the fibre the polymer molecules are assumed to lie in the plane of the surface because in this configuration the van der Waals and hydrophobic interactions will be at their maximum.

22) For application of chitosan adsorption to the paper making system, conditions must be initially optimised for maximum adsorption prior to sheet formation.

CHAPTER 5

EXPERIMENTAL

5.1 Materials used

5.1.1 Chitosan

The chitosan used in this work was supplied by Hercules Incorporated (Kytex H, Lot SPX 5350, and Kytex L, Lot XA390-75-1).

5.1.2 Pulps

The cellulose used for the adsorption experiments was purified cotton. Other pulps were of commercial types, the nature of which are given in Table 33.

5.1.3 Chemicals

The chemicals and solvents used were of General Purpose Reagent grade, except those used in the radioisotope studies which were of radiochemical grade with respect to isotope activity, at a 99% chemical purity. The dyes used were commercial samples and were purified as described in Section 5.5.1. The water was singly distilled.

5.2 Spectroscopic methods

Infrared spectra were recorded on a Perkin Elmer 137 sodium chloride spectrophotometer.

Reflectance measurements were made on a single beam Pye-Unicam SP500 spectrophotometer and on a ICS Micromatch abridged spectrophotometer.

Uv and visible spectra and single wavelength measurements were carried out on a Perkin Elmer 551S spectrophotometer.

Proton magnetic resonance spectra were recorded on a Jeol JNMC-60HL 60 MHz spectrophotometer with tetramethylsilane as the internal standard.

Liquid scintillation counting was carried out using a Packard Tri-carb Liquid Scintillation spectrometer.

5.3 Dilute solution viscometry

Viscometric measurements were made using a suspended level dilution viscometer with a sintered glass filter, as supplied by Polymer Consultants Ltd. All measurements were carried out in a thermostatically controlled water bath at $25^{o}C \pm 0.05^{o}C$ and timed by a stop watch accurate to 0.2 seconds.

5.4 Characterisation of starting materials

5.4.1 Chitosan

Due to process variations in the production of chitosan the products often have different characteristics of N-acetyl content and molecular weight and thus require characterisation.

5.4.1.1 Molecular weight

The molecular weight of both Kytex H and Kytex L were determined by measurement of their Limiting Viscosity Number in a 0.1M acetic acid/0.2M sodium chloride solvent using the suspended level dilution viscometer. Repeat measurements of flow times were made until readings agreed to within 0.2 of a second. The Limiting Viscosity

Numbers were related to molecular weight using the viscometric constants determined in Section 3.1 and subsequently reported by the author[29]. The Limiting Viscosity Numbers (LVN's) were found from plots (Figures 10 and 54) of Viscosity number versus Concentration, expressed in g cm^{-3}, by extrapolation to zero concentration. The values for the LVN's were found to be 1140 and 281 respectively for the Kytex H and Kytex L, and correspond to molecular weights ($\bar{M}v$) of 1.72 $\times 10^6$ g and 3.82 $\times 10^5$ g respectively.

5.4.1.2 Degree of N-acetylation

The degree of N-acetylation for Kytex H and Kytex L was determined using the infrared method of Moore and Roberts[40], the accuracy of which has been established by correlation with two independant techniques (see Section 3.2). They were found to be 16% for Kytex L and 21% for Kytex H.

5.4.2 Cellulose

5.4.2.1 Carboxyl content

The oxidised celluloses in their free acid form were characterised with respect to their carboxyl content using a modification of the method of Samuelson and Wenerblom[135].

The pulp (0.2 g, accurately weighed) was placed in a flask with a 0.01M sodium hydroxide/0.43M sodium chloride solution (40 cm^3). The flask was stoppered, shaken, and allowed to stand for 48 hours at room temperature. The whole sample was then titrated against a 0.01M hydrochloric acid solution to a phenolphthalein end point. The titrated solution was filtered and an aliquot (20 cm^3) further titrated against the hydrochloric acid using a mixed indicator of

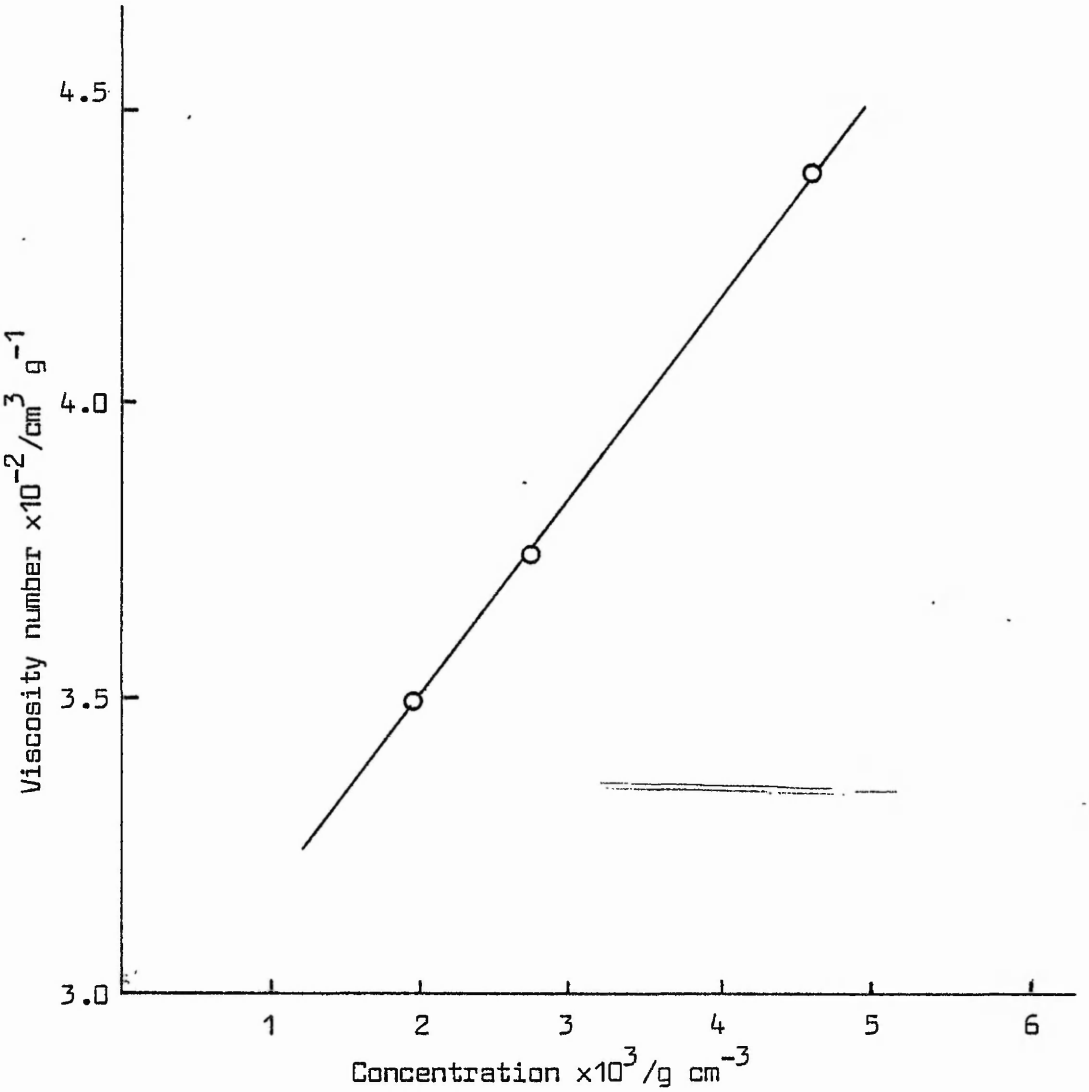


Figure 54 Viscosity number versus Concentration for Kytex L in 0.1M acetic acid/0.2M sodium chloride.

1 g dm^{-3} methyl red/1 g dm^{-3} bromocresol green to the end point where a colour change to red was stable for a few seconds. The solution was then boiled for two minutes and the titration completed to a neutral grey end point. With suitable allowance for the volume of solution used in the second titration, the hydrochloric acid used corresponds to the excess sodium hydroxide left in solution after reaction with the carboxyl groups on the pulp. The volume of standard hydrochloric acid consumed by the original alkaline salt solution (40 cm^3) was also determined separately, titrating in exactly the same way except that no filtration was required. The moisture content of the pulp was also determined by heating to constant weight.

5.4.2.2 Sulphonic acid content

The sulphonic acid content of the celluloses dyed with C.I. Reactive Red 13 were determined by spectroscopic analysis of the dye content of these pulps.

To the pulp (0.1 g, accurately weighed) was added a 70% (v/v) sulphuric acid solution (25 cm^3). The solution was allowed to stand for two hours after which the absorbance intensity at 573 nm was recorded in 1 cm cells against a standard prepared in an identical manner from a purified cotton sample. The moisture contents of the pulps were determined by heating to constant weight. The absorbance at 573 nm can be related to the dye concentration through a molar extinction coefficient determined for the dye at the same wavelength and in an identical solvent. This was found to be 29,957.

5.5 Extinction coefficient determinations

A considerable number of molar extinction coefficients for various compounds in various solvent systems were determined.

Unless otherwise stated these were found by purification of the particular compound and preparation of an accurate solution in the desired solvent system. The uv/visible absorbance intensity at the wavelength of interest was then recorded for these solutions. The molar extinction coefficient was found from either a single concentration absorbance reading (ratio of absorbance to concentration) or from the slope of a plot of Absorbance versus Concentration in mol dm^{-3}.

5.5.1 Purification of compounds

The majority of the organic compounds investigated were purified by recrystallisation (see individual preparation Sections). However for the dyes studied, other techniques were used.

5.5.1.1 Purification of C.I. Reactive Red 13

The commercial C.I. Reactive Red 13 (1 g) was dissolved in methanol (100 cm^3), and the resulting solution filtered, and evaporated to dryness on a steam bath. The solid obtained was dissolved in distilled water (50 cm^3) and passed down a cation exchange column containing Amberlite IR 120 (H^+) (30 cm^3), at a flow rate of 1 cm^3 per minute. The solution was repeatedly passed through the column until it was free of sodium ions as indicated by a negative flame test result, and then allowed to evaporate to dryness at room temperature.

5.5.1.2 Purification of Methylene Blue

This was purified by soxhlet extraction of the dye (1 g) with methanol (150 cm^3) for 4 hours. After this the solvent was evaporated on a steam bath, and the solid obtained was reextracted until

free from salt as shown by a negative chloride ion test using acidified silver nitrate.

5.6 Preparation of chitosan

5.6.1 Powder form

Chitosan (1 g) was dissolved in 1% aqueous acetic acid (100 cm^3). The resulting solution was filtered through a monofilament polyester cloth and then poured into methanol/0.880 ammonia (7/3 (v/v), 200 cm^3). The white precipitate was stirred thoroughly to ensure complete neutralisation and filtered off. It was washed on the polyester filter pad with copious quantities of methanol until the filtrate was neutral to litmus, then with ether (100 cm^3). The product was allowed to dry in a stream of warm air, during which time it was ground into a fine powdery form.

5.6.2 Film form

Chitosan (1 g) was dissolved in 1% aqueous acetic acid (100 cm^3) and filtered through a monofilament polyester cloth. The viscous solution was then poured onto a glass plate and spread as thinly as possible. The plate was covered and the solvent allowed to evaporate, after which it was immersed in a large tank containing methanol/0.880 ammonia (7/3 (v/v)), for 16 hours and then into a tank containing a 10% solution of sodium hydroxide for 3 hours. This last treatment caused the film to separate from the plate, after which it was removed and washed by immersion in water and then in methanol before being dried between filter papers.

5.7 Preparation and reaction of arylhydrazine derivatives of chitosan

5.7.1 Arylosazones of chitosan

5.7.1.1 Chitosan phenylosazone

Chitosan (1 g) was dissolved in 1% (v/v) aqueous acetic acid (175 cm^3) and phenylhydrazine (2.5 cm^3) dissolved in methanol (10 cm^3), and sodium acetate (1 g) were added to the solution. The reaction mixture was kept at 70^{o}C in a stoppered flask for 48 hours, after which the chitosan phenylosazone was precipitated out by pouring the solution into methanol/0.880 ammonia (7/3 (v/v), 200 cm^3). The product was filtered on a monofilament polyester cloth, washed well with methanol until the filtrate was neutral to litmus and then with ether (100 cm^3). The yellow solid thus obtained was air dried before being soxhlet extracted with methanol (150 cm^3) for 4 hours, after which it was refiltered and allowed to dry.

Yield = 0.95 g; λ max (1% HBr/methanol) 395 nm

5.7.1.2 Chitosan p-nitrophenylosazone

This was prepared using a similar procedure to the one employed for the chitosan phenylosazone, except that due to the poor solubility of the p-nitrophenylhydrazine reagent a saturated solution of it was prepared in methanol (100 cm^3) and this was added to the chitosan solution.

Yield = 0.9g; λ max (1% HBr/methanol) 385 nm, 430 nm (sh)

5.7.2 Arylhydrazones

5.7.2.1 Chitosan phenylhydrazone

A chitosan film (0.1 g) was steeped in methanol (200 cm^3) to which was added phenylhydrazine (3 cm^3). After 5 hours the film was removed and washed with methanol (4 x 50 cm^3) and then allowed to dry between filter papers.

λ max (thin film) 280 nm; λ max (1% HBr/methanol) 280 nm

5.7.2.2 Chitosan p-nitrophenylhydrazone

A chitosan film (0.1 g) was steeped in methanol (200 cm^3) saturated with p-nitrophenylhydrazine. After 16 hours the film was removed and treated in a similar manner to the phenylhydrazone product.

λ max (thin film) 395 nm; λ max (1% HBr/methanol) 385 nm

5.7.3 Formazan derivatives

5.7.3.1 Arylhydrazone formazans

The formazan derivative of both chitosan phenylhydrazone and chitosan p-nitrophenylhydrazone were prepared in the same way:

Aniline (1.3 g) was dissolved in 18% (v/v) hydrochloric acid (10 cm^3) and the solution cooled to 0^oC. To this was added, by drop-wise addition, a solution of sodium nitrite (1.3 g) in water (5 cm^3) with the temperature maintained at 0^oC. To the resulting diazonium solution was added methanol (80 cm^3) and pryidine (20 cm^3).

A chitosan arylhydrazone film (0.05 g) was then steeped in this solution for 24 hours at 0^oC. The film was removed, washed with methanol (4 x 50 cm^3) and allowed to dry between filter papers.

chitosan phenylhydrazone formazan:

λ max (thin film) 280 nm, 420 nm (sh)

λ max (1% HBr/methanol) 265 nm, 420 nm (sh)

chitosan p-nitrophenylhydrazone formazan:

λ max (1% HBr/methanol) 265 nm, 400 nm (sh)

5.7.3.2 Attempted formazan formation from chitosan phenylosazone

Chitosan phenylosazone was initially obtained in film form according to the method outlined in Section 5.6.2. The film was reacted under similar conditions to those described in Section 5.7.3.1 for arylhydrazones. A second film was reacted in an identical diazonium solution which also contained 2M ethanolic potassium hydroxide (20 cm^3).

5.7.3.3 Preparation of D-glucose-p-nitrophenylhydrazone formazan

D-glucose-p-nitrophenylhydrazone[153] (1 g) was dissolved in ethanol (40 cm^3) and pyridine (60 cm^3), and cooled to 0^oC. Aniline (1.3 g) was dissolved in 18% (v/v) hydrochloric acid (10 cm^3) at 0^oC. To this was added, by dropwise addition, a solution of sodium nitrite (1.3 g) in water (5 cm^3) also at 0^oC. The resulting diazonium solution was added, by dropwise addition, to the D-glucose-p-nitrophenylhydrazone solution and the whole system stirred for 30 minutes, after which it was poured onto crushed ice (1500 cm^3). The resultant precipitated solid was filtered off and recrystallised from n-butanol saturated with water.

Yield = 0.6 g; λ max (ethanol) 395 nm, 285 nm

5.8 Preparation of phenylosazone derivatives of model compounds

5.8.1 From N-acetyl-D-glucosamine

To N-acetyl-D-glucosamine (0.5 g) was added the minimum of water required for dissolution. To this was added phenylhydrazine (1.25 cm^3) in methanol (5 cm^3), glacial acetic acid (1 cm^3) and sodium acetate (0.5 g). The solution was heated in a water bath at 40°C for 2 hours after which the resultant precipitate was filtered off and washed well with water.

Yield = 0.09 g; m.p. = 206°C; ν max (KBr) 1605 cm^{-1}, 1505 cm^{-1} (phenyl ring), 1540 cm^{-1} (N-H), 1575 cm^{-1} doublet (N=N)

5.8.2 From sodium D-glucuronic acid

To sodium D-glucuronic acid (1 g) dissolved in the minimum of water was added phenylhydrazine (1.5 cm^3) in methanol (5 cm^3), glacial acetic acid (2 cm^3) and sodium acetate (1 g). The solution was heated at 60°C for 2 hours. On cooling, crystallisation occured. The crystals were filtered off and washed with chloroform (100 cm^3). Recrystallisation was carried out using a 50/50 (v/v) water/ethanol system.

Yield = 0.8 g; m.p. = 110°C; ν max (KBr) 1605 cm^{-1}, 1500 cm^{-1} (phenyl ring), 1540 cm^{-1} (N-H), 1585 cm^{-1} (N=N)

5.9 Rate of phenylosazone formation

5.9.1 Relative rates of formation from D-glucose and N-acetyl-D-glucosamine

To D-glucose (0.41 g) and N-acetyl-D-glucosamine (0.5 g), each

dissolved in water (10 cm^3), were added phenylhydrazine (0.75 cm^3) in methanol (5 cm^3), glacial acetic acid (1.25 cm^3) and sodium acetate (0.5 g). Both solutions were diluted to 100 cm^3 in flasks fitted with reflux condensers and placed in a water bath at 40°C. A series of aliquots (5 cm^3) were removed from each solution after various intervals and the uv/visible absorbance intensities of the removed aliquots recorded at 395 nm in 1 cm cells at 20°C.

5.9.2 Rate of phenylosazone formation from chitosan

Chitosan (3 g) was dissolved in 1% (v/v) aqueous acetic acid (450 cm^3), to which was then added phenylhydrazine (6 cm^3) and sodium acetate (3 g). The solution was heated at 70°C under reflux and aliquots (50 cm^3) of the solution were removed after various reaction times and the chitosan phenylosazones precipitated according to the procedure described in Section 5.7.1.1. The product (0.1 g, accurately weighed) was dissolved in 1% (v/v) hydrobromic acid (50 cm^3) and the uv/visible absorbance intensity recorded at 395 nm in 1 cm cells.

5.10 Preparation of a series of phenylosazones from degraded chitosan

Chitosan (4 g) was dissolved in 10% (v/v) aqueous acetic acid (400 cm^3). The solution was heated under reflux, at the boil, and aliquots (65 cm^3) were removed at 12 hourly intervals. Each aliquot was treated by the addition of phenylhydrazine (3 cm^3), water (135 cm^3) and sodium acetate (1 g) after which it was heated at 70°C for 48 hours. The chitosan phenylosazone was precipitated from solution and purified as described in Section 5.7.1.1. The samples produced were labelled 1-6.

5.10.1 Chemical reduction of chitosan and subsequent degradation and preparation of a series of phenylosazones

Chitosan (6 g) was slurried in methanol (300 cm^3) and run into a 2 cm diameter column. The column was eluted with a methanol/5% acetic acid (95/5 (v/v)) solution until the chitosan particles were swollen. The product was then rinsed with methanol and air dried. The solid obtained was dissolved in 1% (v/v) aqueous hydrobromic acid and cooled to 0^oC, after which sodium borohydride (10 g) in water (200 cm^3) was slowly added to the vigourously stirred solution. Simultaneously a 10% (v/v) hydrobromic acid solution (100 cm^3) was added at a rate sufficient to maintain the pH at about 3. After addition of both solutions was complete the chitosan was reprecipitated by the addition of methanol/0.880 ammonia (7/3 (v/v), 1200 cm^3), filtered on a monofilament polyester cloth and washed with methanol until the filtrate was neutral to litmus. The methanol-damp solid was then dissolved in 10% (v/v) aqueous acetic acid (600 cm^3) and the solution maintained at 70^oC in a flask fitted with a reflux condenser. At various intervals aliquots (85 cm^3) were removed and treated as described in Section 5.10 to give a series of chitosan phenylosazones labelled 7-13.

5.11 Molecular weight and viscometric determinations

5.11.1 Spectroscopic determination of molecular weight ($\bar{M}n$)

The procedure for all the degraded chitosan phenylosazones was identical. A known weight of material (about 0.1 g) was dissolved in a known volume of a methanol/0.1M hydrobromic acid (7/3 (v/v)) solution (usually 50 cm^3) and the uv/visible absorbance intensity

recorded at 395 nm in 1 cm cells against the standard methanol/HBr solvent. The absorbance intensity can be related to the number average molecular weight (see Results).

5.11.2 Viscometry

Dilute solution viscometric measurements were made using the degraded chitosan phenylosazones, samples 1-13, in 0.1M acetic acid/ 0.2M sodium chloride and on samples 7, 9, 11 and 12 in a 0.1M acetic acid/0.02M sodium chloride solvent. The conditions and procedure were as outlined in Section 5.3, except that dilutions were not made by addition of solvent to the polymer in the viscometer. Instead the samples were initially prepared at four different concentrations. After the flow time of the most dilute solution was measured it was removed from the viscometer by suction, which was then washed twice with aliquots (10 cm^3) of the next most dilute solution, before flow time measurements were made on a third aliquot of that solution.

5.12 Preparation of a series of chitosans of varying degree of N-acetylation

These were prepared by homogeneous N-acetylation of chitosan according to the general method of Moore and Roberts[100]. Chitosan (6.25 g) was dissolved in 1% (v/v) aqueous acetic acid (625 cm^3). This solution was divided into 5 equal portions to each of which was added methanol (150 cm^3) and then separately the following volumes of a 2% (v/v) solution of acetic anhydride in methanol (3.5 cm^3, 12 cm^3, 15 cm^3, 20 cm^3 and 30 cm^3). After 1 hour the polymer in each solution was precipitated by pouring onto methanol/0.880 ammonia (7/3 (v/v), 100 cm^3), filtered off and washed well with methanol,

until free from ammonia, and then with ether (100 cm^3) before being air dried.

5.13 Preparation of N-(2,4-dinitrophenyl)-chitosan

5.13.1 Heterogeneous reaction

A chitosan film (0.1 g) was steeped in methanol (200 cm^3) containing 2,4-dinitrofluorobenzene (DNFB) (1 cm^3), for 7 days. The film was removed from the solution and washed with methanol (2 x 25 cm^3) and soxhlet extracted with methanol (150 cm^3) for 4 hours.

ν max (thin film) 1620 cm^{-1}, 1520 cm^{-1} (phenyl ring), 1590 cm^{-1}, 1330 cm^{-1} ($-C-NO_2$)

5.13.2 Homogeneous reaction

To chitosan (0.6 g) was added a 1% (v/v) solution of acetic acid (60 cm^3) and methanol (150 cm^3) containing DNFB (1 cm^3). After the onset of gelation one half of the system was removed and blended with methanol (100 cm^3). The resulting slurry was added to ether (100 cm^3) to aid precipitation of the polymer. The solid was filtered off, washed with methanol (200 cm^3) and ether (100 cm^3) before being allowed to air dry. The resultant product was soxhlet extracted with methanol (150 cm^3) for 6 hours, filtered and dried.

The second half of the reaction mixture was allowed to undergo syneresis and was then treated in an identical manner to the first portion.

ν max (KBr) 1620 cm^{-1}, 1520 cm^{-1} (phenyl ring), 1690 cm^{-1}, 1330 cm^{-1} ($-C-NO_2$)

5.14 Preparation of model N-(2,4-dinitrophenyl)-derivatives

5.14.1 From D-glucosamine

D-glucosamine (1 g) was dissolved in a minimum quantity of water to which was added DNFB (2 cm^3) in ethanol (100 cm^3). The solution was left to stand in a stoppered flask for 2 days at room temperature after which it was evaporated to dryness on a vacuum rotary evaporator at the boil. The product was recrystallised from hot water, filtered and washed with a small amount of cold water.

Yield = 1.2 g; m.p. = 179°C; λmax (water) 360 nm (εmax 16,500)

5.14.2 From cyclohexylamine

Cyclohexylamine (1 g) was dissolved in methanol (50 cm^3) containing DNFB (2 cm^3). The solution was heated for 2 hours at 60°C and allowed to cool, which caused crystallisation. The yellow crystals were filtered off and washed with cold water. They were recyrstallised from a minimum of 50/50 (v/v) methanol/water.

Yield = 0.8 g; m.p. = 147°C; λmax (methanol) 349 nm (εmax 16,350)

5.14.3 From morpholine

To morpholine (1 g) was added DNFB (2.5 cm^3) dissolved in methanol (100 cm^3). The solution was shaken and allowed to stand until the product separated out as an oil and it was then placed in an ice box for 16 hours to induce crystallisation. The solid was filtered off, washed with a little water and recrystallised from hot methanol.

Yield = 1.3 g; m.p. = 116°C; λmax (acetone/water) 380 nm (εmax 13,417)

5.14.4 From diethanolamine

To diethanolamine (1 g) was added DNFB (1.75 cm^3) dissolved in methanol (100 cm^3). The solution was allowed to stand for 2 hours after which a further addition of methanol (250 cm^3) was made. It was then placed into an ice box for 16 hours which caused crystallisation to occur. The product was filtered off and recrystallised from hot methanol.

Yield = 0.8 g; m.p. = 98°C; λmax (acetone/water) 390 nm (εmax 13,684)

5.14.5 2-amino-2-deoxy-sorbitol

A sample of freshly prepared D-glucosamine (2.5 g) was dissolved in the minimum of water. To this was added, by dropwise addition, with stirring, a solution of sodium borohydride (0.6 g) in water (15 cm^3). After the addition was complete the solution was left to react for 20 minutes after which it was neutralised (as indicated by a pH meter) using concentrated sulphuric acid. The liquid was evaporated to near dryness at room temperature after which water (100 cm^3) and DNFB (3 cm^3) in methanol (100 cm^3) was added. The resulting solution was allowed to stand at room temperature for 16 hours, before being evaporated to dryness under vacuum in a rotary evaporator at 50°C. The solid obtained was washed with cold methanol and recrystallised from hot methanol.

Yield = 1.6 g; m.p. = 208°C; λmax (water) 360 nm

5.14.6 Uv/visible absorbance stability of N-(2,4-dinitrophenyl)-derivatives

Two general procedures were used for these studies:

a) The DNP derivative of interest was dissolved in the selected solvent at a concentration sufficient to give an on scale uv/visible absorbance reading (between 0 and 1) at the position of λmax. A large volume of the solution (>250 cm^3) was used to test the effect of temperature (under reflux) and time on its stability, with small aliquots (5 cm^3) being removed for each measurement of its uv/visible absorbance spectrum in the region 400 nm to 300 nm. The following systems were investigated in this way:

N-(2,4-DNP)-D-glucosamine in water;
N-(2,4-DNP)-D-glucosamine in methanol;
N-(2,4-DNP)-D-glucosamine in 50/50 (v/v) and 20/80 (v/v) 9N HBr/water;
N-(2,4-DNP)-cyclohexylamine in 90/10 (v/v) methanol/water;
N-(2,4-DNP)-diethanolamine in water.

b) For N-(2,4-DNP)-D-glucosamine and N-(2,4-DNP)-2-amino-2-deoxy-sorbitol, solutions were again prepared at concentrations giving on-scale uv absorbance readings at λmax. However instead of taking small aliquots from a large volume the system was treated by preparing a series of identical solutions (15 cm^3) in graduated boiling tubes. These tubes were heated at 90°C in a water bath and sealed with suba-seal caps. At various times a tube was removed and cooled rapidly with ice to room temperature. If any solvent was lost by evaporation it was replaced by making up to the graduated 15 cm^3 mark, before the uv/visible absorbance spectrum was run between 500 nm and 200 nm. The following systems were investigated in this way:

N-(2,4-DNP)-D-glucosamine in water;
N-(2,4-DNP)-2-amino-2-deoxy-sorbitol in water;
N-(2,4-DNP)-2-amino-2-deoxy-sorbitol in water + excess D-glucose;

N-(2,4-DNP)-2-amino-2-deoxy-sorbitol in water + stoichiometric D-glucose;

N-(2,4-DNP)-2-amino-2-deoxy-sorbitol in water + excess formaldehyde.

5.15 Salicylaldehyde reactions

5.15.1 Stability of salicylaldehyde

The stability of salicylaldehyde in various solvent systems was tested by preparation of salicylaldehyde solutions in these solvents at concentrations in the region of 10^{-2}M. After standing for various times the solutions were diluted to the concentration range of 10^{-6}M and the uv/visible absorbance intensity recorded at 255 nm in 1 cm cells. The solvent systems studied were: methanol; 90/10 (v/v) methanol/water; 50/50 (v/v) methanol/water; 80/20 (v/v) methanol/1% acetic acid and 80/20 (v/v) methanol/0.5M hydrobromic acid.

5.15.2 Hetergeneous reaction with chitosan and analysis of the residual salicylaldehyde

The procedure for heterogeneous reaction and subsequent residual analysis is dependant on the approximate extent of N-acetylation of the chitosan sample. For high degrees of N-acetylation (50% or more) a 0.02M solution of salicylaldehyde in 80/20 (v/v) methanol/1% acetic acid (15-20 cm^3) is used to steep a dried sample of the chitosan (0.1 g, accurately weighed). After 48 hours both the initial stock salicylaldehyde solution and the filtered residual solution were diluted 400 times and their absorbances at 255 nm, in 1 cm cells, were recorded against a standard 80/20 (v/v) methanol/1% acetic acid solution. For lower extents of N-acetylation, more of the 0.02M salicylaldehyde was required (25-40 cm^3) for the initial steeping.

5.15.3 Homogeneous reaction of amines with salicylaldehyde

5.15.3.1 Chitosan

Several homogeneous reaction systems involving chitosan and salicylaldehyde were set up, using varying concentrations of chitosan, salicylaldehyde and acetic acid. For each specific case the procedure is similar and is described here for the reaction of a 0.1% chitosan solution with salicylaldehyde at a 1:5 chitosan free amine:salicylaldehyde ratio. The chitosan solution was prepared using acetic acid and Kytex L at a molar ratio of acid:chitosan of 3:2.

To the chitosan solution (10 cm^3) was added methanol (20 cm^3). The resulting solution was mixed well and allowed to cool to room temperature. To this was added a freshly prepared 1% (v/v) solution of salicylaldehyde in methanol (3.3 cm^3). The resulting system was then diluted with methanol to 50 cm^3 and the uv/visible absorbance intensity monitored as a function of time, at 410 nm in 4 cm cells, against a standard containing an equivalent amount of salicylaldehyde in a similar solvent. The absorbance intensity was followed until it became constant. For other experiments at the same or lower chitosan:salicylaldehyde ratios, it was assumed that the reaction had reached equilibrium after 5 hours. All investigations were carried out in an overall 80/20 (v/v) methanol/water solvent system.

5.15.3.2 D-glucosamine

This was carried out both in the presence and absence of acetic acid. Two solutions of D-glucosamine in water were prepared both at a concentration of 0.00107M but with one containing acetic acid at a

molar ratio of D-glucosamine:acid of 7:2. Both solutions were then treated in a similar manner.

To the solution (10 cm^3) was added methanol (20 cm^3) and a freshly prepared 1% (v/v) solution of salicylaldehyde in methanol (3.3 cm^3). The solution was diluted to 50 cm^3 with methanol and the uv/visible absorbance intensity was monitored as a function of time until constant (at 396 nm in the absence of acid, and at 410 nm in the presence of acid) against an equivalent salicylaldehyde standard in 1 cm cells.

5.15.4 General heterogeneous reaction between chitosan and salicylaldehyde

The heterogeneous reaction of chitosan with salicylaldehyde was carried out on chitosan, both in film form and when adsorbed on a substrate.

The chitosan sample in film form (0.1 g), or when adsorbed on a substrate (0.25 g), was steeped in a 1% (v/v) solution of salicylaldehyde in methanol (100 cm^3). After 48 hours the sample was removed and washed with methanol (4 x 25 cm^3) and then steeped in methanol (50 cm^3) for 16 hours, after which it was soxhlet extracted with methanol (150 cm^3) for a further 4 hours in the presence of dispersed chitosan powder (1 g) which acts as a salicylaldehyde scavenger.

5.15.5 Acid hydrolysis of N-salicylidene chitosan

5.15.5.1 Calibration for chitosan

Four samples of chitosan (Kytex L) in film form ranging in dry weight (accurately weighed) from 0.005 g to 0.03 g were heterogeneously reacted with salicylaldehyde and treated as described in Section 5.15.4.

Each sample was then steeped in a solution of 80/20 (v/v) methanol/ 0.5M HBr (20 cm^3) for 16 hours in a stoppered flask. The solutions were then diluted by a factor of 50 and their uv absorbance intensity at 255 nm in 1 cm cells recorded against a similar solvent as the reference.

5.15.5.2 Hydrolysis of N-salicylidene chitosan-papers

Chitosan treated papers were reacted with salicylaldehyde and treated as outlined in Section 5.15.4. A portion of the N-salicylidene chitosan-paper was used for a moisture determination measurement by drying to constant weight, whilst a second portion (0.2 g accurately weighed) was steeped in 80/20 (v/v) methanol/0.5M HBr solvent (20 cm^3) for 16 hours in a stoppered flask. The solution was then diluted to give an on scale uv absorbance reading at 255 nm, in 1 cm cells, against a similar reference solvent. (For chitosan concentrations between 0 and 0.5% o.w.f. the solution was usually diluted 5 times).

5.15.6 Preparation of N-salicylidene-D-glucosamine

D-glucosamine (1 g) was dissolved in the minimum of water. To this solution was added methanol (50 cm^3) and salicylaldehyde (2 cm^3). The mixture was leftto stand for 3 hours at room temperature, after which the solvent was evaporated off under vacuum on a rotary evaporator. The solid obtained was washed with chloroform and recrystallised from 50/50 (v/v) chloroform/methanol.

Yield 1.2 g; m.p. 181^{o}C; λmax (water) 393 nm (εmax 6680)

5.15.7 Preparation of chitosan treated papers for reaction with salicylaldehyde

5.15.7.1 Deposition of chitosan from a solution of known concentration

A piece of oven-dried (100°C) paper hand sheet (0.2 g, accurately weighed) was steeped in a chitosan acetate solution (100 cm^3) of known concentration. The sheet was removed from the liquid after 30 seconds and the excess solution allowed to drain away. The sheet was then pressed between blotters to remove the remaining excess solution, after which it was immediately reweighed and oven dried at 60°C.

5.15.7.2 General deposition of chitosan

This method was used on paper samples that were to be subsequently analysed by reaction with the salicylaldehyde followed by the hydrolysis of the Schiff's base formed (see Section 5.15.5.2).

A sample of the required hand sheet (0.4 g) was steeped in a chitosan acetate solution (100 cm^3) for 30 seconds. (The concentration of the chitosan solution does not have to be known accurately, but in general the higher the concentration the more chitosan deposited. The concentrations were usually in the range 0.25 - 1 g dm^{-3}). The sheet was removed and the excess liquid drained off, before it was pressed between two blotters. It was then hung up to dry in a cold air stream, in a position where the paper thickness was in the horizontal plane. This was achieved by the use of clips in a ladder arrangement. When dry the sheet was steeped in a large excess of methanol/0.880 ammonia (7/3 (v/v)) for 16 hours, followed by washing with copious quantities of methanol and finally drying between filter papers.

5.15.8 Reflectance measurements on N-salicylidene chitosan-paper samples

Reflectance measurements were carried out on the SP 500 single beam apparatus at 408 nm, against a magnesium carbonate block or the untreated substrate as standard, and also on the I.C.S. Micromatch at 16 different wavelengths from 400 nm at 20 nm intervals using the substrate as a reference. In both cases a triple thickness of sample was used and measurements were taken on both sides at four different regions of the sample.

5.16 Preparation and reaction of chitosan salts

5.16.1 Chitosan hydrobromide

Chitosan (0.5 g) was dissolved in 0.2M hydrobromic acid (100 cm^3). To this solution was added 9N hydrobromic acid (50 cm^3) which caused precipitation of the hydrobromide salt of chitosan. Complete precipitation was ensured by vigorous stirring. The resulting "colloidal-like" slurry was centrifuged at 2000 rpm for 30 minutes, after which the supernatent was removed and methanol (200 cm^3) added. The system was shaken and recentrifuged for a further 30 minutes at 2000 rpm. After the supernatent was decanted off the chitosan hydrobromide was filtered and washed well with methanol until the filtrate was neutral to litmus. The solid was then washed with ether (100 cm^3) and allowed to air dry. When dry it was reslurried in methanol for 10 minutes and then filtered. This process was repeated three more times, after which the product was finally washed with ether (100 cm^3) and dried in a vacuum desiccator over calcium chloride.

5.16.1.1 Titration of hydrobromide salts

The chitosan hydrobromide (0.2 g, accurately weighed) was dissolved in water (100 cm^3). This was then titrated against a standard 0.1M

sodium hydroxide solution using a microburette and with phenolphthalein as indicator, until a permanent red tint was observed.

5.16.2 Chitosan hydrochloride

This was made in an identical way to the hydrobromide salt (see Section 5.16.1) except the chitosan was initially dissolved in 0.2M hydrochloric acid and precipitated by the addition of 36% hydrochloric acid.

5.17 Radioisotopic studies

5.17.1 Quench correction for acetic anhydride on ^{14}C-labelled hexadecane

The standard 1.17×10^6 dpm g^{-1} ^{14}C-hexadecane (0.4 g, accurately weighed) was dissolved in the scintillation fluid T (250 cm^3). Nine solutions were prepared each containing the hexadecane/scintillation solution (10 cm^3) and varying amounts of unlabelled acetic anhydride (from 0 - 1.5 cm^3). The scintillation counts per minute for each of these solutions was then measured at two different channel widths of 50-1000 and 50-100.

5.17.2 Determination of quench for ^{14}C-labelled acetic anhydride diluted with unlabelled acetic anhydride

The standard 1000 μCi/100 cm^3 ^{14}C-labelled acetic anhydride (10 cm^3) was diluted to 27 cm^3 with unlabelled acetic anhydride. This solution (1 cm^3) was further diluted to 100 cm^3 with more unlabelled reagent.

To this solution (1 g, accurately weighed) was added the scint-

illation fluid T (10 cm^3) and the scintillation counts per minute for this system was measured at the 50-1000 and 50-100 channel widths.

5.18 Adsorption of chitosan onto cellulose and modified celluloses

5.18.1 Preparation of pulps

5.18.1.1 Oxidation of purified cotton

The purified cotton was oxidised to various extents by altering the ratios of reagents to cellulose (see Table 49). Other than this the procedure for all the samples was the same and is described here for the production of sample A.

Purified cotton (10 g) was dispersed in a 0.01M solution of sodium periodate (500 cm^3) for 24 hours in the absence of light. During this time the sytem was agitated at regular intervals. The pulp was removed from the suspension by filtration and slurried four successive times in water (500 cm^3) for 10 minutes, being filtered after each treatment. Finally it was suspended in water (500 cm^3) for 16 hours, filtered and redispersed in a 0.2M sodium chlorite/1M acetic acid solution (500 cm^3) for 72 hours. After this time the pulp was filtered off and washed on the filter pad with water (1000 cm^3).

The oxidised cellulose was then steeped in a 1M sodium hydroxide solution (500 cm^3) for 16 hours, filtered and washed with water (1000 cm^3), followed by treatment with 1M hydrochloric acid (500 cm^3) for a further 16 hours. The pulp was filtered and washed on the filter pad with water until the filtrate was neutral to litmus. It was then allowed to air dry at room temperature. After determination of its carboxyl content (see Section 5.4.2.1) the pulp was stored at 0^oC until required.

5.18.1.2 Reactive dyeing of purified cotton

Reactive dyeings were carried out at the 1%, 5% and 10% levels based on the weight of the pulp. The method described here is for the 1% level.

Purified cotton(10 g) was suspended in a solution (400 cm^3) containing commercial C.I. Reactive Red 13 (0.1 g) and sodium chloride (32 g). The pulp was initially vigorously stirred for 20 minutes and left for 72 hours with occasional stirring. The cotton was filtered and suspended in a solution (500 cm^3) containing sodium carbonate (10 g). The suspension was heated at 80^{0}C for 5 hours on a water bath. During this time water was added to the solution to offset that lost by evaporation.

The pulp was filtered and washed on the filter pad with water until the filtrate was colourless. It was then soxhlet extracted with water (200 cm^3) to leach out any remaining unreacted dye and finally allowed to air dry.

5.18.1.3 Basic dyeing of oxidised pulps

A sample of blended pulp C (2.5 g) was treated in a solution (100 cm^3) containing purified Methylene Blue (1 g), 1M sodium hydroxide (3 cm^3) and potassium dihydrogen phosphate (0.2 g) for 72 hours at room temperature. The resulting pulp was filtered and washed on the filter pad with water until the filtrate was colourless, and then allowed to air dry.

5.18.1.4 Direct dyeing of purified cotton

Purified cotton (10 g) was suspended in a solution (400 cm^3)

containing commercial C.I. Direct Yellow 12 (0.5 g) and sodium chloride (2 g). The suspension was constantly stirred at the boil for 40 minutes, after which it was filtered and washed by successive treatments with water (400 cm^3), the pulp being filtered after each treatment. Due to the poor wash fastness of direct dyed cotton the removal of excess dye was assumed to be complete when the addition of the pulp to the water did not cause an immediate colouration of that water. The product was then allowed to air dry.

5.18.1.5 Mechanical treatment of oxidised celluloses

The oxidised cellulose (2 g) was suspended in water (20 cm^3) and treated in a Waring blendor at the high speed setting for 30 seconds. The pulp was filtered and allowed to air dry.

5.18.2 Preparation of solutions for adsorption studies

5.18.2.1 Chitosan acetate solutions

Solutions were prepared based on the dry weight of chitosan (Kytex L) found by moisture determinations. Chitosan (1 g) (regardless of its degree of N-acetylation) was dissolved in a 1% (v/v) aqueous acetic acid solution (45 cm^3) after which it was diluted to close to the required volume (depending on the required concentration) with water. To this was added, by dropwise addition, a 10% (v/v) aqueous acetic acid solution until the pH of the system reached 4, as indicated by a pH meter. The resulting solution was then made up exactly to the required volume based on the three concentrations used of 0.25, 0.5 and 1 g dm^{-3} (accurately determined).

5.18.2.2 Solutions with added electrolyte

These were prepared either at the 1 g dm^{-3} chitosan concentration

level by addition of the required amount of the solid salt to the stock acetate solution or at the 0.5 g dm^{-3} level by a two fold addition of a 1 g dm^{-3} solution of either chitosan acetate or hydrochloride, using a stock 2M salt solution and water, to vary the overall salt concentration between 0 and 1M.

5.18.2.3 Chitosan hydrochloride solutions

A desiccated sample of chitosan hydrochloride (0.27 g,accurately weighed) was dissolved in water (250 cm^3). This solution was initially calibrated against a standard chitosan acetate solution, by metachromic analysis, to determine its concentration in terms of its chitosan rather than chitosan hydrochloride content.

5.18.2.4 Preparation and degradation of adsorption solutions and determination of their molecular weights

A sample of dried chitosan (Kytex H) (0.9 g, accurately weighed) was dissolved in 1% (v/v) aqueous acetic acid (100 cm^3). The solution was diluted to 1 litre with water in a flask. The flask was placed into an oven at 70°C, allowed to adjust to the temperature, and the flask sealed to prevent solvent evaporation. Before heating and after 72, 144 and 216 hours aliquots were removed for adsorption and molecular weight studies. A sinlge concentration viscometric determination was carried out on the degraded solutions to determine the viscosity number at a chitosan concentration of about 0.45 g dm^{-3}. This required a dilution of the chitosan solution (10 cm^3) with a solution (10 cm^3) containing sodium chloride (0.234 g) and acetic acid (0.11 g). This addition adjusts the overall acid and salt concentrations to 0.1M acetic acid/0.2M sodium chloride.

5.18.3 Chitosan solution analysis

The residual amount of chitosan remaining after adsorption onto cellulose was determined using a metachromic dye analysis technique. The dye, C.I. Acid Red 88, was initially characterised with a standard chitosan solution of the same degree of N-acetylation and molecular weight as the adsorption chitosan solution. Residual analysis was then carried out under identical conditions of dye concentration. A large number of these analyses were performed but the procedure is similar for each and is described here for adsorption onto pulp A at a liqor ratio of 40:1 and a temperature of 20°C, using a chitosan concentration of 0.962 g dm^{-3}.

Calibration of dye

The standard 0.962 g dm^{-3} chitosan solution was diluted 10 times using a 0.6% (v/v) acetic acid solution. Seven solutions were then prepared each containing a stock 0.398 g dm^{-3} purified C.I. Acid Red 88 solution (5 cm^3), and varying amounts of the diluted chitosan solution (20, 15, 12, 8, 6, 4 and 2 cm^3 respectively). In each case the solutions were made up to 250 cm^3 with 0.6% (v/v) acetic acid. The absorbance intensity of each solution at 505 nm was then recorded in 1 cm cells against a 0.6% (v/v) acetic acid solution as a reference. The volumes of the chitosan solutions used were chosen so that at least 2 of the absorbance readings at the higher addition levels were constant, which corresponds to the region where all the dye has interacted with the chitosan. The point at which the absorbance readings initially become constant (found by extrapolation) was taken as the end point and corresponds to the exact amount of chitosan required for the dye.

Residual chitosan analysis

The whole procedure was repeated on the chitosan solution after adsorption.

From time to time it was necessary to prepare fresh dye solutions. These were not prepared at the same concentration and often in order to increase the absorbance intensity were only diluted to 100 cm^3 before uv/visible analysis. In general for on scale absorbance readings at 505 nm, concentrations of dye of the order of 10^{-2} g dm^{-3} were required. The actual concentration is irrelevant so long as the particular dye solution was initially calibrated using a standard chitosan solution, prior to its use for residual analysis.

5.18.4 Adsorption experiments

Adsorption experiments were carried out on various cellulose substrates (1 g, 0.5 g or 0.33 g) in boiling tubes fitted with Suba-seal caps, by addition of the required volume of the appropriate chitosan solution to the tube. After the tubes were sealed they were placed in a water bath at the specified temperature and removed at various time intervals for rate studies, or after 48 hours for equilibrium adsorption studies. Prior to residual analysis the solutions were filtered through glass wool.

5.19 Miscellaneous methods

The following methods have been used in the study to prepare required materials, and are based on procedures reported by other workers.

5.19.1 Preparation of di-O-phenylcarbamate-N-benzylidene chitosan and di-O-phenylcarbamate-N-phenylureido chitosan

These were prepared using a sample of Kytex H according to the method of Moore and Roberts[94].

5.19.2 Preparation of D-glucosamine

This was prepared from D-glucosamine hydrochloride using the method of Breuer[111].

5.19.3 Preparation of N-acetyl-D-glucosamine

This was prepared from D-glucosamine hydrochloride by the method of Inouye[76].

5.19.4 Preparation of arylhydrazine derivatives

5.19.4.1 D-glucosephenylosazone

This was prepared according to the method of Richmyer[153].

5.19.4.2 D-glucose-p-nitrophenylhydrazine

This was made by the method of Van Ekerstein and Blanksma[89].

5.19.5 N-acetylation of partially derivatised N-(2,4-dinitrophenyl)-chitosan film

The procedure used was identical to the method outlined by Moore and Roberts [57] for the N-acetylation of chitosan in film form.

5.19.6 Preparation and testing of handsheets

Handsheets were made at the PIRA laboratories according to TAPPI standards[115] and those used for testing were conditioned according to the specifications in standard T402 OS-70[120]. The physical testing of pulps was carried out by PIRA technicians.

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APPENDIX I

Viscosity data for chitosan phenylosazone samples in 0.1M acetic acid/0.2M sodium chloride at 25°C.

Sample	Concentration $\times 10^4$/g cm^{-3}	Flow time t/sec	Solvent flow time, to/sec	η sp	η sp/C /cm^3 g^{-1}
1	2.10	122.6	108.9	0.126	600
1	4.20	137.6	"	0.264	630
1	8.39	171.7	"	0.577	690
1	12.59	209.6	"	0.925	735
1	20.98	305.0	"	1.800	860
2	4.58	119.9	108.9	0.104	226
2	9.16	130.9	"	0.204	223
2	18.33	156.2	"	0.437	238
2	22.91	170.2	"	0.568	248
3	4.48	122.1	108.7	0.124	276
3	8.96	137.3	"	0.263	294
3	17.92	171.6	"	0.578	323
3	22.40	190.3	"	0.750	335
4	9.76	128.2	108.7	0.179	183
4	11.39	131.9	"	0.213	187
4	13.66	137.1	"	0.261	191
4	17.03	144.7	"	0.322	194
5	13.29	133.2	108.7	0.133	100
5	19.94	130.4	"	0.200	100
5	26.59	138.6	"	0.266	100
5	33.24	145.0	"	0.334	100

Sample	Concentration $\times 10^4$/g cm^{-3}	Flow time t/sec	Solvent flow time, to/sec	η sp	η sp/C /cm^3 g^{-1}
6	8.26	120.4	108.7	0.107	130
6	12.39	125.9	"	0.159	128
6	16.52	133.65	"	0.230	139
6	20.65	140.2	"	0.290	140
7	1.03	224.5	207.35	0.083	802
7	4.13	283.6	"	0.368	892
7	6.19	329.6	"	0.590	953
7	10.31	434.5	"	1.095	1063
8	3.18	247.6	207.35	0.265	611
8	4.23	262.2	"	0.433	625
8	6.35	297.2	"	0.794	682
8	10.58	312.2	"	0.194	751
9	5.53	260.6	207.35	0.257	465
9	8.30	292.4	"	0.410	494
9	11.07	326.7	"	0.576	520
9	13.84	368.7	"	0.778	562
10	5.98	243.3	207.35	0.174	290
10	8.97	264.5	"	0.276	307
10	11.97	284.7	"	0.373	312
10	14.95	306.8	"	0.480	321
11	7.08	237.3	207.35	0.144	204
11	10.63	253.1	"	0.220	207
11	14.17	268.3	"	0.294	207
11	17.71	285.7	"	0.378	213

Sample	Concentration $\times 10^4$/g cm^{-3}	Flow time t/sec	Solvent flow time, to/sec	ηsp	η sp/C /cm^3 g^{-1}
12	10.89	242.7	207.35	0.171	156
12	18.15	265.9	"	0.288	156
12	21.78	277.8	"	0.339	156
12	27.23	295.4	"	0.425	156
13	13.65	228.0	207.35	0.100	73
13	15.92	231.8	"	0.118	74
13	19.10	236.7	"	0.141	74
13	23.88	244.8	"	0.181	76

Viscosity data for chitosan phenylosazone samples in 0.1M acetic acid/ 0.02M sodium chloride at 25°C.

Sample	Concentration $\times 10^4$/g cm^3	Flow time t/sec	Solvent flow time, to/sec	η sp	ηsp/C /cm^3 g^{-1}
7	1.51	252.0	206.2	0.249	1469
7	3.02	304.6	"	0.477	1529
7	4.53	364.9	"	0.770	1698
7	6.04	430.5	"	1.090	1800
9	2.48	243.7	206.2	0.182	734
9	3.71	265.4	"	0.287	772
9	4.95	289.6	"	0.404	816
9	6.19	314.7	"	0.526	850
11	2.83	221.2	206.2	0.072	257
11	5.67	236.5	"	0.147	259
11	8.50	253.9	"	0.232	272
11	14.17	289.4	"	0.403	285

Sample	Concentration $\times 10^4$/g cm^{-3}	Flow time t/sec	Solvent flow time, to/sec	η sp	η sp/C /cm^3 g^{-1}
12	2.65	216.2	206.2	0.049	183
12	5.29	276.3	"	0.078	184
12	7.99	236.5	"	0.147	185
12	13.24	257.5	"	0.249	188

APPENDIX II

Gravimetric and reflectance analysis data for deposition of chitosan onto various paper handsheets.

Pulp = Stora 32

Dry weight of paper/g	Weight of paper + solution/g	Solution concentration/%	Concentration o.w.f./%	% 8 point average reflectance
0.1988	0.3727	0.2386	0.209	64.3
0.1299	0.2370	"	0.197	68.9
0.1776	0.3275	0.4679	0.420	52.0
0.2108	0.4082	"	0.438	52.7
0.1621	0.3335	1.1932	1.260	33.8
0.1666	0.3365	"	1.220	30.5
0.2176	0.4120	0.4679	0.394	54.0
0.1963	0.4120	"	0.514	50.2
0.2038	0.4590	"	0.586	46.0
0.1863	0.3470	"	0.403	53.5
0.1728	0.3470	0.5530	0.558	47.5
0.1861	0.3696	"	0.545	48.7
0.2193	0.4106	0.2765	0.240	65.7
0.1598	0.2905	"	0.227	65.6
0.1943	0.3680	0.8295	0.740	46.6
0.1852	0.3675	"	0.816	39.8

Pulp = Stora 61

0.1937	0.3600	0.2765	0.240	64.8
0.1979	0.3520	"	0.215	68.0
0.2624	0.5200	0.5530	0.540	51.8

Pulp = Stora 61 (continued)

Dry weight of paper/g	Weight of paper + solution/g	Solution concentration/%	Concentration o.w.f./%	% 8 point average reflectance
0.2049	0.4215	0.5530	0.580	50.8
0.2049	0.4215	0.8245	0.960	43.0
0.2623	0.5603	"	0.936	44.0
0.2754	0.5929	1.106	1.270	38.0
0.2681	0.5722	"	1.250	36.9

Pulp = Tyee

0.1966	0.3631	0.2963	0.251	73.3
0.1942	0.4967	"	0.426	57.3
0.1453	0.2786	0.5609	0.515	62.9
0.1558	0.2777	"	0.439	69.3
0.1598	0.3296	0.7688	0.817	51.9
0.1550	0.3135	"	0.784	54.4
0.1600	0.3504	1.0241	1.24	42.6
0.1823	0.4590	"	1.58	37.6

Pulp = New Bern

0.2230	0.3940	0.2751	0.211	81.6
0.2226	0.3913	"	0.208	77.3
0.2375	0.4057	0.5505	0.390	67.4
0.2671	0.4669	"	0.412	67.6
0.2701	0.5075	0.8292	0.729	59.8
0.2526	0.4708	"	0.716	56.4
0.2644	0.5172	1.0876	1.040	46.5
0.2770	0.5612	"	1.116	40.5

Pulp = Port Hudson

Dry weight of paper/g	Weight of paper + solution/g	Solution concentration/%	Concentration o.w.f./%	% 8 point average reflectance
0.2501	0.4400	0.2751	0.209	78.6
0.2309	0.4162	"	0.231	78.5
0.2422	0.4216	0.5505	0.400	67.7
0.2098	0.3686	"	0.417	67.5
0.2605	0.4738	0.8292	0.679	55.9
0.2427	0.4515	"	0.713	53.1
0.2510	0.4823	1.0876	1.062	43.5
0.2772	0.5015	"	0.880	50.3

APPENDIX III

Computer program for determination of the chitosan content in paper by means of hydrolysis of N-salicylidene chitosan-paper systems and for determination of the reflectance function for such systems. Written in Sinclair ZX-81 Basic.

```
 10  Print "Input extinction coefficient, E";
 20  Input E
 25  Print E
 30  Print "Input percentage moisture of paper, M";
 40  Input M
 45  Print M
 50  Print "Input moist weight of pulp, W";
 60  Input W
 65  Print W
 70  Print "Input Vol. of acidic methanol used, V";
 80  Input V
 85  Print V
 90  Print "Input dilution factor, D";
100  Input D
105  Print D
110  Print "Input absorbance, A";
120  Input A
125  Print A
126  Let WD = W - (W x M/100)
130  Let S = A x D x V x 1.513/(E x 1000)
140  Let B = WD - S
150  Let C = (100 x A x D x V/(E x 1000))/B
```

```
160  Print "Concentration of chitosan o.w.f. is"; C
170  Print "Log concentration is"; LN C/LN 10
180  Print "Input sample reflectance, R";
190  Input R
200  Print R
210  Print "Input substrate reflectance, RT";
220  Input RT
230  Print RT
240  Let X = (1 - (R/100)) xx2/(2 x (R/100))
250  Let Y = (1 - (RT/100)) xx2/(2 x (RT/100))
260  Let FR = X - Y
270  Print "F(R)"; FR
280  Print "Log F(R) is"; LN FR/LN 10
290 STOP.
```

APPENDIX IV

Metachromic dye analysis data to determine the amount of chitosan adsorbed onto cellulose substrates.

$$\text{Amount of chitosan adsorbed} = \left[C_2 - \frac{C_1 V_1 D_2}{D_1 V_2}\right] \frac{V_3}{1000}$$

where C_2 = concentration of chitosan solution used for adsorption in g dm^{-3}

C_1 = concentration of chitosan solution used to calibrate the dye in g dm^{-3}

V_1 = metachromic end point for calibration solution in cm^3

V_2 = metachromic end point for residual solution in cm^3

V_3 = volume of original solution used for adsorption in cm^3

D_1 = dilution factor for calibration solution

D_2 = dilution factor for residual solution

1. Adsorption-time data for adsorption onto various substrates at a liquor ratio of 30:1 at 20°C.

Sample	Time/h	C_2	C_1	V_1	V_2	V_3	D_1	D_2	Dry pulp weight/g	Conc. o.w.f./%
Purified cotton	1	0.949	0.9516	9.77	10.14	15	20	20	0.5055	0.095
"	5	"	"	"	10.25	"	"	"	0.5063	0.124
"	20	"	"	"	10.29	"	"	"	0.5088	0.134
"	48	"	"	"	10.44	"	"	"	0.5061	0.173
"	68	"	"	"	10.44	"	"	"	0.5057	0.173
B	1	0.949	0.9516	9.77	10.76	15	20	20	0.4963	0.256
B	5	"	"	"	11.70	"	"	"	0.4969	0.466
B	20	"	"	"	13.67	"	"	"	0.5057	0.798
B	48	"	"	"	16.61	"	"	"	0.5076	1.15
B	68	"	"	"	16.70	"	"	"	0.5084	1.16

Sample	Time/h	C_2	C_1	V_1	V_2	V_3	D_1	D_2	Dry pulp weight/g	Conc. o.w.f./%
A	1	0.9516	0.9516	9.29	9.81	30	10	10	1.0253	0.150
A	5	"	"	11.3	12.56	"	20	20	1.0097	0.283
A	16	"	"	"	12.98	"	"	"	1.0239	0.361
A	30	"	"	"	13.36	"	"	"	1.01865	0.432
A	72	"	"	5.5	6.54	"	"	"	1.033	0.435
D	1	0.949	0.9516	9.77	10.66	15	20	20	0.5016	0.230
D	5	"	"	"	10.9	"	"	"	0.5009	0.290
D	20	"	"	"	11.08	"	"	"	0.5028	0.330
D	48	"	0.949	10.03	11.39	"	"	"	0.5004	0.340
D	69	"	"	"	11.41	"	"	"	0.5028	0.340

2. Adsorption data for the effect of salt on equilibrium uptake of chitosan on pulp C, at a liquor ratio of 30:1 at 20°C.

Salt conc./M	C_2	C_1	V_1	V_2	V_3	D_1	D_2	Dry pulp weight/g	Conc. o.w.f./%
0.025	0.4762	0.9524	9.40	14.67	15	19.54	10.13	0.4973	0.488
0.05	"	"	"	15.66	"	"	9.96	0.4920	0.565
0.10	0.4745	0.949	10.03	35.49	"	20	19.96	0.4964	0.576
0.20	"	"	"	18.01	"	"	10.32	0.5031	0.600
0.30	0.4753	"	"	18.58	"	"	10.42	0.5023	0.620
0.50	0.4745	"	"	35.49	"	"	19.86	0.5053	0.618
0.75	"	"	"	34.91	"	"	20.0	0.4981	0.576
1.0	"	"	"	16.98	"	"	10.10	0.5029	0.571

3. Adsorption data for the effect of salt on the equilibrium uptake of chitosan hydrochloride on pulp C, at a liquor ratio of 30:1 and at 20°C.

Salt conc./M	C_2	C_1	V_1	V_2	V_3	D_1	D_2	Dry pulp weight/g	Conc. o.w.f./%
0.0	0.4583	0.9166	10.32	14.49	15	20.69	10.0	0.4970	0.431
0.1	"	"	"	18.51	"	"	10.34	0.4977	0.611
0.5	"	"	"	17.69	"	"	10.0	0.4968	0.604
0.75	"	"	"	17.51	"	"	9.83	0.4958	0.610
1.0	"	"	"	16.50	"	"	9.86	0.4961	0.560

4. (a) Equilibrium uptake for various pulps, at a liquor ratio of 30:1 at 20°C.

Pulp	C_2	C_1	V_1	V_2	V_3	D_1	D_2	Dry pulp weight/g	Conc. o.w.f./%
B	0.949	0.9516	9.77	16.61	15	20	20	0.5063	1.15
C	"	"	10.03	12.04	"	"	"	0.5039	0.47
D	"	0.949	"	11.39	"	"	"	0.5004	0.34
E	"	"	"	15.28	"	"	20.68	0.3341	0.91
F	"	"	"	13.38	10	"	20.18	0.3242	0.785
Purified cotton	"	9.77	9.77	10.44	15	"	20	0.5061	0.173

(b) Equilibrium uptake for various blended pulps, at a liquor ratio of 30:1 st 20°C.

C	0.9524	0.9524	9.40	13.88	15	19.54	20.78	0.5024	0.80
E	"	"	"	24.40	"	"	20.27	0.4987	1.72
F	"	"	"	17.72	"	"	19.82	0.5058	1.32
B	"	"	"	34.40	"	"	20.10	0.5060	2.03
D	"	"	"	11.81	"	"	20.17	0.4951	0.52

5. Adsorption-time data for uptake of chitosan on pulp A at different chitosan concentrations, from a liquor ratio of 30:1 at 20°C.

Time/h	C_2	C_1	V_1	V_2	V_3	D_1	D_2	Dry pulp weight/g	Conc. o.w.f./%
1	0.4758	0.9516	11.3	13.12	30	20	10	1.0376	0.191
5	"	"	"	13.51	"	"	"	1.0430	0.224
16	"	"	"	13.89	"	"	"	1.0336	0.257
29	"	"	"	14.76	"	"	"	1.0344	0.323
72	"	"	"	15.10	"	"	"	1.0440	0.344
1	0.2379	"	4.88	12.28	15	"	"	0.4813	0.151
5	"	"	"	12.60	"	"	"	0.4827	0.166
24	"	"	"	14.25	"	"	"	0.4809	0.233
72	"	"	"	15.37	"	"	"	0.4808	0.270

6. Adsorption-time data for uptake of chitosan on pulp D at a liquor ratio of 30 :1 at 30°C and 40°C.

Temp/°C	Time/h	C_2	C_1	V_1	V_2	V_3	D_1	D_2	Dry pulp weight/g	Conc. o.w.f./%
30	1	0.949	0.949	10.03	11.00	15	20	20	0.5013	0.250
30	5	"	"	"	11.01	"	"	"	0.5001	0.253
30	24	"	"	"	11.39	"	"	"	0.5021	0.343
30	48	"	"	"	11.41	"	"	"	0.5016	0.353
30	70	"	"	"	11.44	"	"	"	0.5006	0.355
40	1	"	"	"	10.56	"	"	19.68	0.4976	0.187
40	5	"	"	"	11.25	"	"	19.86	0.4973	0.328
40	21	"	"	"	11.65	"	"	20	0.4987	0.397
40	48	"	"	"	11.65	"	"	19.84	0.4970	0.418

7. Equilibrium uptake data for chitosan on pulp E at 20°C at varying liquor ratios.

Liquor ratio	C_2	C_1	V_1	V_2	V_3	D_1	D_2	Dry pulp weight/g	Conc. o.w.f./%
20:1	0.9524	0.9524	9.40	30.65	10	19.54	20.13	0.4994	1.31
25:1	"	"	8.40	20.84	12.5	20.06	20.42	0.4995	1.41
30:1	"	"	9.40	24.40	15	19.54	20.27	0.4987	1.72
35:1	"	"	8.40	16.09	17.5	20.06	20.30	0.5004	1.57
40:1	"	"	9.40	15.53	20	19.54	19.49	0.5014	1.51
45:1	"	"	9.40	14.10	25	19.54	20.55	0.5009	1.43

8. Equilibrium uptake data for chitosans of varying degrees of N-acetylation on pulp C at a liquor ratio of 30:1 at 20°C.

Sample	C_2	C_1	V_1	V_2	V_3	D_1	D_2	Dry pulp weight/g	Conc. o.w.f./%
19	0.949	0.949	10.03	12.04	15	20	20	0.5039	0.470
21	0.9885	0.9885	15.84	24.19	"	19.65	20.68	0.4989	0.923
22	0.9865	0.9865	12.69	20.92	"	10.07	10.27	0.4991	1.132
20	0.9959	0.9959	11.61	17.49	"	17.41	19.0	0.4967	0.827

9. Equilibrium uptake for chitosans of varying molecular weights on pulp C at a liquor ratio of 30:1 at 20°C.

Sample	C_2	C_1	V_1	V_2	V_3	D_1	D_2	Dry pulp weight/g	Conc. o.w.f./%
H1	0.8997	0.8997	11.49	12.59	15	21.03	20.35	0.5020	0.317
H2	"	"	10	10.82	"	"	20.12	0.4979	0.311
H3	"	"	"	11.08	"	"	19.82	0.4995	0.400
H4	"	"	8.03	10.81	"	"	20.58	0.4954	0.745

10. Miscellaneous data for equilibrium uptake of chitosan at a liquor ratio of 30:1 at 20°C.

Description	C_2	C_1	V_1	V_2	V_3	D_1	D_2	Dry pulp weight/g	Conc. o.w.f./%
Pulp C in 0.1M NaSCN	0.4762	0.9524	9.40	19.47	15	19.54	10.20	0.5006	0.708
Sample R1	0.9524	"	8.40	8.53	"	20	19.54	0.4958	0.110
Sample R5	"	"	6.64	7.26	"	19.78	20.97	0.5048	0.086
Sample R10	"	"	"	7.87	"	"	22.70	0.5137	0.080
Direct Yellow dyed sample in 0.5M salt	"	"	"	7.96	"	"	20.51	0.4859	0.397
Purified cotton in 0.5M salt	"	"	"	8.19	"	"	21.21	0.4953	0.376
Methylene Blue dyed blended pulp C	"	"	8.4	10.96	"	19.54	20.21	0.5053	0.643

APPENDIX V

Part of this thesis is contained in the following publication copied from the International Journal of Biological Macromolecules, 1982, 4, 374-377.

Determination of the viscometric constants for chitosan

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The viscometric constants a *and* K_m *in the Mark–Houwink equation have been determined for chitosan in 0.1 M acetic acid–0.2 M sodium chloride solution, using the approach of Sharples and Major. The number-average molecular weights were determined by absorbance measurements on solutions of the phenylosazone derivatives. The values obtained* $a = 0.93$, $K_m = 1.81 \times 10^{-3}$ *cm*^{3} *g*$^{-1}$ *differ considerably from those reported previously by Lee but are in agreement with values found for other ionic polysaccharides having related* β-(1→4)-*linked structures.*

Keywords: Viscosity measurement; chitosan; molecular weight; viscometric constants

Introduction

One of the simplest and most rapid methods for determining the molecular weights of polymers is viscometry, although this is not an absolute method and requires the determination of constants through correlation of limiting viscosity numbers (*LVN*) with molecular weights determined by an absolute method. The most commonly used equation relating *LVN* values with molecular weights is the Mark–Houwink equation:

$$[\eta] = K_m \bar{M}_w^a \qquad (1)$$

where a and K_m are constants that are independent of molecular weight over a wide range of molecular weights. These constants are normally evaluated from a plot of $\log[\eta]$ *versus* $\log \bar{M}_w$ for a number of carefully prepared fractions having very narrow molecular weight distributions. When subsequently applying the values of a and K_m thus obtained to the determination of molecular weights of whole polymer samples, the weight-average molecular weight term ($\bar{M}_w$) in the above equation must be replaced by the viscosity-average molecular weight ($\bar{M}_v$) if a is not unity and the polymer sample is polydisperse.

There is only one report[1] in the literature dealing with the determination of a and K_m values for chitosan. The values obtained are $a = 0.71$ and $K_m = 8.93 \times 10^{-2}$ cm^3 g^{-1}. The value for a is very low, compared with the values in the literature for other ionic β-(1→4)-linked polysaccharides e.g. sodium carboxymethyl cellulose[2] and sodium cellulose sulphate[3], whilst the value for K_m is high. Furthermore, only three fractions of chitosan were used in the determination and far from having narrow molecular weight distributions, as required for determination of the constants in equation (1), one fraction had $\bar{M}_w/\bar{M}_n = 1.76$ and another had $\bar{M}_w/\bar{M}_n = 3.51$. In view of these facts it was considered useful to redetermine the values of a and K_m.

Experimental

Materials

The chitosan used was a sample of Kytex H (Hercules Inc.) that had previously been characterized[4] using the values obtained by Lee[1] for the viscometric contents. The reagents used were of GPR grade and were used as supplied.

Determination of LVN *values*

Viscometric measurements were carried out at 25±0.05°C using a suspended-level viscometer containing an internal sintered glass filter to prevent gel and/or dust particles entering the capillary.

Preparation of chitosan phenylosazone

(a) Chitosan (1 g) was dissolved in dilute acetic acid (1% v/v; 175 cm^3) and phenylhydrazine (2.5 cm^3) and sodium acetate (1 g) were added to the solution. The reaction mixture was kept at 70°C, in a stoppered flask, for 48 h, after which the chitosan phenylosazone was precipitated out by pouring the reaction mixture into methanolic ammonia (methanol/0.88 ammonia, 70:30; 200 cm^3). filtered off, washed well with methanol followed by diethyl ether, and air-dried at room temperature. The yellow solid obtained was extracted with methanol in a Soxhlet extraction unit for 4 h, filtered off and dried.

(b) A series of chitosan phenylosazones of various molecular weights were prepared by dissolving chitosan (4 g) in acetic acid (10% v/v; 400 cm^3) and heating the solution under reflux. Aliquots of the solution (65 cm^3)

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were removed at intervals. Each aliquot was treated with phenylhydrazine (3 cm^3), sodium acetate (1 g) and distilled water (135 cm^3) and heated at 70°C for 48 h, after which the chitosan phenylosazone was precipitated out of solution and purified as described above.

(c) Chitosan (6 g) was slurried in methanol (300 cm^3), run into an ion-exchange column, washed with methanol/5% acetic acid (95:5) until highly swollen then rinsed with methanol and air dried. The solid obtained was dissolved in 1% HBr (600 cm^3) and cooled to 0°C, after which sodium borohydride (10 g) in water (200 cm^3) was slowly added to the vigorously stirred solution. Simultaneously, 10% HBr (100 cm^3) was added at a rate sufficient to maintain the pH of the solution at ~3. After both solutions had been added the chitosan was precipitated by addition to excess methanol/ammonia solution, filtered off and washed well with methanol. The methanol-damp solid was dissolved in 10% acetic acid (600 cm^3) and the solution maintained at 70°C for ~400 h, aliquots (85 cm^3) being removed at intervals. These were treated as described in (b), above, to give a second series of chitosan phenylosazones of various molecular weights.

Results and discussion

As mentioned in the Introduction, determination of a and K_m by the normal procedure requires the preparation of a series of fractions of the polymer, each having a narrow molecular weight distribution. Attempts at fractionating chitosan by the technique of Doczi[5] were unsuccessful, only polydisperse fractions being obtained[6]. It was therefore decided to use the approach first proposed by Charlesby[7] and developed by Sharples and Major[8]. This considers the properties of the molecular weight distribution resulting from random degradation of polymer chains and for this equation (1) is modified and expressed as

$$\bar{M}_v = K'[\eta]^{1/a} \qquad (2)$$

where

$$K' = \frac{1}{K_m^{1/a}}$$

Consideration of the molecular weight distribution resulting from random degradation gives

$$\bar{M}_v = \bar{M}_n[\Gamma(2+a)]^{1/a} \qquad (3)$$

where Γ = gamma function.

Combining equations (2) and (3) and expressing it in the logarithmic form gives:

$$\log \bar{M}_n = (1/a)\log[\eta] + \log K' - (1/a)\log[\Gamma(2+a)] \qquad (4)$$

Hence a plot of $\log \bar{M}_n$ *versus* $\log[\eta]$ should give a straight line of slope $1/a$, and intercept equal to $\log K' - (1/a)\log[\Gamma(2+a)]$. Acid hydrolysis of chitosan in solution, under relatively mild conditions, should give rise to random degradation leading to products having the required molecular weight distributions for use with equation (4).

Molecular weight determination

The most common method for determining $\bar{M}_n$ values of polymers is osmometry, but this technique is difficult to apply to polyelectrolytes because of the decreased activity of the counterions relative to their activity in solutions of simple salts. It was therefore decided to use the technique of end-group analysis and to measure the concentration of end groups through formation of the phenylosazone derivative by reaction of the reducing chain ends with phenylhydrazine. The reaction between cellulose and phenylhydrazine was previously studied by one of the authors, who found[9] that the product is similar to D-glucose phenylosazone in spectral properties and chemical behaviour. This and related reactions were extensively studied by Blair and coworkers[10-14] who showed that there was a correlation between the molecular weights of the hydrocellulose samples, as determined by viscosity, and the absorption intensity of the phenylosazone derivatives[10].

A similar correlation would be expected to hold for the product from the reaction between chitosan and phenylhydrazine since both D-glucosamine[15] and *N*-acetyl-D-glucosamine[16] yield D-glucose phenylosazone on reaction with phenylhydrazine in the presence of acetic acid. Thus, regardless of whether the amine group of the terminal hexose unit is free or acetylated, reaction with phenylhydrazine should give a phenylosazone derivative. This has been confirmed by the authors, who have found[17] that chitosan phenylosazone is similar to D-glucose phenylosazone, having the spectra and chemical reactions characteristic of a chelated phenylosazone[18].

Barry *et al.*[19] have reported that the absorption coefficients of a large number of monosaccharide and disaccharide phenylosazones, measured on fresh solutions in methanol, are very similar and are independent of molecular weight. The average value obtained for the absorption coefficient (ε) was 20 200. Although chitosan is not soluble in methanol, its solutions in dilute acid can tolerate dilution with considerable volumes of methanol. A mixed solvent medium of methanol/0.1 M HBr (7:3) was chosen as the standard solvent for absorption measurements and a series of solutions of D-glucose phenylosazone at different concentrations was prepared in it. The absorbance values were measured at 395 nm (λ_{max}) and a plot of absorbance *versus* concentration gave the absorption coefficient $\varepsilon = 20\,200$, identical to that for D-glucose phenylosazone in methanol alone.

One of the experimental difficulties encountered by Blair and Cromie[11], namely the gradual oxidative degradation of the phenylosazone chromophore in the strongly alkaline Cadoxen solution used as solvent for the hydrocellulose phenolysazones, would not apply in the present case. However, phenylosazones are susceptible to acid hydrolysis and so the stability of the chromophore in the methanol/0.1 M HBr solvent at 25°C was checked. No change in the absorbance was observed over a period of 20 h, indicating that the phenylosazone is sufficiently stable under these conditions of acidity and temperature to be used for the determination of molecular weights, using the relationship:

$$\bar{M}_n = \frac{20\,200 \times c \times l}{A}$$

where c = concentration of the chitosan phenylosazone in g dm^{-3}, l = path length in cm and A = absorbance of the solution at 395 nm.

Correlation between $\bar{M}_n$ *and* LVN *values*

Prior to determining the *LVN* for each chitosan phenylosazone sample, the value for the undegraded polymer was measured to ascertain that the solvent system used, 0.1 M acetic acid–0.2 M sodium chloride, gives a linear plot for viscosity number *versus* concentration. This solvent system was used in preference to that of Lee[1], 0.2 M acetic acid–0.1 M sodium chloride–4 M urea, because of the greater simplicity in working with the former system, and was found to give the necessary straight line plots.

A plot of log $\bar{M}_n$ *versus* log $[\eta]$ for the first series of chitosan phenylosazones did not give a straight line, the points showing considerable scatter. Consideration of the experimental technique led to the identification of one possible source of error. In view of the strongly alkaline conditions normally used in the production of chitosan from chitin[20], it is probable that many, if not all, of the reducing end groups originally present in the chitin are converted to either lactone or sodium carboxylate groups, neither of which would form a phenylosazone derivative. Thus, only the new end groups formed during acid hydrolysis would undergo the phenylosazone reaction, giving rise to errors in the measured number-average molecular weight. Phenylosazone derivatives of a further series of randomly degraded chitosan samples were therefore prepared from chitosan previously treated with sodium borohydride under acid conditions to reduce any oxidized end groups to aldehyde groups[21]. The number-average molecular weights and the corresponding *LVN* values were determined for each phenylosazone derivative. The results are given in *Table 1* and the viscosity number *versus* concentration plots in *Figure 1*.

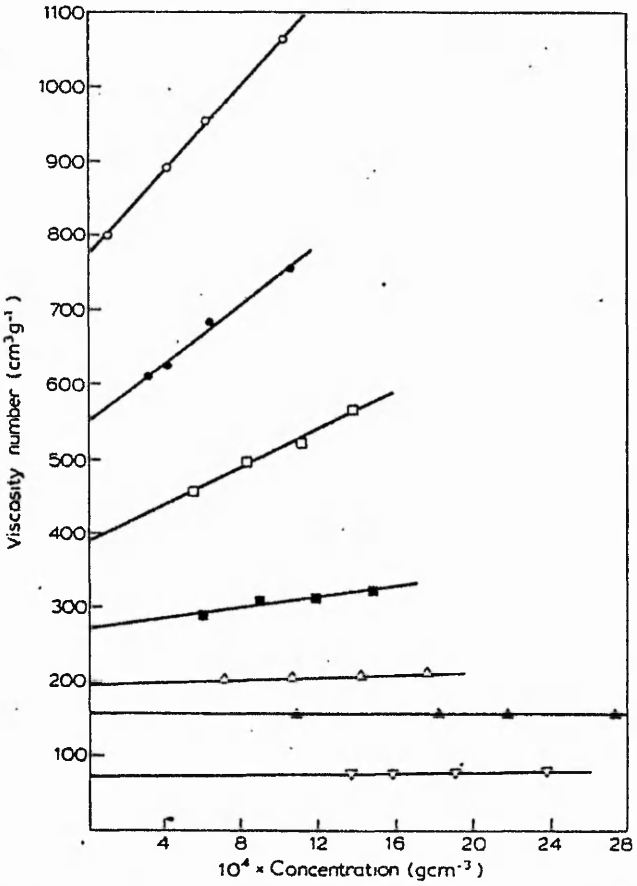

Figure 1 Plots of viscosity number *versus* concentration for chitosan phenylosazones prepared from sodium borohydride-reduced chitosan

Table 1

Sample number	Hydrolysis time (h)	$\bar{M}_n$	*LVN* (cm^3 g^{-1})
7	40	630050	780
8	64	450775	549
9	136	241575	389
10	164	180250	272
11	200	156620	198
12	250	96585	156
13	380	47820	75

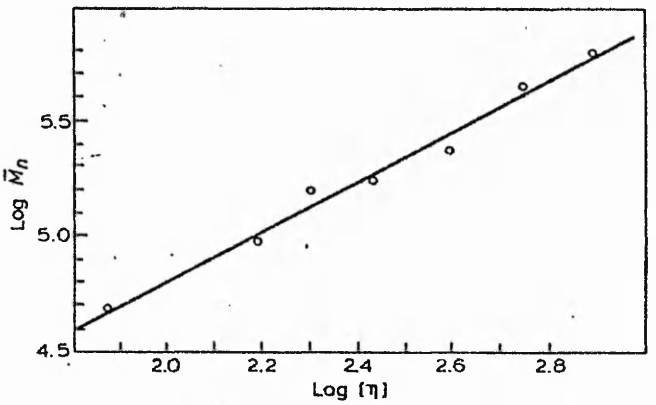

Figure 2 Plot of $\bar{M}_n$ *versus* log $[\eta]$ for chitosan phenylosazones prepared from sodium borohydride-reduced chitosan

A plot of log $\bar{M}_n$ *versus* log $[\eta]$ for this second series of samples is shown in *Figure 2*, from which it can be seen that the points give a good linear relationship. Regression analysis of the data gives a line of best fit having a slope of 1.075 and an intercept of 2.655. Thus

$$a = 1/1.075 = 0.93$$

and $$2.655 = \log K' - \log[\Gamma(2+0.93)] \times 1/0.93$$

Therefore $$K' = 888$$

and since $$K_m^{1/a} = 1/K'$$

$$K_m = 1.81 \times 10^{-3} \text{ cm}^3 \text{ g}^{-1}$$

The *LVN* values were also measured in 0.1 M acetic acid–0.02 M sodium chloride, and similar treatment of the results gave $a = 1.26$, $K_m = 3.04 \times 10^{-5}$ cm^3 g^{-1}. The values of a and K_m obtained in the present work differ considerably from those obtained by Lee[1] but, as mentioned in the Introduction, the latter values are out of line with those obtained for other ionic polysaccharides having related β-(1→4)-linked structures. The values reported here are much closer to those of these related polymers, as can be seen from the values in *Table 2*.

AV–3

Table 2

Polymer	Salt concentration (M)	a	K_m ($cm^3\ g^{-1}$)
Chitosan acetate	0.2	0.93	1.81×10^{-3}
Chitosan acetate	0.02	1.26	3.04×10^{-5}
Sodium carboxymethyl cellulose[2]	0.1	0.91	1.23×10^{-4}
Sodium carboxymethyl cellulose[2]	0.01	1.2	6.46×10^{-6}
Sodium cellulose sulphate[3]	0.5	0.93	7.91×10^{-2}

Conclusions

The viscometric constants a and K_m in the Mark–Houwink equation have been determined for chitosan in 0.1 M acetic acid–0.2 M sodium chloride solution using a technique that utilizes the properties of the molecular weight distribution resulting from random degradation of the polymer chains, in this case from acid hydrolysis of the polymer in solution. Number-average molecular weights were determined from absorbance measurements on the phenylosazone derivatives of the hydrolysed samples. The values obtained for a and K_m differ from those obtained previously but are similar to those reported for related ionic polysaccharides. Thus, the Mark–Houwink equation for chitosan in 0.1 M acetic acid–0.2 M sodium chloride can be expressed as:

$$[\eta] = 1.81 \times 10^{-3}\ \bar{M}_v^{0.93}$$

and in 0.1 M acetic acid–0.02 M sodium chloride as:

$$[\eta] = 3.04 \times 10^{-5}\ \bar{M}_v^{1.26}$$

Acknowledgements

The authors wish to thank Hercules Inc. for supplying the chitosan used in this work, and Mrs F. A. Wood for assistance in the preparation of the paper for publication. One of the authors (JGD) thanks the Science and Engineering Research Council and the Paper Industry Research Association for the award of a CASE Research Studentship.

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