

A spectroscopic and viscometric study of the metal ion-induced gelation of the biopolymer chitosan

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The gelation of the biopolymer chitosan in solution through crosslinking reactions with Pt(II) has been investigated by simultaneously monitoring both the viscosity of the system and the number of metal–amine bonds formed per chitosan chain, as probed spectroscopically. The variables studied in the investigations included the chitosan to Pt(II) concentration ratio, the pH of the reaction solution, and the concentration, molecular weight and degree of deacylation of chitosan. A kinetic model for the time dependence of the formation of crosslinks and a spectral model for the time evolution of the spectra were developed for the gelation process. These models included the effects of light scattering, steric hindrance, pH and variation of pK_a as a function of the fraction of monomer units charged, and they were tested successfully against the experimental spectra. The time of gelation was found to decrease with increases in the following experimental parameters while holding all the other parameters constant: (1) concentration of Pt(II), (2) pH of the reaction solution, (3) molecular weight of chitosan, and (4) concentration of chitosan in solution. In accordance with percolation theory, the calculated crosslink/chain ratios at the time of gelation were found to be quite consistent for the various reaction conditions and to be consistent also with predictions of thresholds for percolation of rigidity by network theories. © 1997 Elsevier Science Ltd.

(Keywords: gelation; chitosan; metal ion)

INTRODUCTION

Chitosan is a well known type of polymer^{1,2} obtained by deacetylating the biopolymer chitin, which is found in the skin or shell of arthropods. It is a copolymer containing both β -(1 → 4)-2-acetamido-2-deoxy-D-glucose and β -1(1 → 4)-2-amino-2-deoxy-D-glucose units, and its structure is shown in *Figure 1* along with that of chitin. The amine group of the deacetylated units of chitosan can form coordinate covalent bonds to various metal ions by complexation in solution³ or by exposure of solid chitosan films or particles to metal ion-containing solutions⁴.

These metal–chitosan coordinative interactions are thought to be important, since the viscosities of some metal–chitosan solutions are quite different from those of the chitosan itself under the same conditions. This usually is attributed to metal ion-induced association, and often specifically to coordinate covalent bond formation, but the relationship between coordination complex formation and solution properties is not clear. Even for the most widely studied solution complexation reaction of chitosan with Cu(II) there is no agreement regarding even the number of amine groups bound to Cu(II) in these complexes, even at one given pH^{3–6}. Moreover, while the viscosities of some metal(II)–chitosan solutions increase dramatically as the pH of the solution changes, the increase can occur at different pHs for different metal

ions. For example, the viscosity increases at pH 4.7 for Cu(II)–chitosan solutions while the visible spectra indicate that there is significant coordination of Cu(II) to chitosan amine groups⁷. For Ni(II)–chitosan solutions the viscosity increases at pHs greater than 6.2, but in this case the spectra indicate that relatively little coordination of Ni(II) to chitosan has occurred⁷. Furthermore, chitosan solutions without added metal ion-containing solutions precipitate at pHs greater than 6.5, which is approximately the pK_a of the amine group in chitosan⁶, or when electrolyte is added to chitosan solutions⁸ above pH 5.3.

Clearly, the gelation of metal ion–chitosan solutions is quite complicated and can be due to contributions from hydrogen bonding, crosslinking reactions with metal ions and changes in electrostatic repulsion. However, if the pH, ionic strength, concentration and other variables are held constant while the metal-to-chitosan coordinate covalent bonds are formed, and if their formation causes a chitosan solution to gel, it should be possible to relate this bond formation to gelation. In fact, if the bond formation and gel formation can be followed simultaneously and independently, this relationship can be placed on a quantitative basis. Indeed, bond percolation theory predicts that there is critical concentration of crosslinks required to bring about gelation regardless of the reaction conditions.

Following the early development of the mean field theory of gelation by Flory and Stockmayer⁹, experiments have been reported relating the number of chemical bonds (crosslinks) being formed and the solution in a

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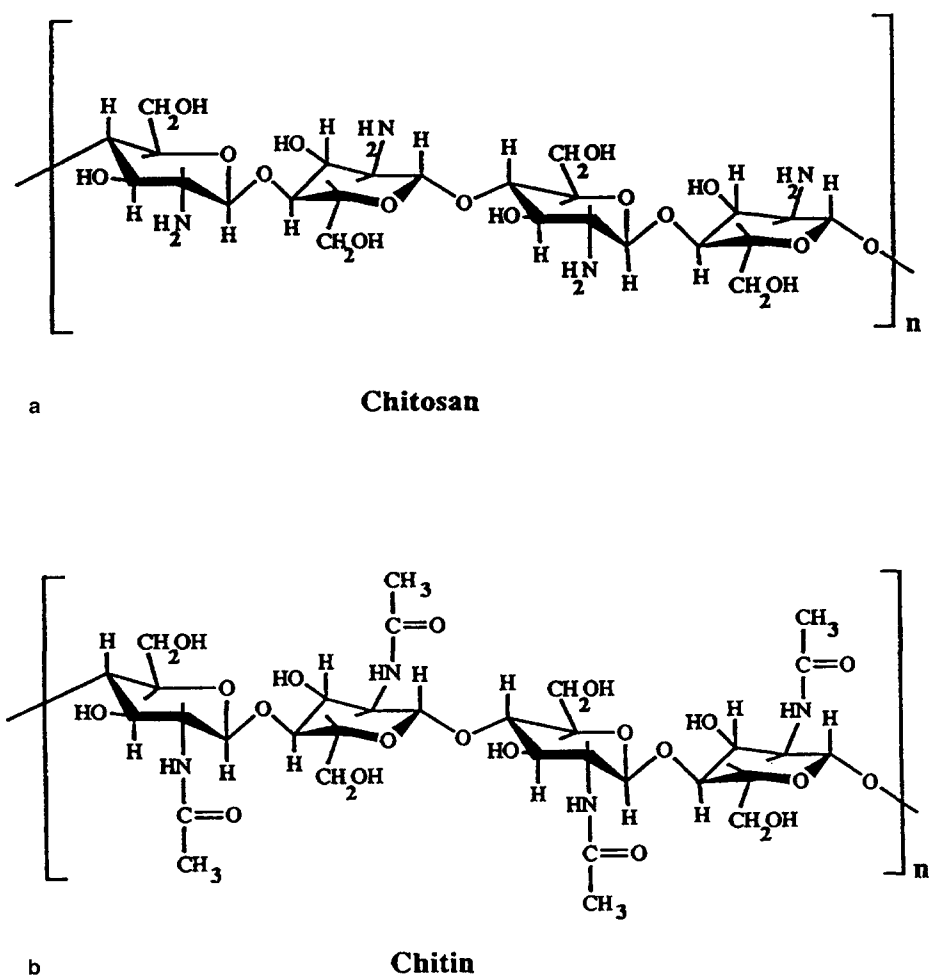


Figure 1 Chemical structure of (a) 100% deacetylated chitosan and (b) chitin

polymer system undergoing a sol–gel transition. Examples include polyesterification and other condensation reactions^{10–16} and thermoreversible sol–gel transitions of aqueous gelatin solutions¹⁷. The first examples are polymerization reactions of monomers in solution, and the second example involves the formation of alpha helices as crosslinks in a biopolymer. In contrast, the present work concerns the formation of crosslinks between already formed polymers by means of complexation reactions with transition metal complexes. Another approach to studying gelation is to make a kinetic model for the polymerizing system based on the known reactivities of the monomer species¹⁸. Gupta and Kumar¹⁹ have reviewed kinetic treatments of that type concerning the polymerization of monomer species rather than the crosslinking of already formed polymers. Typically, only the changes in concentration of reactant species and molecular weight of the polymer molecules formed were modelled in those investigations. In the present study, spectral changes, as well as concentration changes in the reactant species, as a function of time, are modelled. This feature makes it possible to use spectral measurements to confirm independently the validity of the model, as will be discussed later.

The gelation of the Pt(II)–chitosan system has been studied in this work. The Pt(II)–chitosan system is ideally suited for studying the correlation between increases in the macroscopic property of viscosity as a function of time with those of the microscopic property of chemical

bond formation during the gelation process. Platinum(II) can make kinetically inert, stable complexes with chitosan by slowly forming Pt(II)–amine bonds; once formed they remain in place for a long time. The relatively slow complex formation allows both the viscosity of the system and the number of metal–amine bonds formed, as probed spectroscopically, to be monitored over the relatively long time periods required for gelation.

While this is an ideal system to study, some of the complexities of studies of reactions of biopolymers with transition metals are important in the full analysis of the data. In order to interpret the spectra taken throughout the course of the reaction in terms of the number of crosslinking bonds, it is necessary to know the spectra of individual species formed during the reaction and the rates of Pt(II)–chitosan complexation reactions. A greater number of the substitution reactions of Pt(II) with Cl^- , H_2O and NH_3 and the associated rate constants and spectra of the resulting species have been reported^{20–27}. By selecting a system for which extensive data are available, it is possible to develop a kinetic model for the gelation process and then test the model with experimental spectra. As a result, these experiments should make it possible to determine quantitatively the relationship between gel formation and the formation of crosslinks through transition metal ions.

Information concerning the kinetics of gelation, the effects of the various reaction parameters on gelation time and the types of interactions and reactant species

that induce it are useful in the many areas in which the gels and the gelation of metal-containing polysaccharides²⁸ and other polymer²⁹ solutions can have important applications. Such gels are used for controlling rock permeability in the petroleum and mining industries, and in this application it is crucial to have a kinetic or empirical model for predicting the time of gelation. Since *cis*-diamine Pt(II) complexes with other polymer systems³⁰ and with the chitosan 'monomer unit', glucosamine³¹, are of interest as analogues to the anticancer drug cisplatin, analogous biopolymer forms, such as that with chitosan, may be of value.

EXPERIMENTAL

Materials

High and medium molecular weight 80% deacylated chitosan materials (nos 22743 and 22742, Fluka Chemie AG) were studied. In addition, approximately 100% deacylated chitosan samples were obtained from them using the method of Domard and Rinando³². The percentage deacylation of the four different chitosan materials studied here was determined from infra-red spectral measurements using the method of Miya *et al.*³³, and the molecular weights were determined by viscometry using a Corning Ubbelohde viscometer 200 (no. 47). The sample designations, percentage deacylations and viscosity average molecular weights of the chitosans studied are: (a) HMW-X-80, which has 81% deacylation and M_w of $2.1 \times 10^6 \text{ g mol}^{-1}$; (b) MMW-X-80, with 80% deacylation and M_w of $7.3 \times 10^5 \text{ g mol}^{-1}$; (c) HMW-X-100, with 99% deacylation and M_w of $1.0 \times 10^6 \text{ g mol}^{-1}$; and (d) MMW-X-100, with 99% deacylation and M_w of $3.0 \times 10^5 \text{ g mol}^{-1}$.

Reactions

Chitosan solutions were prepared with acetic acid and triple distilled water. Aliquots of 1.00 wt% chitosan solutions in 2.00 wt% acetic acid solution were titrated with 5.0 M NaOH and diluted with water to give a desired pH and a stock solution molarity [$X_{\text{d.a.}}$] of $3.69 \times 10^{-2} \text{ M}$ in terms of moles of deacylated 'mer' units per litre. For some experiments, chitosan solutions in 2.00 wt% acetic acid having different initial concentrations were used to prepare final titrated and diluted solutions of different concentration [$X_{\text{d.a.}}$]. The molarity, $X_{\text{d.a.}}$, is defined as the total number of moles of chitosan amine groups, in both the protonated and unprotonated forms, per litre of solution. The total acetic acid and sodium acetate concentration for the final titrated and diluted solutions was typically 0.25 M. Freshly prepared chitosan solutions were used for all the gelation experiments.

The concentrations of chitosan used in the various gelation experiments are given in Table 1, which also gives related information to specify the solutions. The calculation of the chain concentration, [chain], from the concentration of monomer units with acetyl, unprotonated amine or protonated amine functionality [X_{total}], the weighted average molecular weight of the 'mer unit', $M_{w.m.u.}$; and the viscosity average molecular weight of the polymer M_v , is given in equation (1):

$$[\text{chain}] = [X_{\text{total}}] \times M_{w.m.u.} / M_v \quad (1)$$

The weighted average molecular weight of the 'mer' unit, $M_{w.m.u.}$ is given as an example for the 80% deacylated chitosan in equation (2):

$$M_{w.m.u.} = (0.80) \times (161.1 \text{ g mol}^{-1}) + (0.20) \times (203.2 \text{ g mol}^{-1}) \quad (2)$$

The molecular weight of the 'mer' unit of chitin is 203.2 g mol^{-1} and that of 100% deacylated chitosan is 161.1 g mol^{-1} .

Kinetic and spectral studies of the reaction of K_2PtCl_4 with model small molecules were carried out in a pH 5.9 and 0.25 M acetic acid/sodium acetate buffer solution with an X/Pt ratio of 10:1. The model small molecules included the sugar amines, glucosamine (Sigma, reagent grade), galactosamine (Sigma, reagent grade) and *N*-acetyl-D-glucosamine (Sigma, reagent grade).

A typical gelation reaction of chitosan with Pt(II) was initiated by dissolving the appropriate mass of K_2PtCl_4 (Johnson Mathey Electronics, 99.9% purity on a metals basis) in a $3.69 \times 10^{-2} \text{ M}$ X_{total} chitosan solution to give the desired [$X_{\text{d.a.}}$] to [metal] ratio, which is called X/Pt. Reactions were carried out under a range of conditions and a range of X/Pt values, including 10:1, 20:1 and 40:1, although sometimes other ratios were used also. The reaction was followed viscometrically and spectroscopically at $25 \pm 1^\circ\text{C}$, as described below. All reactions of K_2PtCl_4 with chitosan or model small molecules were carried out at a constant temperature of 25°C using a water bath to control the temperature.

Using methods similar to those described for the reaction of chitosan with Pt(II) in the form of K_2PtCl_4 , a number of complimentary reactions were carried out to clarify certain aspects of the reactions of chitosan with Pt(II). These complementary reactions include the reaction of chitosan with: (1) prehydrolysed K_2PtCl_4 , (2) *cis*- and *trans*-diamine Pt(II) complexes, and (3) the tetraamine Pt(II) complex. The purpose of the first reaction was to determine the relative importance of the hydrolysis step in the reaction mechanism. The second reactions were carried out to examine the influence of steric

Table 1 Concentration of functional groups and chains in chitosan/ K_2PtCl_4 gelation experiments

Chitosan material	wt%	[$X_{\text{d.a.}}$] M RNH ₂ and RNH ₃ ⁺	[X_{total}] M RNH ₂ , RNH ₃ ⁺ , and acetyl	M_w of chain M_v (g mol ⁻¹)	[chains] M
HMW-X-100	0.78	3.68×10^{-2}	3.68×10^{-2}	1.0×10^6	5.9×10^{-6}
HMW-X-80	0.78	3.68×10^{-2}	4.61×10^{-2}	2.1×10^6	3.8×10^{-6}
MMW-X-100	0.78	3.68×10^{-2}	3.68×10^{-2}	3.0×10^5	2.0×10^{-5}
MMW-X-80	1.15	5.41×10^{-2}	6.76×10^{-2}	7.3×10^5	1.5×10^{-5}
MMW-X-80	0.78	3.68×10^{-2}	4.61×10^{-2}	7.3×10^5	1.0×10^{-5}
MMW-X-80	0.53	2.48×10^{-2}	3.10×10^{-2}	7.3×10^5	7.0×10^{-6}

effects on the gelation process. The third reaction was carried out in order to confirm the inertness of the Pt(II)-ammonia (amine) bonds once they are formed. Additional reactions of chitosan with the PdCl_4^{2-} complex, which has a similar coordination chemistry to that of PtCl_4^{2-} , but which reacts faster, were carried out in order to confirm that the formation of metal-amine complexes was responsible for bringing about gelation.

Measurements

Ultraviolet/visible (u.v./vis.) spectra and viscosities were measured simultaneously on different aliquots of the reacting solution at various times throughout the reaction. The viscometry measurements during gelation were carried out using Corning Ubelhode 500 number 80 viscometers. The u.v./vis. spectra were measured on a computer-interfaced Perkin Elmer Lambda 3b in the 200–700 nm region. The reference cuvette in all of these measurements contained a reference solution of chitosan identical to the reaction solution except that no K_2PtCl_4 was added. The reference and reaction solutions were maintained in between spectral measurements at a constant temperature of 25°C in a water bath.

Light scattering in selected gelation experiments was studied via turbidity and nephelometry measurements. The previously mentioned u.v./vis. spectrometer was used to measure the turbidity in the range 600–900 nm, where the Pt(II) complexes are transparent. Nephelometry measurements were carried out at 90° in the region of 350–650 nm on a Spex F111 XI Fluorimeter with a quartz cell. The nephelometry data were corrected for the spectral response of the instrument and solution. Nephelometry measurements were conducted on solutions containing K_2PtCl_4 and either the chitosan monomer unit glucosamine and or the HMW-X-100 chitosan.

RESULTS

Reactions between chitosan and Pt(II) were carried out under the experimental conditions described above and specified in Table 1. In each case, the viscosity and the u.v./vis. spectrum were measured at times from the initiation of the reaction to a time past the gelation. In some cases the reaction also was followed by light scattering measurements to establish its contribution to the u.v./vis. spectra. The gelation time, t_{gel} , for each of the reactions was taken to be the time at which the most rapidly rising part of the viscosity *versus* reaction time curve extrapolated to the time axis. Typical gelation data are shown in Figure 2 for the gelation reaction at various pHs. The gelation times were found to be quite reproducible with a precision of *ca* 1%. Representative gelation times are presented in Table 2.

The data are selected and arranged in Table 2 to show how gelation time varies (a) with molecular weight of chitosan (HMW-X-100 *versus* MMW-X-100 and HMW-X-80 *versus* MMW-X-80), (b) with X/Pt ratio (10:1, 20:1 and 40:1 at pH 5.3), (c) with chitosan concentration (0.53 and 0.78 wt% at pH 5.3 and X/Pt of 20:1), (d) with pH (X/Pt of 20:1 at pH 5.3, 5.6 and 5.9), (e) with degree of deacylation of MMW chitosan (MMW-X-100 and MMW-X-80 at 0.78 wt% chitosan, X/Pt of 20:1 and pH 5.3), (f) with degree of deacylation of HMW-chitosan (HMW-X-100 and HMW-X-80 at 0.78 wt% chitosan, X/Pt of 20:1 and pH 5.3), and (g) with degree of deacylation of chitosans having similar molecular weights

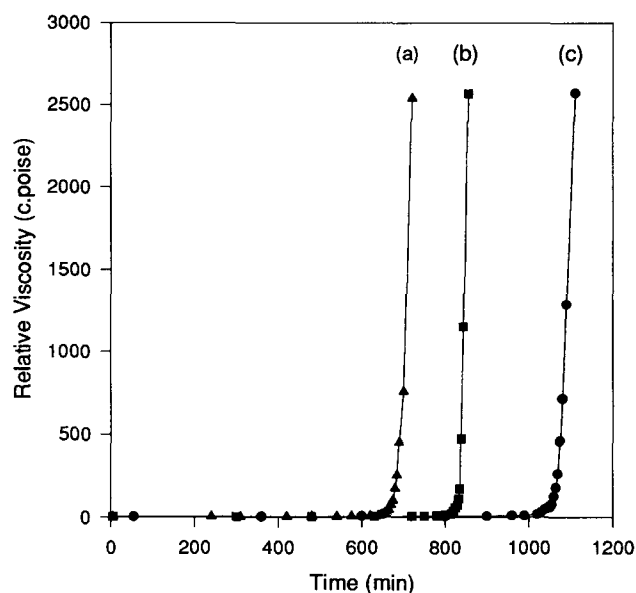


Figure 2 Viscosities as a function of time for 0.78 wt% MMW-X-80 chitosan/ K_2PtCl_4 solutions at an $X/\text{Pt(II)}$ ratio of 10:1 and pH of (a) 5.9, (b) 5.6 and (c) 5.3

Table 2 Gelation times for selected chitosan/ K_2PtCl_4 solutions

Chitosan material	wt%	pH	$X_{\text{d.a.}}/\text{Pt(II)}$	Gelation time (min) ^a
MMW-X-100	0.78	5.3	20:1	2.20×10^3 a1,d1
MMW-X-80	0.53	5.3	10:1	1.78×10^3 b1
			20:1	4.02×10^3 bf,c
			40:1	5.40×10^3 b1
	0.78	5.3	20:1	1.96×10^3 a2,c,d1,e
		5.6	10:1	8.25×10^2 b2
		5.6	20:1	1.53×10^3 b2,d1,e
		5.9	20:1	1.20×10^3 c
	1.15	5.3	30:1	2.06×10^3 e
HMW-X-100	0.78	5.3	20:1	1.59×10^3 a1,d2,d3
HMW-X-80	0.78	5.3	20:1	8.80×10^2 a2,d2,d3

^a Gelation times are average values; uncertainty is estimated statistically to be $\pm 1\%$

The entry markings of a,b,c,d or e indicate that the entry is part of a series. The variations are of: (a) gelation times as a function of the molecular weight of chitosan; (b) gelation times as a function of $X_{\text{d.a.}}/\text{Pt(II)}$ concentration ratio; (c) gelation times as a function of chitosan concentration; (d) gelation times as a function of percentage deacylation of chitosan; (e) gelation times as a function of the reaction pH

(HMW-X-100 and MMW-X-80 at 0.78 wt% chitosan, $X/\text{Pt(II)}$ of 20:1 and pH 5.3). Several effects are exhibited by these data. First, for the same conditions of pH, $X/\text{Pt(II)}$ ratio and percentage deacylation of the chitosan, the gelation time decreases as the molecular weight increases. Second, the gelation times increase as the $X/\text{Pt(II)}$ ratio increases at constant chitosan concentration. Third, the gelation time decreases with increasing chitosan concentration when all other reaction parameters are held constant. Fourth, the gelation times are shorter for reactions at higher pH, if the other reaction parameters are held constant. Finally, the gelation time is shorter for more highly deacylated chitosans of similar molecular weight, e.g. in comparing HMW-X-100 with MMW-X-80. The comparisons of HMW-X-100 with HMW-X-80 or of MMW-X-100 with MMW-X-80 cannot be done so readily since both the molecular weight and percent deacylation are varying.

Gelation times for representative complementary

reactions are given in Table 3. A comparison of the prehydrolysed K_2PtCl_4 reaction in Table 3 with its unhydrolysed counterpart in Table 2 indicates that the time of gelation is greatly reduced in the prehydrolysed case. The Pt(II)-diamine and tetraamine complexes also react with chitosan to give gels, but their gelation times are about 100 times longer than those of chitosan with $[PtCl_4^{2-}]$ given in Table 2. Moreover, the *trans* complex forms gels faster than the *cis* complex, and both diamine complexes form gels faster than the tetraamine complex. The Pd(II) complexes induce chitosan gelation much more rapidly than their Pt(II) analogues. The gelation time given in Table 3 is about a factor of 10^5 shorter for the reaction of chitosan with $PdCl_4^{2-}$ than that given in Table 2 for the analogous reaction with $PtCl_4^{2-}$. The reactions of $Pd(NH_3)_4^{2+}$ with chitosan have gelation times which are about 10 times longer than that of the comparable $PdCl_4^{2-}$ reactions, but they are still much shorter than those of any of the Pt(II) reactions.

The u.v./vis. spectra taken at a number of times during a typical reaction of chitosan with K_2PtCl_4 are shown in Figure 3 for reaction times from $t = 0$ to a time just past gelation. The experimental spectra for all of the gelation experiments are observed to have several common features which develop as the reactions proceed. There are

Table 3 Gelation times for selected complementary chitosan gelation reactions

Chitosan (wt%)	$[X_{d.a.}] NH_2$ groups	Metal complex	$X_{d.a.}/Pt(II)$	Gelation time (min) ^a
0.78	3.68×10^{-2}	K_2PtCl_4 (prehydrolysed for 24 h)	10:1	7.20×10^2
0.68	3.23×10^{-2}	<i>cis</i> - $[Pt(NH_3)_2Cl_2]$	10:1	2.16×10^5
0.66	3.10×10^{-2}	<i>trans</i> - $[Pt(NH_3)_2Cl_2]$	10:1	1.44×10^5
0.71	3.38×10^{-2}	$[Pt(NH_3)_4Cl_2]$	10:1	5.33×10^5
0.75	3.54×10^{-2}	K_2PdCl_4	10:1	$< 3 \times 10^{-2}$
0.78	3.54×10^{-2}	K_2PdCl_4	150:1	2.2×10^{-1}
0.79	3.73×10^{-2}	$[Pd(NH_3)_4Cl_2]$	150:1	1.2×10

^a Gelation times are average values; uncertainty is estimated statistically to be $\pm 1\%$

All gelation reactions were carried out using MMW-X-80 chitosan at pH 5.6. All other reaction parameters that are not given here are the same as those given previously for the reaction of chitosan with K_2PtCl_4

two cross-over regions observed at frequencies near 26 500 and 37 000 cm^{-1} . The intense shoulder present initially at about 38 000 cm^{-1} and the small band present initially at about 25 500 cm^{-1} decrease in absorbance with time, and a broad multicomponent band at about 31 000 cm^{-1} increases with time.

Light scattering by the chitosan/ K_2PtCl_4 solutions, as measured by the turbidity and nephelometry experiments, was found to increase with time as the reaction proceeded, especially near the gelation point. The scattering data from both types of experiments showed similar behaviour when measured at the same time of reaction, and no significant light scattering or fluorescence was evident in the case of the reactions of glucosamine and K_2PtCl_4 solutions.

KINETIC MODEL DEVELOPMENT

To explore the relationship between gelation and the formation of crosslinks due to Pt(II)-chitosan complexes, a kinetic model was constructed for calculating the concentrations of crosslinking Pt(II)-diamine species as a function of time. It is based on the known reactions of $PtCl_4^{2-}$ and other Pt(II) complexes with NH_3 , H_2O and Cl^- and, as shown in Figure 4, includes all reasonable products of Pt(II) reactions of $PtCl_4^{2-}$ and derived species in the presence of amines, H_2O and Cl^- .

Evaluating the concentrations of all of the species in the model imposes two main requirements: developing a method of calculation and obtaining values for the rate constants. A program was written to carry out the calculation using a finite element approach in which a very large number of very small steps were computed along all of the reaction paths. It provided for complete feedback into the calculation of the concentrations of all species at all times after each small step in time, complete consistency checks and output of the concentrations of all species at all times. The program is given in ref. 8.

Since the reactions of Pt(II) with NH_3 , H_2O and Cl^- have been studied extensively for many years, most of the rate constants in this model are known. The few that are not have been estimated from the rates of similar Pt(II) reactions or by order of magnitude change from known Pd(II) rate constants. The rate constants for the

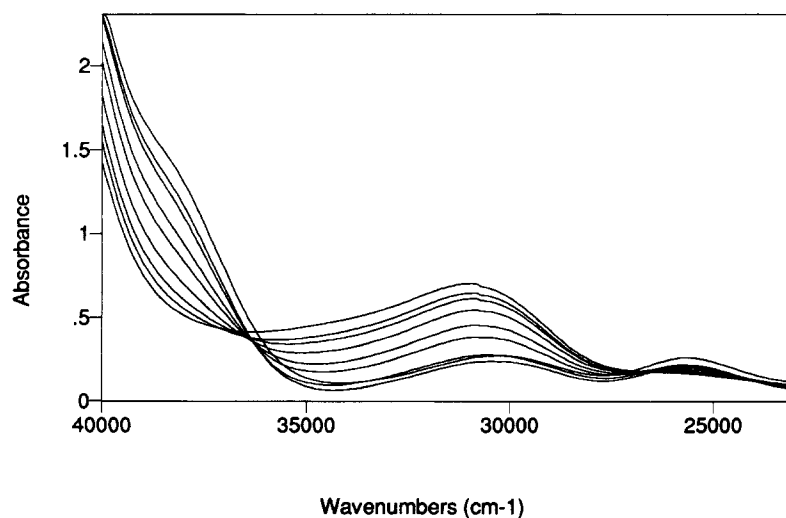


Figure 3 U.v./vis. spectra as a function of time for 0.78 wt% MMW-X-80 chitosan/ K_2PtCl_4 solutions at pH 5.9 and an $X/Pt(II)$ ratio of 10:1. The spectra sequence when viewed bottom to top at 33 000 cm^{-1} is for spectra measured at $t = 0, 40, 150, 290, 360, 480, 540, 610, 720, 800$ and 850 min

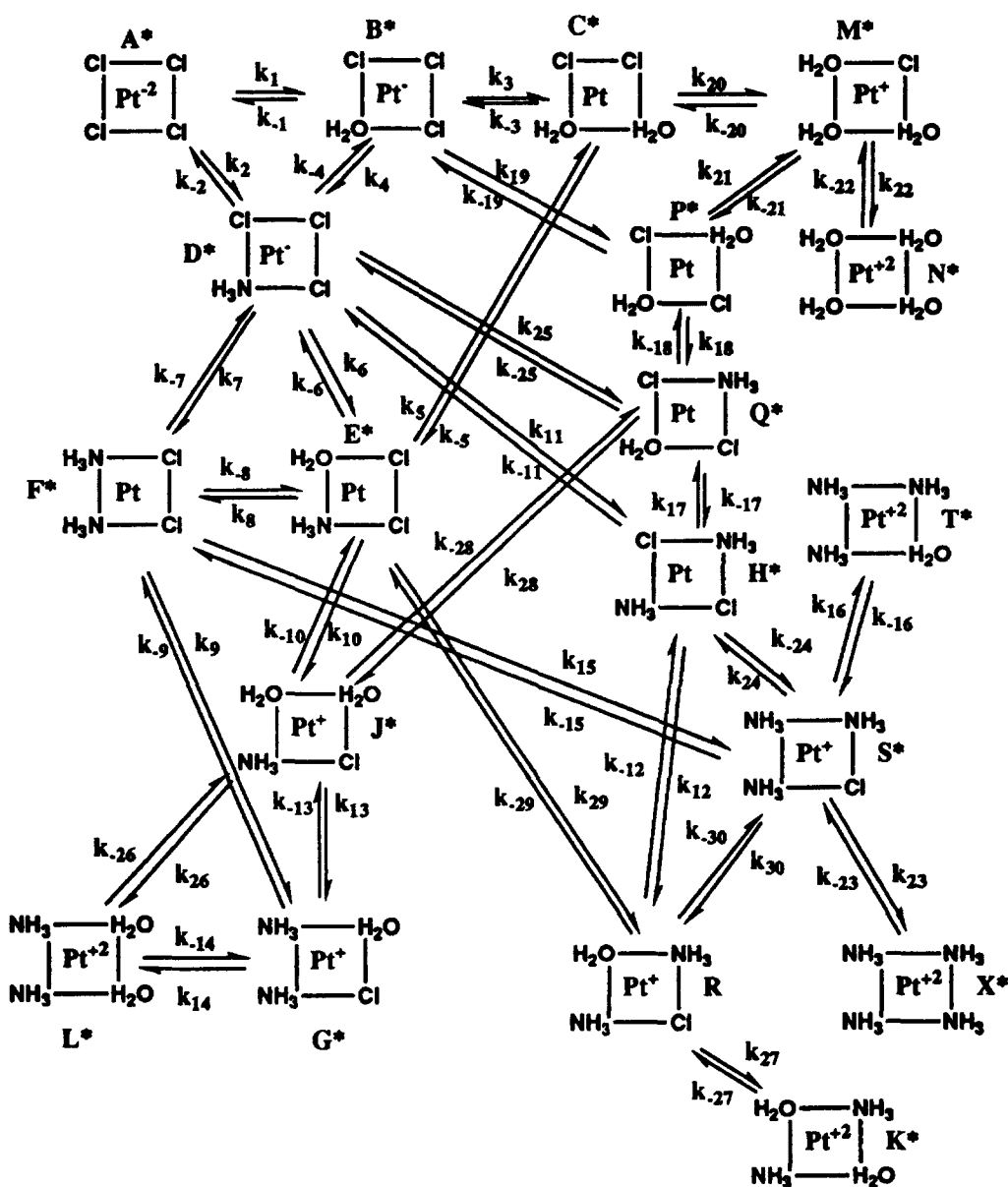


Figure 4 Model for the reaction of K_2PtCl_4 with chitosan based on its reactions with H_2O , NH_3 and Cl^-

amination reactions of $Pt(II)$ initially were taken to be the same as the known rate constants for $Pt(II)-NH_3$ reactions, but they later were taken to be an order of magnitude lower than the ammoniation rate constants. The final rate constants are given in Table 4. Note that the reaction orders are reflected in the model, and the units of the rate constants are given. The concentration of amine groups available for reaction in the form of RNH_2 at a given pH was calculated on the basis of a pK_a of 6.5 for the amine group of chitosan³⁴. Some additional calculations were done to explore how the results (e.g. number of crosslinks per chain at t_{gel}) vary with pH in even greater detail, using the reported⁶ variation of pK_a with pH for chitosan amine groups.

Another program was written to calculate the u.v./vis. spectrum of the reacting solution at any time t_i using the computed concentrations, C_{ij} , of all the species, j , at that time t_i . The total absorbance $A(t_i, \lambda)$ of the solution at wavelength λ and time t_i was calculated using a Beer's law summation for all the individual species j included in the kinetic model based on the known spectra²⁰⁻²⁷ of

their extinction coefficients $\epsilon_{j\lambda}$ and the cell pathlength (l) of 1 cm.

$$A(t_i, \lambda) = \sum C_{ij}\epsilon_{j\lambda}l \quad (3)$$

Shown in Figure 5 are preliminary (initial) model spectral data calculated on the basis of the kinetic model in which the ammoniation rate constants were used for the amination reactions. These model spectra are from the gelation experiment for which the corresponding experimental spectra are shown in Figure 3. While the model spectra do show changes with time, there are both qualitative (three cross-over regions instead of two) and quantitative mismatches between the model spectra (Figure 5) and the experimental spectral data (Figure 3). This is not surprising since the reactivities of functional groups of polymers are quite often different from that of the same functional groups on small molecules due to phenomena known collectively as 'polymer effects'¹⁸. In the next set of calculations, the rate constants of the amination reactions were set to an order of magnitude lower than

Table 4 k values used in the final PtCl_4^{2-} /chitosan kinetic model

k	Value	Reference	k	Value	Reference
k_1	$3.7 \times 10^{-5} \text{ s}^{-1}$	20–22	k_{-15}	$0.0 \text{ M}^{-1} \text{ s}^{-1}$	—
k_{-1}	$2.8 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$	20,21,23	k_{16}	0.0 s^{-1}	—
k_2	$3.7 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$	22,24 ^a	k_{-16}	$0.0 \text{ M}^{-1} \text{ s}^{-1}$	—
k_{-2}	$2.3 \times 10^{-11} \text{ M}^{-1} \text{ s}^{-1}$	23,24	k_{17}	$0.002 \text{ M}^{-1} \text{ s}^{-1}$	— ^a
k_3	$6.0 \times 10^{-5} \text{ s}^{-1}$	23	k_{-17}	0.0 s^{-1}	—
k_{-3}	$7.5 \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$	23	k_{18}	$0.002 \text{ M}^{-1} \text{ s}^{-1}$	— ^a
k_4	$0.002 \text{ M}^{-1} \text{ s}^{-1}$	— ^a	k_{-18}	0.0 s^{-1}	—
k_{-4}	$0.0 \text{ M}^{-1} \text{ s}^{-1}$	—	k_{19}	$2.8 \times 10^{-8} \text{ s}^{-1}$	21,23
k_5	$0.002 \text{ M}^{-1} \text{ s}^{-1}$	— ^a	k_{-19}	$4.6 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$	21,23
k_{-5}	0.0 s^{-1}	—	k_{20}	$2.0 \times 10^{-7} \text{ s}^{-1}$	21,23
k_6	$5.6 \times 10^{-5} \text{ s}^{-1}$	21	k_{-20}	$2.0 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$	21,23
k_{-6}	$4.3 \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$	21	k_{21}	$1.0 \times 10^{-4} \text{ s}^{-1}$	21,23
k_7	$9.9 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$	24 ^a	k_{-21}	$0.5 \text{ M}^{-1} \text{ s}^{-1}$	21,23
k_{-7}	$0.0 \text{ M}^{-1} \text{ s}^{-1}$	24	k_{22}	$3.0 \times 10^{-7} \text{ s}^{-1}$	21,23
k_8	$0.002 \text{ M}^{-1} \text{ s}^{-1}$	— ^a	k_{-22}	$3.0 \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$	21,23
k_{-8}	0.0 s^{-1}	—	k_{23}	0.0 s^{-1}	—
k_9	$2.5 \times 10^{-5} \text{ s}^{-1}$	21	k_{-23}	$0.0 \text{ M}^{-1} \text{ s}^{-1}$	—
k_{-9}	$7.6 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$	21	k_{24}	$0.0 \text{ M}^{-1} \text{ s}^{-1}$	—
k_{10}	$5.9 \times 10^{-5} \text{ s}^{-1}$	20	k_{-24}	$0.0 \text{ M}^{-1} \text{ s}^{-1}$	—
k_{-10}	$0.099 \text{ M}^{-1} \text{ s}^{-1}$	20	k_{25}	$6.3 \times 10^{-5} \text{ s}^{-1}$	20
k_{11}	$1.2 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$	24 ^a	k_{-25}	$4.8 \times 10^{-4} \text{ s}^{-1}$	20
k_{-11}	$0.0 \text{ M}^{-1} \text{ s}^{-1}$	24	k_{26}	$4.7 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$	— ^a
k_{12}	$9.8 \times 10^{-5} \text{ s}^{-1}$	21	k_{-26}	$0.0 \text{ M}^{-1} \text{ s}^{-1}$	26
k_{-12}	$0.28 \text{ M}^{-1} \text{ s}^{-1}$	21	k_{27}	$5.0 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$	26
k_{13}	$0.02 \text{ M}^{-1} \text{ s}^{-1}$	—	k_{-27}	$0.002 \text{ M}^{-1} \text{ s}^{-1}$	26
k_{-13}	0.0 s^{-1}	—	k_{28}	$7.2 \times 10^{-5} \text{ s}^{-1}$	20
k_{14}	$3.3 \times 10^{-5} \text{ s}^{-1}$	25	k_{-28}	$7.5 \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$	20
k_{-14}	$9.9 \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$	25	k_{29}	$1.2 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$	— ^a
k_{15}	0.0 s^{-1}	—	k_{-29}	$0.0 \text{ M}^{-1} \text{ s}^{-1}$	—

^a These amination rate constants are the final values used in the calculation. They are 10 times lower than the ammoniation rate constants, reflecting polymer effects, as discussed in the text

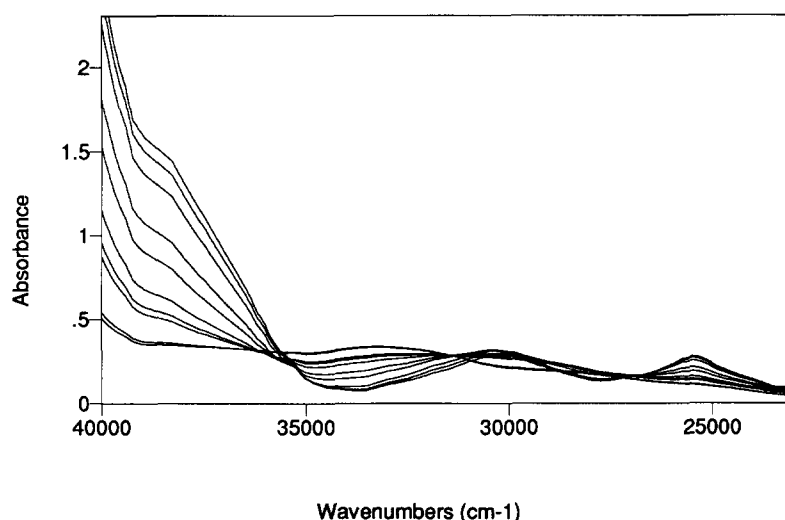


Figure 5 Initial-model spectra calculated as a function of time for the reaction of K_2PtCl_4 with MMW-X-80 chitosan. The reaction conditions and times are the same as those in Figure 3. The rate constants for the initial kinetic model (Table 4) were used. Note: the amination rate constants in Table 4 which are marked with an asterisk are a factor of 10 larger in the initial kinetic model

the reported ammoniation rate constants to compensate for these polymer effects. This produced spectra that were in good qualitative agreement (number and position of cross-over regions) with the experimental spectra. This is shown in Figure 6 for the modelling of the

gelation experiment corresponding to the experimental spectra of Figure 3.

Next, the effect of light scattering was included in the model. This resulted in spectral data that were in both good qualitative and quantitative agreement with the

experimental spectra (Figure 3), as shown in Figure 7. The light scattering was included (Figure 7b) by adding curves of the form $I = a\lambda^{-4} + b$ (Rayleigh type scattering). The values of the constants a and b were chosen so that the ratio a/b and changes in the magnitude of a with time were consistent with the values of a and b obtained from curve fittings of the nephelometry data.

KINETIC MODEL RESULTS

The kinetic model involved two processes. First, a reaction kinetic model was used to calculate the concentrations of all species involved in reactions leading to coordination of Pt(II) at all times. The concentration-weighted calculated u.v./vis. spectra of all species and the light scattering were added and compared to the experimental spectra at equal times of reaction. With the single change of correcting the rate constants as a set, to account for the polymer effects on reactivities of sites on polymers by reducing the rate constants of the amination reactions an order of magnitude relative to their ammoniation counterparts, the calculated and experimental spectra were brought into reasonably good qualitative and quantitative agreement.

In order to explore this behaviour further, other postulated differences between the rate constants for the reactions of Pt(II) with the amine groups of chitosan and those of the reactions of Pt(II) with ammonia were built into the kinetic model and then tested⁸. It was found that decreasing the amination rate constants further, e.g. by two orders of magnitude or more instead of the one order of magnitude used in the final kinetic model, quickly led to significant disagreement between the calculated and experimental model spectra. As another example, it was reasoned that steric effects might reduce the rate constants more for those reactions in which a chitosan amine group is introduced *cis* rather than *trans* into another chitosan amine group ligand that is already present in the Pt(II) coordination sphere. When this hypothesis was tested, significant differences in the positions of the peak maxima and the number of cross-over points and their positions were found upon comparison of the calculated

spectra with the experimental spectra. This was found to be true for all such modelling cases tested in which the amination rate constants for the introduction of a second amine group in the *cis* position were decreased by a different factor than that used to decrease the rate constants for reactions leading to the introduction of a second amine ligand in the *trans* position. In another context, it was postulated that the rate constants of a Pt(II) complex for a second amination reaction might be reduced more than that of the amination reaction to introduce the first amine group due to steric hindrance; however, disagreement was also found between the model and experimental data if the rate constants for the introduction of a second amine group to a Pt(II) coordination sphere were reduced by a different factor than that which had been used to decrease the rate constant of the amination reactions that introduce the first amine group into the coordination sphere.

After the kinetic model and the model spectral data were successfully tested against experimental data for a variety of experimental reaction conditions, the concentrations of Pt(II)-diamine complexes at each gelation time were evaluated. Since there are many Pt(II) diamines in the model, these concentrations were calculated variously as the sum of the *cis*-Pt(II) diamines, the *trans*-Pt(II) diamines or the total Pt(II) diamines. Each can be a crosslink, and from them crosslink/chain ratios at the time of gelation were calculated for the various gelation experiments that had been carried out. These are the total number of Pt(II)-diamine crosslinks formed per chitosan chain. Although there is some scatter in the calculated [crosslink]/[chain] ratios presented in Table 5 for the MMW-X-80 experiments, the following three prominent trends appear in this data set.

- (1) The calculated values for the total number of crosslinks per chain are about the same order of magnitude for experiments with the same $X/\text{Pt(II)}$ ratio and polymer concentration, but different pH. For example, the experiments with an $X/\text{Pt(II)}$ ratio of 10:1 and a chitosan weight percentage of 0.78 all have an average of 3.5 for the number of crosslinks/chain at the time of gelation, with the exception of

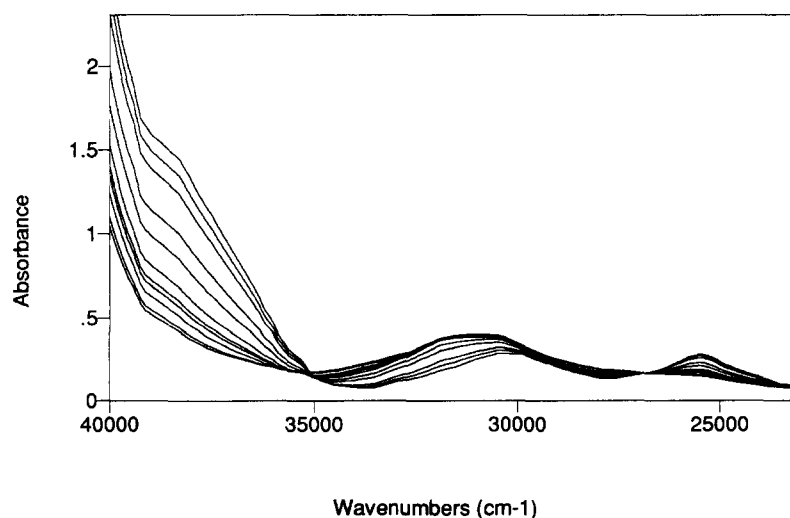


Figure 6 Refined-model u.v./vis. spectra calculated as a function of time for the reaction of K_2PtCl_4 with MMW-X-80 chitosan. The reaction conditions and times are the same as those in Figure 3. The rate constants for the refined kinetic model (Table 4) were used. Note: the amination rate constants in Table 4 which are marked with an asterisk are a factor of 10 smaller in the refined final kinetic model than they are in the initial kinetic model

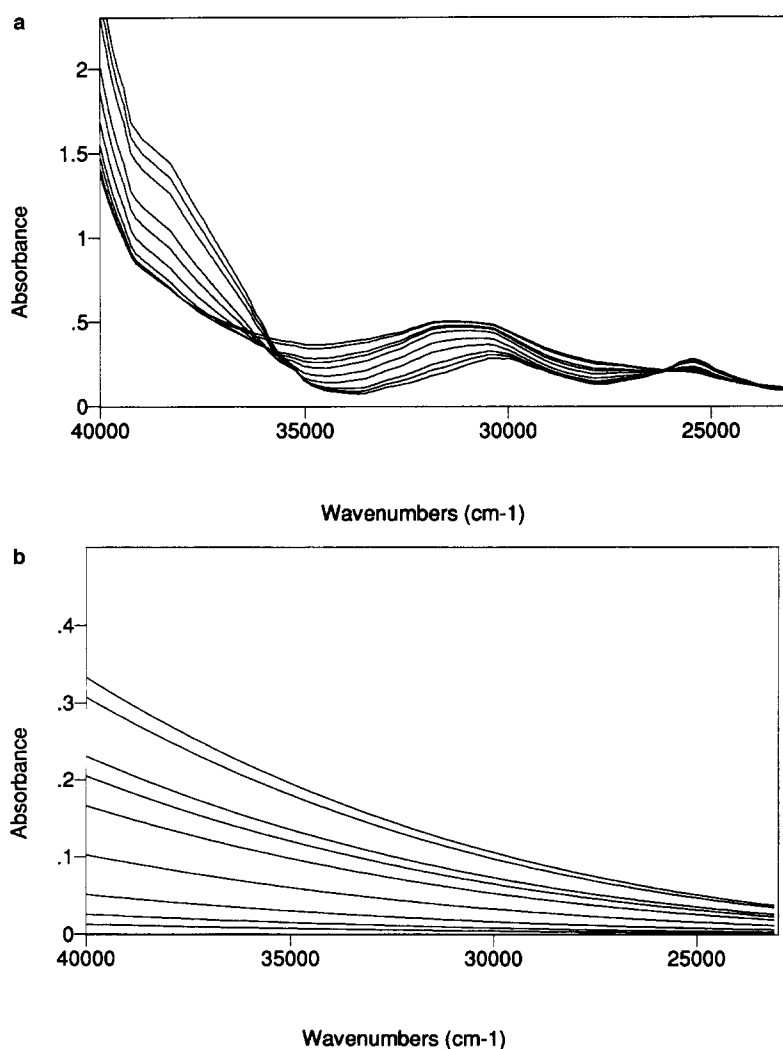


Figure 7 Refined-model u.v./vis. spectra including light scattering calculated as a function of time for the reaction of K_2PtCl_4 with MMW-X-80 chitosan. The reaction conditions and times are the same as those in Figure 3. The spectra in (a) are the same as those in Figure 6 with the addition of light scattering, and (b) shows the light scattering alone

the experiments with the highest molecular weight chitosan material, HMW-X-80, as will be discussed later.

- (2) The calculated number of crosslinks per chain at the time of gelation increases as the $X/Pt(II)$ concentration ratio increases from 10:1 to 20:1 and 40:1 while all other parameters remain constant.
- (3) As the chitosan concentration of the reaction solution in the experiment increases and all other parameters are held constant, the number of crosslinks per

chain at the time of gelation decreases. This trend can be seen by comparing the data from the 0.53 wt% and the 0.78 wt% experiments*.

Table 6 gives the crosslink/chain ratios at t_{gel} for selected experiments in which the kinetic model included the reported variation of pK_a and extent of ionization with pH, molecular weight and percentage deacylation. When the variation of pK_a with pH was included in the model, the crosslink/chain ratio at t_{gel} decreased to a lesser extent with increasing pH than for the case of the modelled experiments in which a constant pK_a was assumed. In fact, in some cases, the trend was actually reversed and a small increase in calculated crosslink/chain ratio at the time of gelation was observed with increasing pH.

DISCUSSION

Gelation times

The hypothesis that the kinetics of the gel formation are controlled by the formation of coordinate-covalent bonds to $Pt(II)$ is confirmed by two observations. First, the gelation times for analogous reactions of $Pd(II)$ with chitosan are about a factor of 10^4 – 10^5 shorter than those

* Comparing the calculated crosslink/chain ratios for the 1.15 wt% experiments to those from the 0.78 and the 0.53 wt% experiments is more difficult, because 30:1 and 60:1 $X/Pt(II)$ ratios were used in the case of the 1.15 wt% experiments instead of 10:1, 20:1 and 40:1. However, the calculated crosslink/chain ratios at t_{gel} for the 30:1 $X/Pt(II)$ and 1.15 wt% chitosan experiment are nearly as low as those from the corresponding 20:1 $X/Pt(II)$ and 0.78 wt% chitosan experiment. In order to examine this behaviour in more detail, extrapolated crosslink/chain ratios for the hypothetical case of 10:1 $X/Pt(II)$ and 1.15 wt% chitosan gelation experiments are also given in Table 4. The extrapolation was performed on the basis of variation of the crosslink/chain ratio with $X/Pt(II)$ ratio from the 0.78 and 0.53 wt% chitosan experiments. These extrapolated values indicate that this trend of decreasing crosslink/chain ratios at t_{gel} continues with increasing chitosan concentration

of the corresponding Pt(II) species⁷. More directly, the observed gelation times vary systematically with the $X/Pt(II)$ ratio when all other parameters are held constant. The approximately 100-fold increase in the gelation times when chitosan is reacted with the tetraamine complex of Pt(II) instead of its tetrachloride complex confirms the hypothesis that, once formed, the Pt–N bonds are kinetically inert and that further reaction of the tetraamine complex is relatively unimportant in the kinetic model. The very long gelation times of chitosan when it is reacted with either the *cis*- or the *trans*-diamine Pt(II) complex do not show any clear steric effects and instead further confirm the hypothesized kinetic inertness of the Pt–N bonds and the relative unimportance of the further reactions of the diamine complexes in the kinetic model.

The decrease in gelation time for the reactions of solutions as the pH is increased, and all other parameters remain constant, is likely to be due primarily to the greater number of amines in the unprotonated form. This pH dependence is taken into account in the calculations using our kinetic model. The higher RNH₂ concentration increases the rate of the amination reactions of Pt(II) and thus the rate of formation of crosslinking species, and it would be expected to increase also the extent of inter- and intra-chain hydrogen bonding. The decrease in the average charge per 'mer' segment (from 0.64 to 0.50 for HMW-X-80 chitosan materials) as the pH increases from 5.3 to 5.9 could have some effect on the gelation and conformation due to differences in intra- and inter-molecular electrostatic repulsion forces. The initial viscosities of the various pH chitosan solutions are quite similar, however, indicating that differences in electrostatic forces over this pH range are small, especially for our solvent and ionic strength conditions. Other workers have reported³⁵ also that chitosan is a random coil polymer under similar conditions of pH, solvent and ionic strength.

The observed decrease in gelation times for solutions using higher molecular weight chitosans can be understood as follows. As crosslinking proceeds, the average size of the growing polymeric clusters, S_{ave} , increases and approaches the limit $S_{ave} \rightarrow \infty$ as gelation occurs³⁶. The chitosans that are of higher molecular weight and thus larger size should approach the limit $S_{ave} \rightarrow \infty$ faster than lower molecular weight chitosans (for the same rate of crosslinking) because larger size units are being joined together by crosslinking in the case of the higher molecular weight polymers.

Evidence for this type of behaviour comes from Figure 8 where the gelation times for the HMW-X-80 and MMW-X-80 experiments are plotted against one another for those experiments having the same conditions of pH and $X/Pt(II)$ ratio. It can be seen that the data fit well to a line with a slope of 2.85, which is the ratio of the molecular weights of the two polymers. Because similar plots can be constructed to compare the data for the HMW-X-100 and MMW-X-80, for example, and show an equally good fit to a line with a slope corresponding to the molecular weight ratio, it appears that the difference in percentage deacylation between the 80 and 100% deacylated chitosans is not important in determining the gelation time. This is because the unprotonated amine groups are in significant excess in comparison with the Pt(II) concentration for all experiments reported here, with the exception of the pH 5.3 10:1 $X/Pt(II)$ ratio experiments.

Table 5 Selected [crosslink]/[chain] ratios at t_{gel}

Chitosan	wt%	pH	$X_{d.a.}/Pt(II)$	[Pt(diamine)] ^a
				[chains]
MMW-X-80	1.15	5.3	30:1 (10:1) ^a	8.8 (<2.8) ^a
	0.78	5.3	10:1	2.8
	0.53	5.3	10:1	4.3
	1.15	5.6	30:1 (10:1) ^a	9.8 (<3.2) ^a
	0.78	5.6	10:1	3.3
	0.53	5.6	10:1	3.9
	1.15	5.9	30:1 (10:1) ^a	20.0 (<6.7) ^a
	0.78	5.9	10:1	7.6
	0.53	5.9	10:1	8.1
HMW-X-100	0.78	5.3	10:1	3.7
	0.78	5.6	10:1	2.9
	0.78	5.9	10:1	3.4
MMW-X-100	0.78	5.3	10:1	2.0
	0.78	5.6	10:1	4.0
	0.78	5.9	10:1	2.6
	0.78	5.9	20:1	5.1
	0.78	5.9	40:1	6.9
HMW-X-80	0.78	5.3	10:1	0.63
	0.78	5.6	10:1	0.75
	0.78	5.9	10:1	0.42

^a Values for 1.15 wt% MMW-X-80 experiments are extrapolated for a $X_{d.a.}/Pt(II)$ ratio of 10:1 based on the dependence of the [crosslink]/[chain] ratio on $X_{d.a.}/Pt(II)$ for other experiments

Table 6 Effect of variations in pK_a with α^a on calculated [crosslink]/[chain] ratios at t_{gel} [$X_{d.a.}/Pt(II)$ ratio of 10:1, 0.78 wt% chitosan]

Chitosan	pH	α	pK_a	[Pt(diamine)]
				[chains]
MMW-X-80	5.3	0.085	6.33	5.4
MMW-X-80	5.6	0.15	6.35	6.5
MMW-X-80	5.9	0.25	6.38	11.2
HMW-X-100	5.3	0.19	5.93	27.9
HMW-X-100	5.6	0.26	6.05	12.4
HMW-X-100	5.9	0.34	6.19	9.1

^a Values of pK_a and α obtained from ref. 6

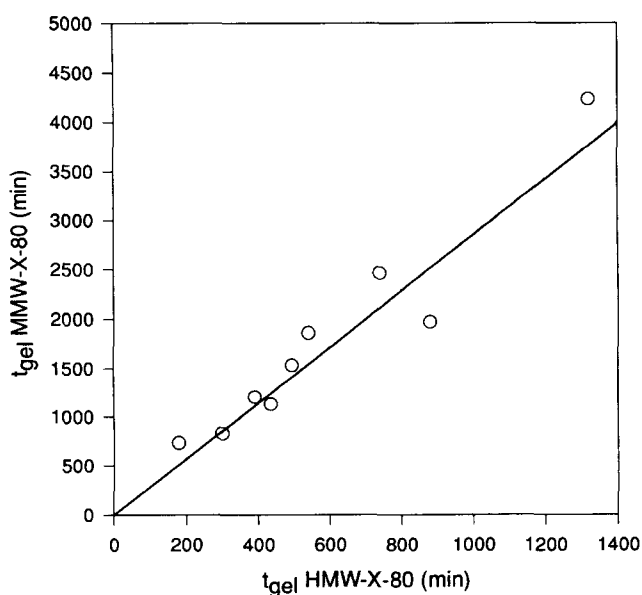


Figure 8 Gelation times for the HMW-X-80 and MMW-X-80 experiments plotted against one another for those experiments having the same conditions of pH, chitosan concentration and $X/Pt(II)$ ratio

Model results

Flory¹⁶ had predicted that, in the case of sufficiently high molecular weight polymers, gelation could occur for a crosslink/chain ratio of 0.5, which is the first point at which all the polymer chains can be connected to one another. This value is somewhat lower than the crosslink/chain ratios at t_{gel} calculated in most of our experiments [e.g. an average of 3.5 for the 10:1 $X/\text{Pt(II)}$ ratio experiments]. Although all the polymer chains can be connected to one another at crosslink/chain levels as low as 0.5, current network theories predict—in contrast to Flory's treatment—that a crosslink/chain ratio of the order of two or three is needed to percolate the rigidity measured as very high viscosity. For example, in the Scanlan–Case elastically active network chain theory^{37,38}, an active junction point of a network in three-dimensional space is considered to be one that is tied to the surface by at least three independent routes. There is also experimental and theoretical model evidence in several cases for glasses that mechanical stability, as evidenced by a softening in the elastic constants, decreases as the average coordination decreases to about 2.4³⁹. This is the prediction of mean field theory for a lattice of any coordination number. It can be concluded then that the order of magnitude of the results in *Tables 5* and *6* appears to be in good agreement with those of current theoretical and experimental data for other systems.

The apparent increase in the calculated crosslink/chain ratios with increases in the $X/\text{Pt(II)}$ ratio is likely due to the weakness of the constant reactivity approximation (implicit in our use of the same rate constants throughout the course of the reactions) in the highly viscous pregel region. In that region, the mobility of the polymer decreases rapidly and the chains become more constrained⁴⁰. The higher $X/\text{Pt(II)}$ concentration ratio experiments approach the gel point much more slowly and thus spend more time in the highly viscous pregel region than do the experiments with lower concentration ratios. Since the mobility is reduced at high viscosity, it would be expected also that the reaction rates are reduced. For this reason, it would be expected then that the constant reactivity approximation would be less accurate—and the calculated crosslinks per chain at the time of gelation overestimated—for those experiments conducted with higher $X/\text{Pt(II)}$ ratios (lower $[\text{Pt(II)}]$) compared to those with lower $X/\text{Pt(II)}$ ratios (higher $[\text{Pt(II)}]$). Support for this hypothesis comes from the observation that the variation between the calculated crosslink/chain ratio for the 10:1 and 40:1 experiments is a factor of three smaller just before the highly viscous pregel region is entered compared to the variation between the crosslink/chain ratios later at t_{gel} .

The decrease in calculated crosslink/chain ratios at t_{gel} as the concentration of chitosan increases can be ascribed⁴¹ to the preferential formation of intermolecular crosslinks as opposed to intramolecular crosslinks as the concentration of polymer chains increases. This is presumed to be due to the increased probability of collision and reaction between functional groups from separate chains as the concentration of chains increases.

The relative contribution of entanglements to gelation could also be a factor in understanding the observed decrease of the crosslink/chain ratio at the time of gelation, with increasing chitosan concentration, when all

other factors are held constant. The number of entanglements and thus the resulting number of junction zones has been found to increase with increasing concentration or molecular weight of polymer⁴⁷. Thus, another reason for the apparent decrease in the crosslink/chain ratios with increasing polymer concentration may be that there is a greater contribution to gelation from entanglement junctions as the polymer concentration is increased. This also may partly explain why the calculated crosslink/chain ratios are somewhat lower for the HMW-X-80 experiments than for all the experiments with the lower molecular weight chitosans.

A final consideration in this discussion of the calculated crosslink/chain ratios at gelation concerns our assumption that the $\text{p}K_{\text{a}}$ of the amine group of chitosan is constant for our experimental conditions. It was found that, with very few exceptions, the calculated crosslink/chain ratio at the time of gelation decreases slightly with increasing pH, all other parameters being constant (*Table 5*). When the reported variation⁶ of $\text{p}K_{\text{a}}$ with pH (or the extent of ionization, α , where $\alpha = 1$ when all of the chitosan amine groups are unprotonated, and $\alpha = 0$ when all of the amine groups are protonated) was included in the model, the crosslink/chain ratio at t_{gel} decreases to a lesser extent, or in some cases even slightly increases (*Table 6*), with increasing pH in comparison with the modelled experiments in which a constant $\text{p}K_{\text{a}}$ was assumed (*Table 5*). The comparison of the calculated crosslink/chain results of *Table 6* with those of *Table 5* indicate though only qualitatively the effect that variations in $\text{p}K_{\text{a}}$ with pH could have on the calculated crosslink/chain ratios at t_{gel} . Because the acid solvent system and its ionic strength used in ref. 6 were different from that used in this study, our chitosan $\text{p}K_{\text{a}}$ may vary with pH in a somewhat different manner from that in the cited work.

Small pH effects on gelation due to differences in the extent of hydrogen bonding or electrostatic repulsion with pH cannot be ruled out of course. The two main observations which indicate that variations in pH-dependent hydrogen bonding or electrostatic interactions are not important factors, however, in determining the gelation point under our experimental conditions are: (1) the lack of viscosity variation as a function of pH (over the range pH 5.3–5.9) in the initial chitosan solutions prior to addition of Pt(II) complexes, and (2) the small variations in calculated crosslink/chain ratios at t_{gel} for both the model having a constant $\text{p}K_{\text{a}}$ (*Table 5*) and the one including variations of $\text{p}K_{\text{a}}$ with extent of ionization (*Table 6*). From these results it would appear that although the pH is important in determining the concentration of unprotonated amines and thus the relative rate of amination for a particular $X/\text{Pt(II)}$ ratio, pH does not have a large effect on the number of crosslinks per chain at the time of gelation, at least in the pH range of this investigation. One must keep in mind that a factor of three variation in the concentration of RNH_2 is relative and that the actual percentage of 'mer' units having an unprotonated RNH_2 group varies at most from a level of about 6% to about 18% (assuming a constant $\text{p}K_{\text{a}}$). Perhaps electrostatic and hydrogen bonding interactions are less important in determining the gelation point in this investigation due to the high ionic strength of the solution and the smaller bond strength and greater reversibility of hydrogen bonding interactions compared to covalent ones.

SUMMARY

In accordance with bond percolation theory, a critical number of crosslinks per chain was found to be required to bring about the gelation of chitosan solutions through complexing reactions with Pt(II) complexes. This critical number of crosslinks per chain is of the order of 3.5, in accordance with current network theories, and appears to be independent of the solution pH, percentage deacetylation of the chitosan (in the range investigated) and concentration ratio of chitosan amine groups to Pt(II) [the X/Pt(II) ratio]. It was found that the number of crosslinks per chain required to bring about gelation decreased with increasing concentration or molecular weight of chitosan.

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