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# Physicochemical behaviour of chitin gels

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**Abstract**

Syneresis of chitin gels formed in the course of N-acetylation of chitosan in hydroalcoholic media has been studied. A critical cross-linking density related to a critical acetylation degree for which the gel undergoes weak syneresis and swells in water was shown (degree of acetylation (DA) 88%). Above this value, the weight loss during syneresis increases with DA. Conversely, syneresis decreases on increasing the polymer concentration, but disappears at a macroscopic level for a polymer concentration close to the critical concentration of entanglement in the initial solution. An increase in temperature favours the formation of hydrophobic interactions and new inter- and intramolecular hydrogen bondings. Due to the weak polyelectrolyte character of chitin, the weight of the gel depends on the pH and ionic strength of the media. Swelling–deswelling experiments show that the swelling of the gel is not fully reversible in relation with the formation of new cross-links during the depletion of the network. Our results reveals that the balance between segment–segment and segment–solvent interactions as well as the molecular mobility play the major role. © 2000 Elsevier Science Ltd. All rights reserved.

*Keywords:* Chitin; Chitosan; Physical gels; Syneresis; Swelling; Polysaccharides

## 1. Introduction

Chitosan is a copolymer of (1 → 4)-2-amino-2-deoxy-β-D-glucan and (1 → 4)-2-acetamido-2-deoxy-β-D-glucan with an acetyl content generally below 30%. It has been reported that acetylation of chitosan in hydroalcoholic media allowed the selective modification of the free amino groups and was responsible for a process of gelation [1–8]. The high hydration, the physicochemical and physical properties, as well as the polyelectrolyte behaviour of this kind of gel allow applications especially in the field of waste-water treatment or bioactive dressing for wound healing [9] to be considered. Possible use as a slow-release drug-delivery system is also to be considered [10]. Syneresis is one of the major properties of physical gels, particularly hydrogels [11–22]. It is usually considered as a kinetic and thermodynamic process allowing a more stable state than initially [11,12] to be achieved. It depends on the balance between the segment–segment and segment–solvent interactions and on a process of stress relaxation. It is characterized by a molecular reorganization of the macromolecular network involving both the disruption and the formation of some bonds. This behaviour is critical in the case of food processing and numerous studies were performed to avoid this problem [15,16]. Syneresis of chitin gels has already been observed [1,6], but no particular investigation on

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the mechanism was reported. In this work, several parameters on syneresis were studied, such as the nature of the solvent, ionic strength, pH, the degree of acetylation (DA) of chitosan and the initial concentration of the polymer. We also studied the swelling of the gel in deionized water in relation with the DA of the network and the influence of ionic strength on the formation of the gel.

## 2. Experimental

*Materials.*—The initial chitosan sample was from Aber Technologies (Plouguerneau, France, batch No. A32E03). The DA, determined by $^1$H NMR, and $\overline{DP}_W$ as determined by SEC-MALLS were 2.4% and 810, respectively.

*Gel formation.*—An aq AcOH (0.5% w/w) solution of chitosan (1% w/w) was filtered on Millipore membranes of porosity from 3 to 0.22 μm. This solution (100 g) was mixed with propanediol (80 ml) and the mixture was left to stand overnight for degassing without stirring. A freshly prepared acetylating solution (20 mL of propanediol with the desired amount of $Ac_2O$) was slowly added to the aq alcoholic chitosan solution. The mixture was then stirred for 30 s and transferred to a cylindrical mould. For the study of the influence of ionic strength on gelation, a known amount of KCl was dissolved in the initial aq alcoholic chitosan solution.

*Syneresis and swelling monitoring.*—A known amount of gel ($\approx 20$ g) having cylindrical form ($\varnothing = 28$ mm $H = 33$ mm) was immersed in a thermostatted bath at $22 \pm 0.5$ °C. Syneresis was followed by weighing the gel periodically (the surface of the gel was wiped with paper). Concerning the role of the initial ionic strength (before gelation), syneresis was followed by weighing the exudates instead of the gels, which were too brittle.

The study of the influence of ionic strength after gelation was carried out without washing the gel. The dilution effect on the ionic strength due to the gel phase can be neglected. Concerning the study of the role of pH, gels were washed in order to eliminate the influence of AcOH on pH values. If necessary, pH was adjusted either by means of NaOH or 0.1 M HCl.

*Gas chromatography analysis.*—The composition of the solvent after syneresis was analysed on a DELSI 700 chromatograph with a CP WAX 52 CB Chrompack capillary column coupled to a catharometer as detector. The oven temperature was 110 °C and helium was the flow gas.

*$^1$H NMR spectroscopy.*—After washing, the hydroinsoluble gels were hydrolysed by HCl in $D_2O$ (20% w/w) [8] and analysed on a Bruker AC 250 spectrometer (250 MHz for $^1$H) at 298 K. The DA was determined from the ratio of the area of the methyl protons of (1→4)-2-acetamido-2-deoxy-β-D-glucan to the sum of the areas of the $H_2$ to $H_{6'}$ protons of (1→4)-2-amino-2-deoxy-β-D-glucan and (1→4)-2-acetamido-2-deoxy-β-D-glucan glucosamine residues according to Hirai et al. [23].

*Mechanical properties.*—Dynamic mechanical measurements were performed at room temperature on a Rheometrix RMS 800 rheometer with parallel-plate geometry. The plates were 13 mm in diameter and the gap between the plates ranged from 1 to 2 mm. The strain applied was less than 5% in order to avoid plastic behaviour.

*Laser light scattering.*—The laser-light scattering (LLS) detection of syneresis was carried out in batch by means of a Wyatt DAWN F detector, operating at 632.8 nm and at an angle of 90°.

## 3. Results and discussion

*Syneresis.*—The reorganization of the chain segments during syneresis of chitin gels can easily be shown by the increase of the intensity of the light scattered by the gel. This increase must be related to an increase of the crosslinking density responsible for an increase of the polymer concentration in the gel and then of a stiffening of the material.

In order to check the thermodynamic origin of syneresis, the role of various parameters classified as structural and external parameters was investigated.

### *The role of structural parameters*

*DA*. The DA of chitin in the gel depends on the molar ratio of $Ac_2O$–amine residues ($R$) used for its preparation [8]. As shown in Fig. 1, for a given DA, the decrease of the gel weight is relatively important in the first 100 h and a plateau is achieved after 200 h. The role of DA — in relation to the value of $R$ — in syneresis is reported in Fig. 2. The relative weight loss of the gels after 200 h syneresis increases with DA up to a maximum value close to 70% for a DA of 98%. Conversely, the gels corresponding to DA values close to 80% do not release their solvent. This behaviour must be ascribed to various changes. When the DA increases, the overall charge density due to the presence of ammonium groups decreases simultaneously with the increase of the cross-linking density related to the formation of hydrogen bonding and hydrophobic interactions. The lower limit of DA for which the syneresis does not operate — after the gel point — corresponds to a situation for which a thermodynamic equilibrium between the network and the solvent is already achieved. In this state, the attractive interactions (H bonds, hydrophobic interactions, stress relaxation of the chain segments, etc...) equilibrate the repulsive interactions; this is essentially due to the solvation of the hydrophylic functions and the charges of the ammonium groups. We can conclude that a critical cross-linking density, below which the solvent used for the gel formation becomes a good solvent, exists.

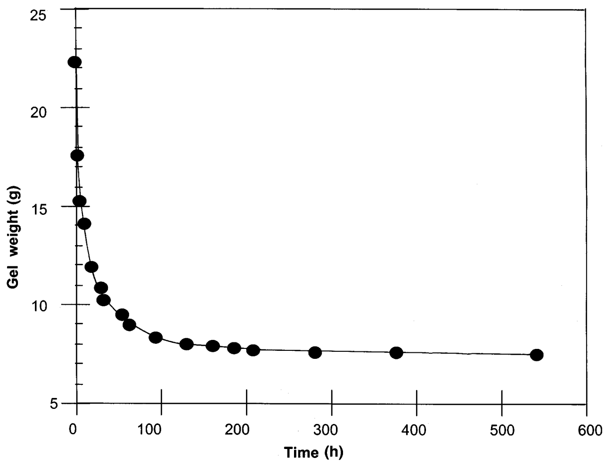

Fig. 1. Variation of the syneresis of a chitin gel (DA 98%) with time, measured from the addition of the acetylating solution (concentration of chitin at the gel point: 0.6%).

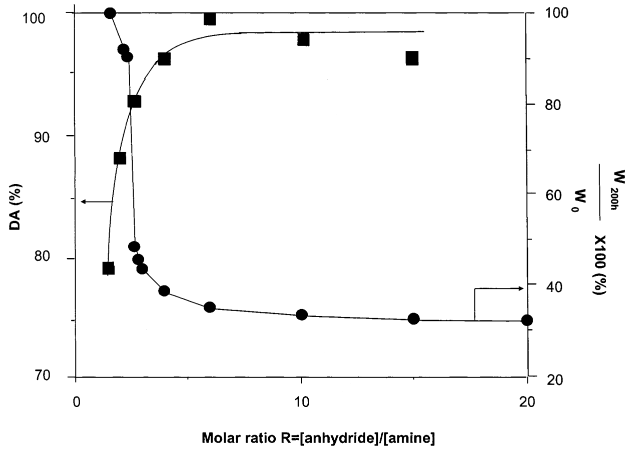

Fig. 2. Syneresis (●) and DA (■) as a function of the molar ratio $R$ ($W_{200\ h}$ = weight of the gel after 200 h of syneresis, $W_0$ = weight of the gel at the gel point; concentration of chitin at the gel point = 0.6%).

Anthonsen et al. [24] showed that two parameters influence the solubility of the polymer when the DA varies: the second virial coefficient, which decreases when DA increases, and the chain stiffness, which increases with DA. When the DA increases from 80 to 98%, the solubility decreases considerably with the disappearance of the charges borne by the chains, whereas the modification of the chain stiffness should be less important. As a consequence, the charge parameter can be considered as playing the major role on the solubility of the polymer chain segments. It is also important to mention the contribution to the osmotic pressure of the gel brought about by the counterions ($AcO^-$), which favours the swelling and then counteracts the syneresis.

The kinetics of gel formation [8] as well as the kinetics of syneresis depend on the DA. Syneresis can be considered as spontaneous for DA values over 88% although it may need several hours to begin to be observed for gels of lower DA values, which are more charged. The composition of the solvent released during syneresis contains information on the interaction changes occurring between the polymer segments and the solvent during the gelation process. The solvent in the gel contains water, propanediol, and acetic acid produced both by the reaction of acetylation and the hydrolysis of the excess of acetic anhy-

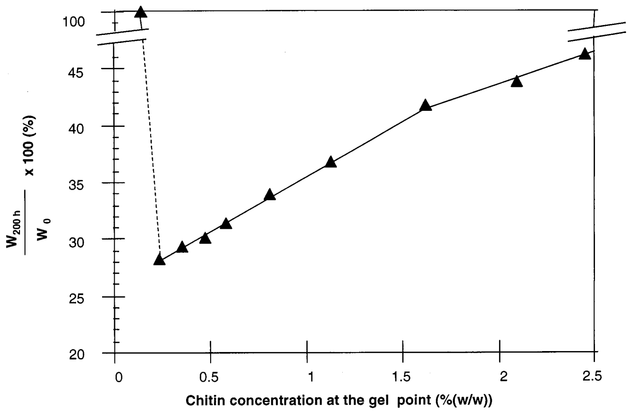

Fig. 3. Syneresis as a function of chitin concentration at the gel point ($W_{200\ h}$ = weight of the gel after 200 h of syneresis, $W_0$ = the weight of the gel at the gel point).

dride [8]. The molar percentages of water and propanediol in the exudates are independent of the value of DA and remain close to 18 and 80% respectively. These values are the same as in the formation solvent of the gel and demonstrate that, whatever the DA, the polymer has no preferential affinity for one of these solvents. The content of acetic acid in the exudates follows the reaction of acetylation quite well (not shown) and increases linearly over the upper limit of acetylation (98 ± 2.5%) achieved from $R = 4$. As a consequence, over this limit the content of acetic acid corresponds exactly to the excess of anhydride fully converted into acetic acid. These results agree with the fact that, for molar ratios over four, the chemical structure is constant whatever the value of $R$. In this case, only the rates of acetylation and gelation are modified when $R$ varies.

*Concentration of polymer*. The initial concentration of polymer has an important effect on the syneresis. Indeed, it allows the control of two important parameters, i.e., the frequency of the interactions between the polymer and the solvent and the cross-linking density of the network. With this in mind, the syneresis for various concentrations of polymer — before syneresis — between 0.24 and 2.46% (wt.%) was studied. The value of $R$ was maintained constant and close to 10, which leads to the maximum acetylation (98 ± 2.5%) [8]. The weight of the gel after 200 h of syneresis ($W_{200\ h}$) divided by the weight of the gel at the gel point ($W_0$) for various concentrations of chitin (calculated after reacetylation) is shown in Fig. 3. As we observed a critical DA, we observe a critical concentration (0.12%) for which the gel does not undergo syneresis. This value must be related to a critical minimal value of the cross-linking density. At this concentration, which is close to $C^*$, the critical concentration of entanglement (0.11%), a macroscopic gel cannot be formed and only a dispersion of microgels or aggregates can occur. In these conditions, the exclusion of solvent responsible for syneresis cannot be observed at a macroscopic level. For concentrations over 0.24%, the syneresis decreases when the polymer concentration increases. This behaviour has already been observed in various other cases, for example for agarose gels [13,14]. An increase of the polymer concentration is responsible for an increase of the cross-linking density. As a consequence, the length of the chain segments between cross-links decreases. Therefore, their degree of freedom decreases as well as the number of possible conformations and then, the stiffness of the chain segments increases. On the other hand, the stress on these chain segments increases, which limits their possible relaxation. In relation to an increase of the local viscosity, the molecular mobility decreases, which contributes to a lower rate of syneresis. Moreover, the number of molecules of solvent retained in the gel increases in relation to the osmosis induced by polymer chains.

The curve in Fig. 3 exhibits typical behaviour. In the first part, for concentrations between 0.24 and 1.6%, the weight loss of the gels is directly proportional to the polymer concentration in agreement with a simple thermodynamic process. This variation also reflects the fact that the affinity of the polymer toward the solvent does not vary with the concentration. Over 1.6%, the slope becomes weaker and seems to tend toward zero. This change of behaviour must be related to the fact that, on increasing polymer concentration, the cross-linking density is necessarily increased up to a plateau. As a consequence, the role of concentration on syneresis becomes ineffective.

We were also interested in studying the influence of the polymer concentration on the composition of the syneresis exudates. The concentration and relative proportion of dialcohol and water were constant whatever the polymer concentration. The amount of anhydride added was increased upon increasing the polymer concentration in order to maintain the molar ratio $R$ equal to 30. As for the study of DA, the composition of the exudates was similar to that of the initial solvent (taking into account the conversion of acetic anhydride into acid). As a consequence, in these conditions, there was no apparent affinity of the polymer for one of the components.

*Role of external parameters*

*Role of the alcohol*. The presence of an alcohol is essential for the gel formation because of its influence on the balance between repulsive and attractive interactions. It also plays two other roles: on one hand, it allows only the acetylation of the amino groups and on the other hand, it decreases the dielectric constant of the media thus favouring the deprotonation of the ammonium groups and then their acetylation. In a previous study [8], we showed that the nature of the alcohol only had an influence on the kinetics of the gel formation. Its role on syneresis has been studied by the comparison between ethanol and propanediol. Whatever the value of $R$, syneresis is more important in the case of ethanol and this difference increases as $R$ decreases. Thus for $R = 2$, 200 h after the gel point, the weight loss is only 20% in the case of propanediol although it attains 57% for ethanol. The dielectric constant of the media can be considered as playing an important role in this difference. The dielectric constants for ethanol and propanediol are 24.3 and 32 [25], respectively. For $R = 2$, DA is close to 85–88% [8] and then, it remains as free ammonium groups on the chains that will be less protonated in the presence of ethanol. As a consequence, the solvation of these groups and of other polar functions of the glucosidic ring is more important in propanediol.

Table 1
Gelation time as a function of KCl concentration in the initial aqueous/alcoholic solution of chitosan for two values of the molar ratio $R$

| [KCl] (M) | Gelation time (min) [a] $R = 2.5$ | $R = 10$ |
|---|---|---|
| 0 | 13 | 2.5 |
| 0.04 | 18 | 3.5 |
| 0.08 | 36 | 4.75 |
| 0.11 | undetermined | 5.66 |
| 0.2 | limit of gelation | 6.75 |

[a] Gelation time was measured from the addition of the acetylating mixture in the aqueous alcoholic solution of chitosan.

*Role of ionic strength*. Ionic strength is well known to play an important role on the behaviour of polyelectrolytes in solution. We studied the role of the concentration of KCl initially present in the hydroalcoholic solution on the gel formation, syneresis and the mechanical properties of the gel for two values of $R$: 2.5 and 10. The results reported in Table 1 show that the time necessary to achieve the gel point increases with KCl concentration independently of $R$. In the absence of salt, the conformation of chitosan in solution is believed to be that of a semi-rigid random coil with a persistence length of between 20 and 30 nm [26,27]. The latter decreases with the increase of the ionic strength of the media [24]. Before gelation, the charge density of the polymer chains is at a maximum and the presence of salt induces a reduction of the screening length of Debye of the ammonium groups with a possible condensation of the counter-ions and then, a decrease of the solubility of the polymer chains. As a consequence, the conformation of the polymer becomes more compact, which disfavours the chain organization responsible for the three-dimensional network formation. This behaviour explains why the gels obtained in these conditions are more opaque (precipitated domains) and have weaker mechanical properties (the shear modulus $G'$ is $1.74 \times 10^3$ Pa in the presence of 0.11 M KCl instead of $3 \times 10^4$ Pa for a frequency of 100 rad/s). This conformational change, associated with a loss of solubility, also disfavours the reaction of acetylation necessary to gelation [8]. Indeed, the DA obtained for $R = 2.5$ in the presence

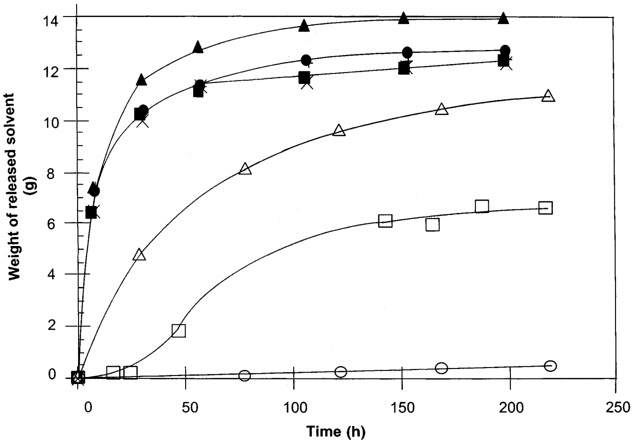

Fig. 4. Variation of syneresis of chitin gels with time for two $R$ values and for different KCl concentrations. $R = 2.5$: 0 M (▲), 0.08 M (+), 0.11 M (●) and 0.2 M (×). $R = 10$: 0 M (△), 0.08 M (□), 0.11 M (○). The time when the acetylating solution was introduced was considered as $t = 0$.

of 0.11 M KCl was only 78 instead of 92% for the same conditions in the absence of salt.

In the presence of salt, the rate of solvent exclusion during syneresis decreases when the concentration of salt increases for the two gels obtained with $R = 2.5$ and 10 (Fig. 4). This behaviour must also be related to a more compact conformation in the presence of salt and is more pronounced in the case of $R = 2.5$. In this case, the number of free amino groups in the ammonium form is more important and then the conformation of the chain segments is more sensitive to the loss of solubility. This behaviour is reinforced by the fact that the cross-linking density is lower, so the average length of the chain segments between two crosslinks is higher.

*Role of temperature.* As syneresis is of thermodynamic origin, temperature necessarily influences the phenomenon. It also plays a particular role in the low-energy interactions involved in the formation of the physical polymer network and as a consequence on the gel volume [28,29]. In order to check the role of temperature on the syneresis of chitin gels, we carried out an experiment on a gel prepared with $R$ close to 10 (DA 98 ± 2.5%), stored at a temperature of −25 °C. At this temperature, the hydroalcoholic solution remains liquid. After 190 h, the weight loss of the gel is only 14% compared with 70% at room temperature. If the gel is then placed at 21 °C, the weight loss attains 65%. This behaviour reveals that syneresis is an endothermic process, so a decrease of temperature limits the syneresis but in a fairly reversible manner. A similar experiment was performed on a gel stored (DA 98 ± 2.5%) for 9 h at 70 °C after its syneresis. In this condition, there is an additional weight loss equal to 7%. If the gel is then placed at room temperature, its weight increases but not in a completely reversible manner in relation to the formation (in this case) of new cross-links at 70 °C, which remain at lower temperature. Therefore, temperature favours both the molecular mobility and the formation of new cross-links.

*Swelling–deswelling.*—According to the circumstances, it can be interesting to control the volume of the gel when it is placed immediately after gelation in an excess of solvent different from the media of formation. Thus, the role of different parameters was investigated.

*Role of the nature of the solvent.* In Fig. 5(a and b), the values of the weight loss when gels corresponding to two different DA values are placed in various environments are updated. In aqueous media, when the volume of the gel decreases, the environment is modified by the solvent desorption. In relation to the release of acetic acid, we verified that at the end of the experiment, the pH was 3.4 and 3.1 for DA values of 92 and 98%, respectively. As a consequence, we must consider that the ionization of the amino sites was not greatly modified, since for high DA values the p$K_0$ of the network can be assumed to be near that of the monomer, i.e., 7.7 [30]. Fig. 5(a and b) shows that whatever the value of the DA, the solvating power is the best for the media of synthesis. Moreover, it normally decreases when the DA increases (see values at the plateau). Solvation by water also decreases when the DA increases in relation to the fact that for high DA values, water is not a good solvent for the system. On decreasing the DA, we attain a limit located near 90% where we begin to observe a swelling of the gel. Thus, for a DA of 88%, we notice a swelling of 50%. This behaviour must be essentially related to the increase of the number of ionic sites and their counter-ions on decreasing the DA, thus favouring the swelling. The difference between the behaviour in the water–alcohol solution and pure water is that for high DA values, the hydrophobic interactions are certainly the most important. Contrary to the case for a DA value of 92%, DMAc–LiCl has the same solvating power as the water–alcohol mixture for a DA value of 98%. This must be related to the fact that DMAc–LiCl becomes a better solvent as the DA increases [31]. If we fully exchange the initial hydroalcoholic solvent of the gel by the new solvent, then we achieve a total dissolution.

*Role of ionic strength.* As already mentioned, the state of the swelling of the gel in water depends on the repulsive electrostatic interactions between the charged segment of the polymer chains. It also depends on osmotic parameters related to the concentration and molecular weight of all the species present in the gel. If we consider the gel as a whole, for high degrees of acetylation (DA values of 92 and 98%), the charge density of the gel is necessarily weak although, locally, some sequences of sufficiently long glucosamine residues could be preserved. As a consequence, the condensation of counter-ions must be regarded as negligible at a macroscopic level. Usually, the presence of salt induces the deswelling of charged gels related both to the decrease of the Debye screening length [32] and to the salting-out effect [33] responsible for the decrease of the solubility of the chain segments. If we consider the results of Fig. 5(a and b), those obtained with KCl at different ionic strengths agree quite well with these assumptions. Thus, the gel that was 98% acetylated, the less charged, is the less sensitive to the variation of ionic strength. On the other hand, for a high ionic strength (0.04 M), the weight of the gel at the plateau becomes independent of DA and a limit for the weight loss is attained. In this case, the screening effect has certainly achieved its limit.

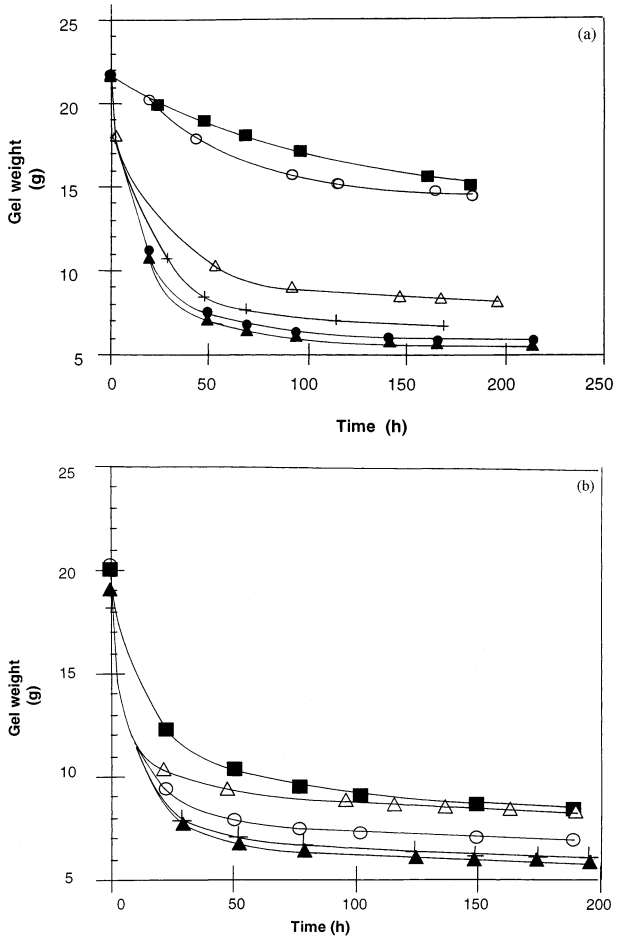

Fig. 5. Variations of syneresis of chitin gels with time for two DA values: (a) 92% and (b) 98%. Measurements were made from the introduction of the gel in different solvents and different aqueous solutions : water (○), KCl 0.02 M (+), KCl 0.04 M (●), KCl 0.1 M (▲), water–propanediol (■) and DMAc–LiCl (△) (volume of bath: 100 mL).

When a gel of initial weight close to 22 g (DA 92%) is placed in 100 mL of distilled water, the deswelling leads to a limit weight close to 11.7 g. After exchange, the release of acetic acid induces a final ionic strength in the bath close to $4 \times 10^{-4}$ M. When the gel is initially placed in 100 mL KCl 0.01 M instead of pure water, the limit weight becomes lower and close to 7.5 g. If afterwards, the gel is placed in 100 mL of pure water, in relation to an important decrease in the ionic strength, we observe its swelling up to 8.60 g, a value much lower than 11.7 g. There also, a part of the inter- and intramolecular interactions produced during the contraction of the network is not reversible. It certainly corresponded to an increase in the number of cross-links related to the acetylated groups.

The role of ionic strength is essentially observed in the polyelectrolyte behaviour of the chain segments and then depends on the charge density and the density of cross-linking and, as a consequence, on DA. When a gel corresponding to a DA of 88% is formed, the weight loss after syneresis is low and the gel weight decreases from 22.45 to 19.75 g. If we

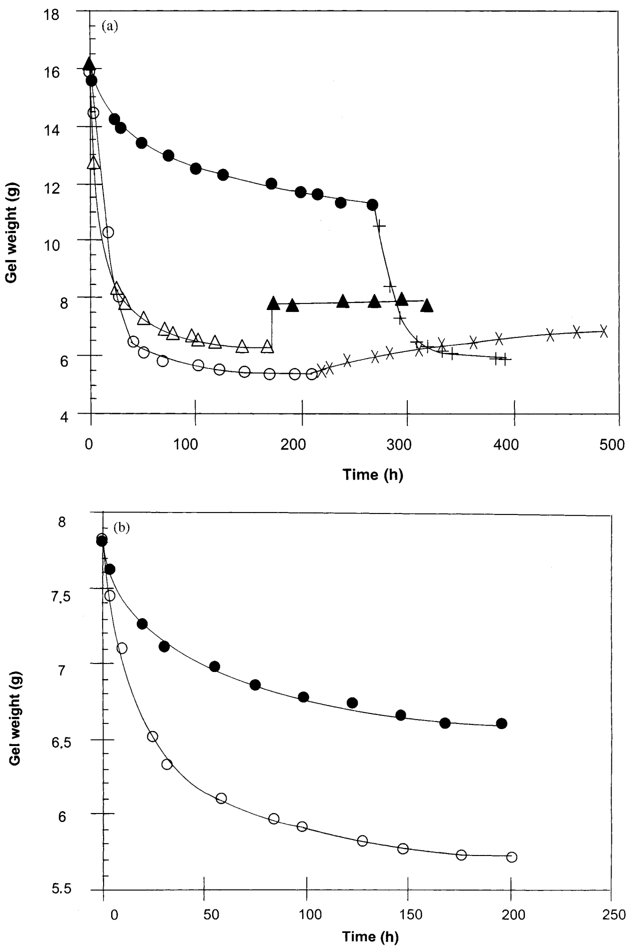

Fig. 6. Variations of syneresis of chitin gels with time for two DA values: (a) 92% and (b) 98%. Measurements were made from the introduction of the gel in baths of different pH values: pH 4 (), pH 2 (△), pH 10 (○), pH increasing from 2 to 4 (▲), pH decreasing from 10 to 4 (×) and pH increasing from 4 to 10 (+) (volume of bath: 200 mL).

consider that cross-links are formed due to the acetylated residues, when the DA decreases, the cross-linking density is certainly lower, the solubility higher, the electrostatic repulsions higher and the syneresis is weaker. When such a gel is placed, after syneresis, in pure water, the gel swells (the weight attains 29.7 g) and the volume at equilibrium is greater than the initial volume. In this case, we can consider that during syneresis, no new cross-links are formed and that, on decreasing the ionic strength, the phenomenon is purely entropic and fully reversible.

*Role of pH.* pH plays an important role in the stability of charged gels placed in water [34,35]. If we consider the results reported on Fig. 6(a and b), the state of swelling of the gels depends considerably on the pH of the environment. Whatever the DA, the depletion is minimal for pH close to 4. At this pH, for a given DA, the apparent charge density of the chain segments is maximum. Indeed, the free amino groups present on these polymer chains are at their maximum protonation, in a medium of relatively low ionic strength. As a consequence, the electrostatic repulsions, the solvation of the ionic groups and the osmotic contribution are maximum, thus contributing to a maximum swelling. This behaviour is confirmed by a lesser effect when the DA is increased. At pH 2, the excess of ionic strength of the bath due to protons and their counter-ions is sufficiently high to reduce the Debye screening length of the ammonium groups and then decrease the role of the parameters involved at pH 4. Therefore, the depletion is more important at this pH and should contribute to the formation of new cross-links. At pH 10, the situation is quite different, the amino groups are completely deprotonated and then contribute to the loss of solubility of the chain segments and to the formation of new cross-links by hydrogen bondings. Local conformational changes are also favoured by a higher free rotation of the free amino groups compared with the ammonium groups [29]. The solubility parameter is thus minimum at this pH and maximum depletion is observed. Contrary to the behaviour observed at pH 4, the depletion in this case is lower when the DA increases (the weight of the gels after syneresis increases from 5.17 to 5.78 g when the DA increases from 92 to 98%, respectively). This behaviour should be due to the increase of the steric hindrance and the stiffness brought about by the acetylated residues limiting the formation of new hydrogen bondings at this pH.

It was also interesting to test the role of pH on the swelling and deswelling behaviour of the gel. When pH is increased from 2 to 4 after stabilization of the gel weight (Fig. 6(a)), the weight increases showing a reversible part, but the new weight level remains relatively far from the one obtained directly at pH 4. The reversible part corresponds to the reversibility of the electrostatic properties of the free amino groups, in particular the modification of the Debye screening length. On the contrary, the partial loss of reversibility must be related to the fact that on decreasing pH from 4 to 2, the formation of new cross-links is favoured due to segment–segment interactions are favoured. This could be related to either hydrophobic or van der Waals interactions or hydrogen bondings essentially involving the acetylated residues. These junctions are then mostly maintained at pH 4. We then operated several cycles of pH change between 2 and 4 (Fig. 7). This kind of experiment was previously carried out for other gels [36]. In order to avoid the problem of salt formation, all increase of pH was performed following successive washings of the gel in pure water. It is interesting to notice that the minimum weight remains constant although the upper limit decreases regularly. This result confirms that each depletion favours the formation of new irreversible links, but does not change the value of the minimum swelling observed at pH 2, which must be then considered as the limit for this kind of gel when considering the pH in acidic media.

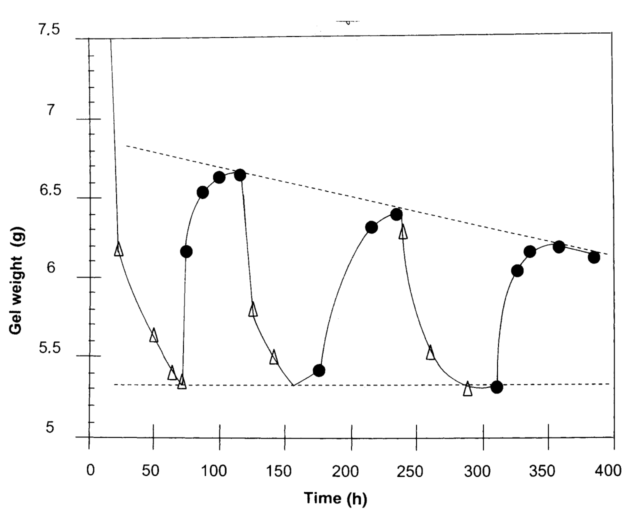

Fig. 7. Swelling and deswelling of a chitin gel (DA 92%) as a function of time for successive steps at different pH values: pH 2 (△) and pH 4 (●) (volume of bath: 200 mL).

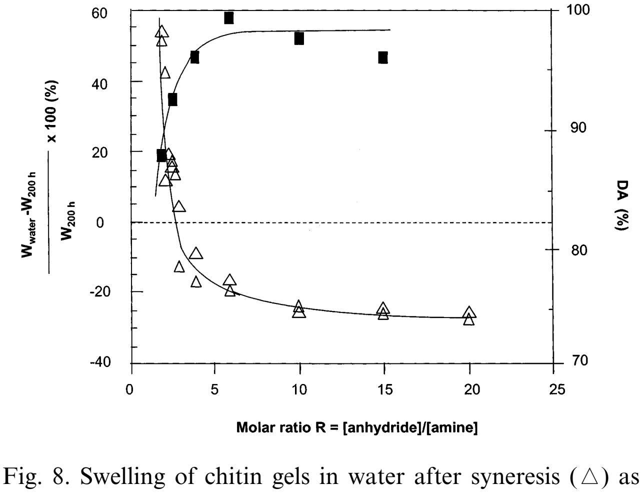

Fig. 8. Swelling of chitin gels in water after syneresis (△) as a function of DA (■) ($W_{200\text{ h}}$ = the gel weight after 200 h of syneresis, $W_{\text{water}}$ = the gel weight after 24 h in water, pH of bath 5.5–5.7, volume of bath: 200 mL).

When pH decreases from 10 to 4 (Fig. 6(a)), we also observed a swelling of the gel but, as above, the value at equilibrium was very far from that obtained directly at pH 4. It is also below the value obtained on increasing the pH from 2 to 4. The situation is quite different since we induce an important change from a state in which the free amino groups are uncharged to a highly charged state. Nevertheless, this contribution to the reversibility of the swelling seems relatively weak and then, the new irreversible junctions formed at pH 10 are necessarily more important and more stable than those that appeared at pH 2. This result confirms the important role played by the amino groups in the formation of new reversible junctions. It is also interesting to notice (Fig. 6(a)) that in this experiment, the rate of swelling on decreasing pH is very low compared with the previous case and must be related to the fact that at pH 10, the molecular mobility is very low and the reorganization of the chain segments becomes more difficult on increasing their solubility.

When, in a final experiment, we increased the pH from 4 to 10, we observed (Fig. 6(a)) an important deswelling with a limit close to the value achieved when the gel was directly placed at pH 10 for the same time. This behaviour confirms the result and interpretation discussed above and then, the limit pH for a minimum swelling corresponds to an alkaline pH. Indeed, at alkaline pH, two kinds of new cross-links can appear: those corresponding to the general process, whatever the pH, and those that are reversible due to the presence of amino groups.

*Role of DA.* If, after syneresis, the gel was placed in deionized water (pH within 5.5–5.7), the results reported in Fig. 8 show that according to DA, we can observe either a swelling or a deswelling with a frontier between the two kinds of behaviour near a DA of 92%. This frontier reveals the separation between the range of DA values where the hydrophilic interactions play the major role ( < 92%) and the one where the hydrophobic interactions are the most important ( > 92%). As discussed above, in the first range, we find a particular case, close to dissolution (DA 88%) where the swelling allows a gel weight over the initial value before syneresis. In the latter case, the swelling is very high but the number of cross-links remains sufficient to preclude dissolution.

## 4. Conclusions

Two essential components seem to regulate the syneresis of chitin gels. The first is related to a purely thermodynamic behaviour depending on the solution properties of the chain segments between two cross-links of the polymer network. This behaviour is necessarily reversible. The second is related to the possible formation of irreversible interactions corresponding to hydrogen bondings and hydrophobic or van der Waals interactions, favoured in particular by an increase of the molecular mobility due to the contribution of external parameters. All the results show that the charge density of the chain segments is an essential parameter and then, all factors that lower this parameter favour the deswelling and reversibility. When the deswelling becomes too high, the reversibility disappears to the benefit of the formation of irreversible junctions, and then the gels which initially can be considered as close to physical gels tend to behave as chemical gels.

This study is of interest with regard to the use of chitin gels in the field of biomedical applications as drug-delivery systems or as materials for surgical devices, especially in the field of cell rebuilding.

## References

[1] S. Hirano, R. Yamaguchi, *Biopolymers*, 15 (1976) 1685–1691.

[2] S. Hirano, Y. Ohe, *Agric. Biol. Chem.*, 39 (1975) 1337–1338.

[3] S. Hirano, Y. Ohe, *Carbohydr. Res.*, 41 (1975) C1–C2.

[4] S. Hirano, S. Kondo, Y. Ohe, *Polymer*, 16 (1975) 622.

[5] S. Hirano, Y. Ohe, H. Ono, *Carbohydr. Res.*, 47 (1976) 315–320.

[6] G.K. Moore, G.A.F. Roberts, *Int. J. Biol. Macromol.*, 2 (1980) 73–77.

[7] G.K. Moore, G.A.F. Roberts, *Int. J. Biol. Macromol.*, 2 (1980) 78–80.

[8] L. Vachoud, N. Zydowicz, A. Domard, *Carbohydr. Res.*, 302 (1997) 169–177.

[9] A. Domard, B. Grandmontagne, G. Sparaka, T. Karibian, H. Tournebise, French Pat. PCT/FR96/01106 (1996).

[10] S. Hirano, K. Tobetto, M. Hasegawa, N. Matsuda, *J. Biomed. Mater. Res.*, 14 (1980) 477–486.

[11] D.A. Rees, *Adv. Carbohydr. Chem. Biochem.*, 24 (1969) 78–80.

[12] G. Tonchava, D. Hadjikinov, I. Panchev, *Food Chem.*, 49 (1994) 29–31.

[13] K. Nagasaka, S. Taneya, *Nippon Shokuhin Kagaku Kaishi*, 43 (1996) 1176–1182.

[14] K. Park, W.S.W. Shalaby, *Biodegradable hydrogels for drug delivery*, Technomic, H. Park, Lancaster, 1993, p. 102.

[15] Y. Nakao, A. Konno, T. Taguchi, T. Tawada, H. Kasai, J. Toda, M. Teresaki, *J. Food Sci.*, 56 (3) (1991) 769–776.

[16] C.-W. Lin, C.-H. Hsieh, H.P. Su, *J. Food Sci.*, 59 (1994) 952–955.

[17] C. Renault, E. Gastaldi, A. Lagaude, J.L. Cuq, B. Tarodo de lalafuente, *J. Food Sci.*, 62 (1997) 907–910.

[18] D. St-Gelais, S. Hache, *Milchwissenschaft*, 50 (1995) 71–75.

[19] C. Tellier, F. Mariette, J.P. Guillement, P. Marchal, *J. Agric. Food Chem.*, 41 (1993) 2259–2266.

[20] Y. Nakao, A. Konno, T. Taguchi, T. Tawada, H. Kasai, J. Toda, M. Teresaki, *J. Food Sci.*, 56 (1991) 769–776.

[21] C. Yook, F. Sosulski, P.R.B. Saskatoon, *Starch/staerke*, 46 (1994) 393–399.

[22] C. Pompei, E. Casiraghi, M. Lucisano, *Milchwissenschaft*, 49 (1994) 562–564.

[23] A. Hirai, H. Odani, A. Nakajima, *Polymer Bull.*, 26 (1991) 87–94.

[24] M.W. Anthonsen, K.M. Vårum, O. Smidsrød, *Carbohydr. Polym.*, 22 (1993) 193–201.

[25] D.W. Van Krevelen, *Properties of Polymers*, Elsevier, Amsterdam, 1990, p. 758.

[26] M. Terbojevich, A. Cosani, G. Conio, E. Marsano, E. Bianchi, *Carbohydr. Res.*, 209 (1991) 251–260.

[27] M. Rinaudo, A. Domard, in G. Skjåk-Bræk, T. Anthonsen, P. Sandford (Eds.), *Chitin and Chitosan*, Elsevier, London, 1989, pp. 71–86.

[28] M.B. Huglin, J.M. Rego, *Polymer*, 32 (1991) 3354–3358.

[29] K. Kataoka, H. Koyo, T. Tsuruta, *Macromolecules*, 28 (1995) 3336–3341.

[30] S. Tsukuda, Y. Inoue, *Carbohydr. Res.*, 88 (1981) 19–38.

[31] F.A. Rutherford, P.R. Austin, in R.A.A. Muzzarelli, E.R. Pariser (Eds.), *Proc. 1st Int. Conf. on Chitin/Chitosan*, 1977, MIT Sea Grant Program Report MITSG 78-7, 1978, pp. 182–192.

[32] P. Debye, E. Huckel, *Physik. Z.*, 24 (1923) 185–206.

[33] M.F. Refojo, *J. Polym. Sci. Part A*-1, 5 (1967) 3103–3113.

[34] D. Harihana, N.A. Peppas, *Polymer*, 37 (1996) 149–161.

[35] L. Brannon-Peppas, N.A. Peppas, *J. Controlled Release*, 16 (1991) 319–330.