ELSEVIER

Preparation and characterization of physical gels and beads from chitin solutions

Elvan Yilmaz[a,*], Murat Bengisu[b]

[a]Department of Chemistry, Eastern Mediterranean University, Famagusta TRNC via Mersin 10, Turkey
[b]Department of Industrial Engineering, Eastern Mediterranean University, Famagusta TRNC via Mersin 10, Turkey

Abstract

A detailed account of physical bulk gel and bead formation from various chitin solutions and nonsolvents is given. Instant gel formation occurs upon contact of chitin solutions in dimethylacetamide (DMAc)/lithium chloride (LiCl) or N-methyl-pyrrolidinone (NMP)/LiCl solvents and nonsolvents such as water, ethanol, or acetone. Ethanol was found to be the optimal nonsolvent for homogeneous spherical bead formation from chitin solutions. Similarly, DMAc-based chitin solutions proved to yield higher quality beads compared to NMP-based solutions. The differences in bead morphology, crystallinity, and thermal degradation are explained in light of the attainment of a balance between attractive hydrogen bonding in the chitin gel network and segment–nonsolvent interactions. The dependence of swelling of chitin gels on pH indicated a maximum of swelling ratio value of 4.3 at pH 11 in aqueous solutions while the equilibrium swelling ratio value of chitin beads formed with ethanol reached a maximum of 2.4. Bulk gels formed under favorable conditions were demonstrated to be recyclable after solvent separation and drying.
© 2003 Elsevier Ltd. All rights reserved.

Keywords: Chitin; Gelation; H-bonding

1. Introduction

Polysaccharides, in general, are capable of forming physical, thermoreversible gels with various solvents. Some examples to gelling polysaccharides are amylose and amylopectin from starch, cellulose derivatives, bacterial polysaccharides such as gellan gum, and marine polysaccharides, such as alginates, carrageenans, and chitin (Clark, 1996). Among gelling polysaccharides, chitin, $(1 \rightarrow 4)$-2-acetamido-2-deoxy-β-D-glucose, is one of the least studied due to its poor solubility. The most abundant polymorphic form of chitin, the α-form, has a tightly compact, highly crystalline structure where the chains are arranged in an anti-parallel way. Intra-molecular and inter-molecular hydrogen bonding exist in the chitin structure through O–H···O and N–H···O linkages (Muzzarelli, 1977). Dimethylacetamide (DMAc)/lithium chloride (LiCl) mixture proved to be a suitable solvent system for

chitin that would allow preparation of chitin solutions (Terbojevich & Cosani, 1997), gels (Bianchi, Marsano, & Tacchino, 1997; Hirano & Horiuchi, 1989; Khor, Wan, Tee, & Hastings, 1997), and fibers (Kumar, 2000). The complex between DMAc and LiCl or N-methyl-pyrrolidinone (NMP) and LiCl dissolves chitin through ion–dipole interactions (Striegel, 1997).

Decreasing the solvent power by destroying the $[DMAc–Li]^+$ or $[NMP–Li]^+$ complex which solubilizes chitin causes gelation of the biopolymer. A three-dimensional network structure is formed at reduced solvent power. The nature of linkages that leads to the formation of the network structure during physical gel formation is not well understood. Several factors may act simultaneously. For example, crystalline microregions may act as crosslinking points or gelation may occur as a result of chain ordering and association (Clark, 1996). The destruction and reorganization of hydrogen bonds are reported to be significant during chitin gel formation (Hirano et al., 1989).

Although the preparation of chitin fibers (Kumar, 2000) and beads (Yusof, Lim, & Khor, 2001) were demonstrated using the nonsolvent coagulation method, gel formation into

* Corresponding author. Tel.: +90-392-630-1086; fax: +90-392-365-1604.
E-mail address: elvan.yilmaz@emu.edu.tr (E. Yilmaz).

film or bulk form is not commonly known or addressed in the literature. Instead, more complicated or time-consuming approaches are generally used, such as solvent evaporation (Khor et al., 1997), thermoreversible gelation (Bianchi et al., 1997), acetylation of chitosan in alcoholic solution (Vachoud, Zydowich, & Domard, 1997), and mixing of chitin solutions with pyridine followed by heating (Hirano & Horiuchi, 1989). In this study, the effect of solvent/ nonsolvent pair and concentration of polymer solution on bulk gel formation and the quality of chitin beads has been investigated. An account of the physical and microstructural properties of chitin beads obtained from different concentrations of DMAc/LiCl and NMP/LiCl solutions on contact with water, ethanol, and acetone is given. Chitin beads formed by this process have potential in biotechnological applications such as drug delivery, wound dressing components, and tissue engineering.

2. Experimental

2.1. Materials

Chitin (Sigma, Germany), DMAc (Aldrich, Germany), NMP (Aldrich, Germany), dimethylformamide (DMF) (Aldrich, Germany), LiCl (Sigma, Germany), LiBr (Sigma, Germany) and NaCl (Aldrich, Germany) were purified as described later. Food grade ethanol (Sema, Northern Cyprus) and analytical grade acetone (Aldrich, Germany) were used as received.

2.2. Purification of chitin

Chitin powder was insoluble as received. Therefore a purification process was applied. Chitin was first treated with 1M NaOH solution at 80 °C for 3 h, neutralized by being washed with water and then digested in 1M HCl solution for 12 h and neutralized again (Mima, Miya, Iwamotoand, & Yoshikawa, 1983). This procedure was repeated twice. Chitin powder recovered was soluble in 5% w/w solution of LiCl in DMAc or NMP.

2.3. Preparation of solvent systems

The solvents DMAc, NMP and DMF were dried for 48 h over molecular sieves (400 Å) activated at 280 °C for at least 4 h. LiCl, LiBr, and NaCl salts were dried at 130 °C. The solvent/salt system was prepared by weighing the salt and adding the solvent such that a 5% w/w solution is obtained. Complete dissolution was possible by stirring the solutions overnight.

2.4. Preparation of chitin solutions

Chitin solutions with concentrations 0.3, 0.5, 1.0 and 1.5% w/v were prepared by dissolving appropriate amounts

of chitin in DMAc/LiCl, and NMP/LiCl solutions. Clear, transparent chitin solutions were obtained after stirring the solutions for at least 48 h at room temperature. The amount of undissolved, swollen material was negligible. Complete dissolution of chitin in DMF/LiCl, DMAc/LiBr, NMP/LiBr, DMF/LiBr, or DMAc/NaCl, NMP/NaCl, DMF/NaCl solutions could not be achieved. Chitin was only partly soluble in these solvent systems. After settling of the suspension, supernatants of these solutions were used for gelation experiments.

2.5. Determination of intrinsic viscosity and molecular weight

Dilute solution viscometry was used to determine the intrinsic viscosity, [η], of chitin in DMAc/LiCl solution at 25 °C. An Ubbelohde type viscometer was used. The solution was filtered through a G4 Gooch crucible before the flow times were measured. Care was taken to avoid the contact of the solution with air as this might cause gelation of the solution. The molecular weight, $\mathbf{M}_v$, was calculated as 3.0×10^5 using the Mark–Houwink equation proposed as (Terbojevich & Cosani, 1997):

$$[\eta] = 2.1 \times 10^{-4} \, \mathbf{M}_v^{0.88} \tag{1}$$

2.6. Determination of the degree of acetylation of chitin

FTIR spectrophotometry was used to determine the degree of acetylation of chitin. FTIR spectra of KBr pellets of chitin were taken using a Mattson 5000 Satellite FTIR spectrophotometer. N-acetylation was determined as 99% from the relationship (Muzzarelli, Rochetti, Stanic, & Weckx, 1997):

$$\%N\text{-acetylation} = \frac{A_{1655}}{A_{3450}} \times 115 \tag{2}$$

where A_{1655} and A_{3450} are the absorbances of amide I band and O–H stretching vibrations of chitin.

2.7. Preparation of chitin gels

Chitin solutions of concentration 0.5, 1.0 and 1.5% w/v were placed in tubes of length 10 cm and diameter 1.2 cm. Chitin gels phase-separated from solution on addition of any one of the nonsolvents, water, ethanol or acetone. A chitin solution:nonsolvent volume ratio of 10:1 was enough to initiate gelation. A 1:1 volume ratio assured complete gelation. Upon addition of sufficient nonsolvent, a gel formed instantaneously with a continuous network spanning the whole sample. Gels thus formed obtained the shape of the test tubes from which they could be easily removed. These gels obtained in this way were freed from any solvent entrapped in the network structure by immersion in water, acetone and

then diethyl ether, replacing each solvent with a fresh sample every 12 h for at least 48 h. Gels were then dried at 60–80 °C in vacuum. Some of the dried gels were purified with acetone, redissolved in DMAc/LiCl or NMP/LiCl, and the solutions were used to test the possibility of repeated gel formation with their original nonsolvents.

2.8. Preparation of Chitin beads

A chitin solution of 0.3, 0.5 or 1.0% in either DMAc/LiCl or NMP/LiCl was taken into a 3 ml plastic transfer pipette and slowly dropped into the nonsolvent that is water, ethanol or acetone. Chitin solution gels in the form of a bead instantaneously. Beads formed by this method were left in the nonsolvent for 24 h to allow solvent exchange. They were then filtered and immersed in the same nonsolvent for another 48 h replacing the nonsolvent every 12 h. The beads were filtered and dried under vacuum at 40 °C to constant weight.

2.9. Thermal analysis

Chitin beads prepared under different conditions were used for thermal analysis. Differential thermal analysis (DTA) was performed with a Perkin Elmer analyzer (model DTA7). Samples were loaded into Pt crucibles and heated at a rate of 10 °C/min under flowing nitrogen. The maximum temperature was 1000 °C. Differential scanning calorimetry (DSC) was conducted with a TA Instruments analyzer (model DSC 2010). Aluminum pans were used and a heating rate of 10 °C/min was applied up to a maximum temperature of 600 °C in air.

2.10. Microstructural analysis

Scanning electron microscopy (SEM) of chitin beads was performed with a Hitachi S-570 scanning electron microscope. A thin Pt layer was coated on the beads for conductivity. Some samples were also analyzed with a Nikon optical microscope.

2.11. X-ray diffraction

X-ray diffraction (XRD) analysis was applied to detect the crystallinity of chitin beads. A Scintag powder diffractometer was used for this purpose between 2θ angles of 5 and 40°. Ni-filtered Cu Kα-radiation was used as the X-ray source. The relative crystallinity of chitin beads was determined from the peaks at the 2θ angle of 19.5° corresponding to the (100) plane (Urbanczyk et al.,1997) and the amorphous fraction (Hermans & Weidinger, 1949). The mass crystallinity was determined from

$$w_c = \frac{I_c}{I_c + KI_a} \tag{3}$$

where I_c and I_a are the intensities of the crystalline and amorphous fractions, and K is a calibration factor which can be set to unity for comparative purposes (Gedde, 1995).

2.12. Swelling behavior

Chitin beads obtained from DMAc/LiCl and NMP/LiCl solutions of chitin gelled in ethanol were soaked in distilled water at room temperature until equilibrium was reached. Bulk chitin gels obtained from DMAc/LiCl solutions mixed with ethanol were soaked in distilled water as well as in hydrochloric acid (pH = 1 and 3) and sodium hydroxide (pH = 9 and 11) solutions. The amount of water absorbed was determined gravimetrically and the swelling ratio, Q, was calculated as follows:

$$Q = \frac{w_s}{w_d} \tag{4}$$

where w_s = weight of swollen material and w_d = weight of dry material.

3. Results and discussion

3.1. Bulk gel formation

The possibility of bulk gel formation through contact of chitin solutions with nonsolvents has not been directly addressed in the literature to our knowledge. Although this is a simple and instant method, other more complicated methods have been preferred (Khor et al., 1997; Vachoud et al., 1997). While chitin fibers and beads were prepared by this process, it was referred to as 'coagulation' in both cases (Kumar, 2000; Yusof et al., 2001).

In order to better understand the nature of the gelation process and the parameters, which have a direct influence on it, a large set of experiments was designed and executed. The parameters that were investigated include the concentration of chitin and types of solvent, salt, and nonsolvent. Table 1 lists these parameters and shows those variables that led to gel formation.

As seen in Table 1, chitin solutions in DMAc/LiCl or NMP/LiCl form instant gels when mixed with water, ethanol, or acetone. Another variable that was analyzed

Table 1
Salt/Solvent/Nonsolvent systems used for gelation experiments

Salt		Solvent		Nonsolvent		Concentratiom% (w/v)	
LiCl	+	DMAc	+	Water	+	1.5	+
NaCl	−	NMP	+	Ethanol	+	1.0	+
LiBr	−	DMF	−	Acetone	+	0.5	+

A successful gelation system should be composed of a salt, solvent and nonsolvent all indicated by +.

was the type of salt in the solution. While DMAc/LiCl and NMP/LiCl solutions were effective solvents for chitin, similar salts such as NaCl and LiBr did not play the same role as LiCl. These results are in agreement with previous studies on various solvent systems for polysaccharides (Striegel, 1997). Limited dissolution of chitin occurred in these alternative solvent systems and no gel formation took place. However, some precipitation occurred in chitin–NMP/LiBr and chitin–DMF/LiBr solutions when mixed with nonsolvents.

The effect of nonsolvent on the quality of chitin beads and the role of the nonsolvent in the gelation process will be discussed in Section 3.2. In terms of bulk gel formation, no significant difference was observed between ethanol and water. Gels formed with acetone, on the other hand, were more opaque and easier to deform. Diethyl ether formed a viscous liquid but not a solid gel. This result implies that the chemical nature and H-bonding capacity of the nonsolvent plays an important role during gelation.

All three concentrations of chitin in DMAc/LiCl solutions resulted in strong gels when mixed with the aforementioned nonsolvents. Future work may be useful in order to find out the lower limit of chitin concentration, which still forms a strong gel for practical use.

In order to discover the possibility of recovering and recycling chitin from the gels that were formed, purified gel samples were vacuum dried at 60–80 °C and tested for dissolution in DMAc/LiCl or NMP/LiCl solution. In general, samples gelled with water as the nonsolvent and dried at 60 °C were only partly dissolved while those that were gelled with acetone dissolved completely. Samples gelled with ethanol and dried at 60 °C usually resulted in better dissolution than those gelled with water but inferior to those gelled with acetone. All samples that were dried at 80 °C, on the other hand, dissolved completely. These solutions formed strong gels when they were mixed with their original nonsolvents for a second time. These results indicate that using the techniques mentioned earlier, the majority of chitin can be recovered from the gels and reused for similar purposes. Purification of the gels and complete drying is necessary for the efficiency of the recycling procedure.

3.2. Gelation and bead morphology

SEM micrographs of beads obtained from DMAc/LiCl solutions of chitin in different nonsolvents are given in Fig. 1(a)–(f). Beads obtained from ethanol, can be observed from Fig. 1(a), (c) and (e), to have a rigid, spherical shape with a smooth outer surface and a low degree of porosity. Some of our preliminary work suggested that better distribution of droplets can be obtained by spraying the solution into the nonsolvent to reduce the possibility of agglomeration. However, dropping the polymer solution into the nonsolvent was preferred in our case since it allowed better control of bead size and uniformity. SEM micrographs reveal that using water as a nonsolvent results in rather irregularly shaped particles, Fig. 1(d) and (f).

It can be observed in Fig. 1(b) that acetone is completely unsatisfactory in a chitin bead forming system. The morphology of the beads obtained in acetone is very similar to that of chitin powder, Fig. 1(g). The differences exhibited in the morphology of the beads formed upon contact of chitin solution with different nonsolvents reveal that the type of nonsolvent plays an important role on the quality of the beads formed. During gelation, the complex between the [DMAc–Li]$^+$ ion and the chitin molecule is broken down through solvation by water, ethanol or acetone molecules. As a consequence of reduced solvent power, chitin molecules phase-separate from solution by forming an associative network through attractive segment–segment interactions namely forming intra- and inter-molecular hydrogen bonding. Physical crosslinks form as a result of hydrogen bonding interactions. Crystalline domains simultaneously develop, which serve as an additional element in network formation. During the gel formation and solvent exchange period, nonsolvent molecules intrude with the degree of intra- and inter-molecular hydrogen bonding among chitin chains. So, a balance between segment–segment, segment–solvent and segment–nonsolvent interactions is needed for successful gelation and bead formation. Among the nonsolvents compared water is the most polar one with the highest hydrogen bonding capacity. The complex between DMAc and Li$^+$ ion is solvated by water molecules very well, resulting in a drastic decrease in solvent power. The polymer tends to phase-separate from the solution quickly. It is possible to observe chitin beads forming very rapidly, swirling on top of water. Having a high hydrogen bonding capacity, water intrudes with intra- and inter-molecular hydrogen bonding of chitin chains during bead formation and solvent exchange periods to an appreciable extent. Phase-separation of chitin chains occurs due to reduced solvent power, but attractive segment–nonsolvent interactions operate instead of repulsive segment–nonsolvent interactions. Lack of repulsive segment–nonsolvent interactions causes deformation of the spherical shape. A balance is established between segment–segment and segment–nonsolvent interactions within the polymer network in the presence of ethanol, which has medium polarity, and medium hydrogen bonding capacity when compared to water and acetone. Intrusion of hydrophilic –OH group of ethanol with the establishment of hydrogen bonding interactions between chitin chains is balanced with the repulsive hydrophobic interaction of the ethyl group with chitin molecules. A network system in which attractive and repulsive forces are balanced leads to most uniform bead formation. Weaker solvation of the [DMAc–Li]$^+$ ion and less powerful destruction of the ion–polymer complex are achieved by acetone which is the least polar nonsolvent used. The carbonyl group of acetone only weakly interacts with –OH and –NH groups of the chitin chain. Repulsive segment–nonsolvent interactions due to two methyl groups on acetone, combined with attractive segment–segment interactions cause total collapse of the polymer with an
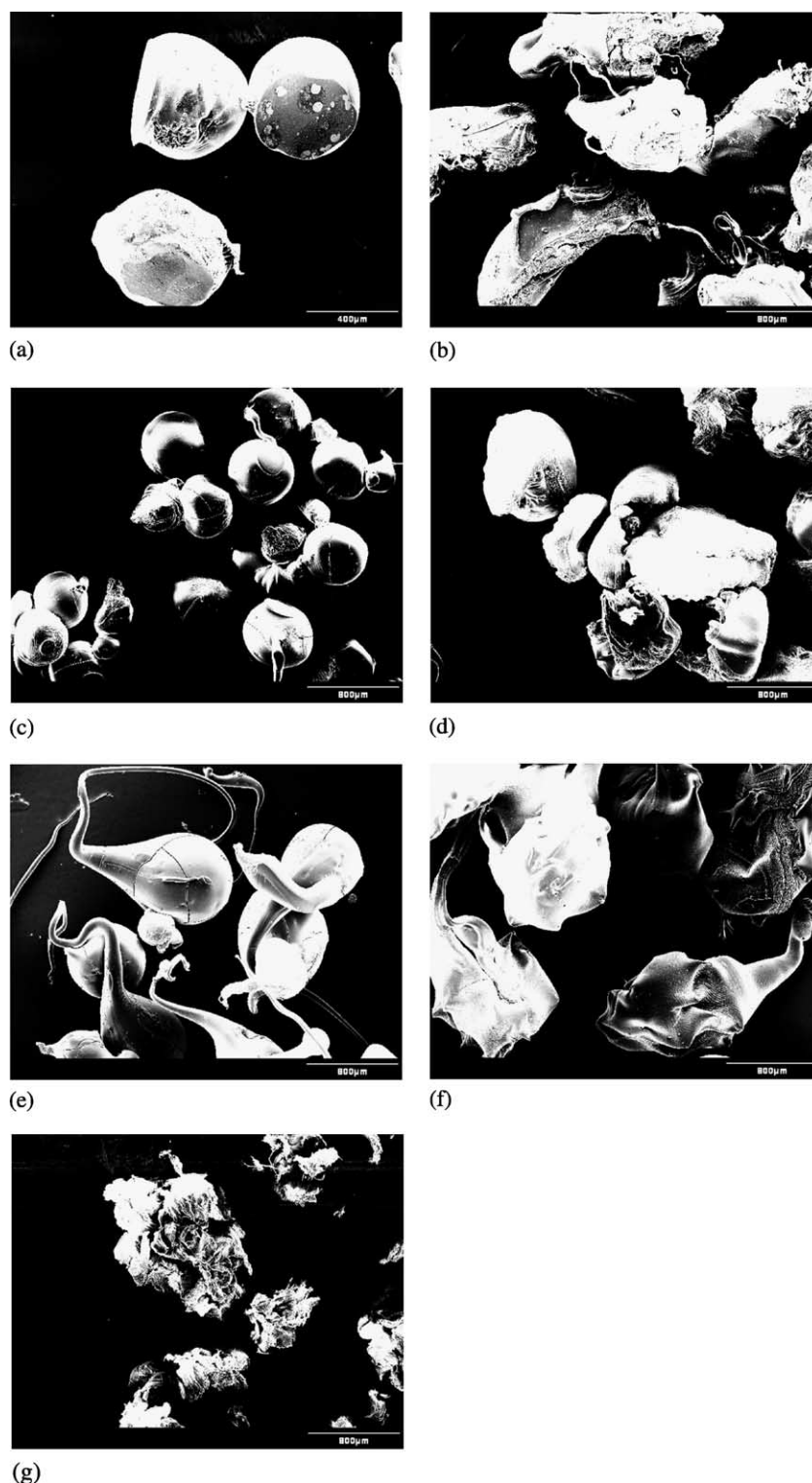
Fig. 1. SEM micrograph of chitin beads of (a) 0.3/DMAc/ethanol (b) 0.3/DMAc/acetone (c) 0.5/DMAc/ethanol (d) 0.5/DMAc/water (e) 1/DMAc/ethanol (f) 1/DMAc/water (g) chitin powder.

irregular shape. The morphology of the beads obtained in acetone is very similar to that of chitin powder, Fig. 1(g).

Beads with the most uniform size and spherical shape are obtained from 0.5% (w/v) chitin solution in ethanol, Fig. 1(c). A lower concentration, namely 0.3% (w/v) results

in a mixture of spherical and irregularly shaped particles, Fig. 1(a). Beads formed from low concentrations of polymer in solution are expected to be weaker. Random collision and contact of spheres causes blending of weaker particles into each other resulting in non-uniform shape. Chitin solution

with 1% (w/v) concentration, on the other hand, is too viscous. This causes the formation of beads with tails on being dropped into ethanol, Fig. 1(e). Cracks may also be observed on the surface of beads obtained from 1% (w/v) solution, Fig. 1(e) and (f). Comparison of various morphologies in Fig. 1(a)–(f) shows that cracking occurs in beads with non-uniform shapes or in agglomerates, as opposed to individual and uniform beads. This indicates that cracking is associated with non-uniform stresses, which develop during drying in non-uniform morphologies.

SEM micrographs of chitin beads obtained from different concentrations chitin/NMP/LiCl solutions in ethanol and water are given in Fig. 2(a)–(e). Especially the beads generated from chitin/NMP/LiCl solutions in ethanol are much less rigid and more irregularly shaped than those obtained from DMAc/LiCl solutions. These observations could be related to the differences in the solvent qualities of NMP/LiCl and DMAc/LiCl towards chitin. The possible contribution of conformational changes to bead formation should not be neglected.

3.3. XRD analysis

Relative mass crystallinities of chitin beads are listed in Table 2. Crystallinities of the beads obtained in either water or ethanol are comparable to each other at any given solution concentration. Beads obtained from chitin solutions of 1 and 0.5% solutions have a relative crystallinity of ∼70%. XRD spectra of the original chitin powder and chitin beads obtained from 0.3% solution of chitin/DMAc/LiCl mixed with acetone, water and ethanol are given in Fig. 3(a)–(d), respectively. XRD analysis showed that the relative crystallinity of the original chitin powder is 83%. The relative crystallinity of the beads generated from 0.3% solutions in ethanol is around 61% and in water 66%. This value increases to 73% when the nonsolvent used is acetone.
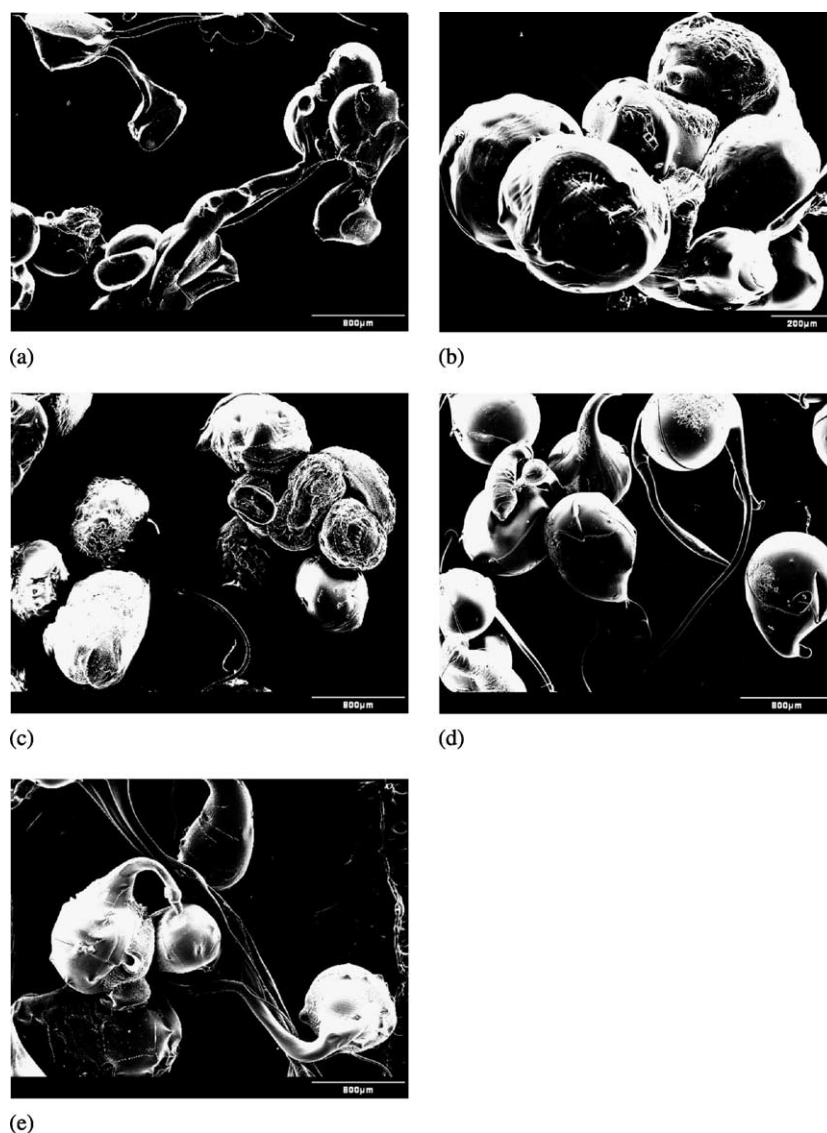

Fig. 2. SEM micrograph of chitin beads of (a) 0.3/NMP/ethanol (b) 0.5/NMP/ethanol (c) 0.5/NMP/water (d) 1/NMP/ethanol (e) 1/NMP/water.

Table 2
Relative mass crystallinity, %w_c, of chitin beads

Bead[a]	% w_c	Bead	% w_c	Bead	% w_c	Bead	% w_c
1/DMAc/Et	72	0.5/DMAc/Et	73	0.3/DMAc/Et	61	0.3/DMAc/Ac	73
1/DMAc/W	72	0.5/DMAc/W	72	0.3/DMAc/W	66	Chitin powder	83
1/NMP/Et	68	0.5/NMP/Et	65	0.3/NMP/Etl	60		
1/NMP/W	70	0.5/NMP/W	72				

Et: ethanol, W: water, Ac: acetone.
[a] Concentration of chitin solution %(w/v)/solvent/nonsolvent.

Acetone having the least hydrogen bonding capacity among the three nonsolvents studied does not interfere to an appreciable extent with the hydrogen bonding interaction among chitin molecules forming the network. Instead, repulsive segment–nonsolvent interactions predominate due to the hydrophobic nature of methyl groups of acetone and hydrophilic nature of −OH groups of chitin as explained above. Hence, ordering of chitin chains can take place without too much intrusion in segment–segment interactions leading to higher crystallinity. Higher probability of segment–segment interactions in more concentrated solutions, namely 0.5 and 1% solutions, results in a greater proportion of crystalline domains. Crystalline regions probably act as junction zones and contribute to the development of a stable network structure. However, a high crystallinity, as in the case of beads obtained in acetone, may be an additional factor disfavoring uniform bead formation due to hindered chain mobility.

3.4. Thermal analysis

DTA curves of chitin beads obtained are given in Figs. 4–7. Three endothermic peaks are observed on these curves. The first peak that appears around 90–100 °C is due to loss of water. The other two peaks, which emerge around 200–260 °C and 300–340 °C, reveal that chitin undergoes thermal decomposition in two stages, the main thermal process taking place around 200–260 °C. Maximum decomposition temperatures of different samples seem to be affected by the preparation conditions of the beads.

Fig. 4 shows the DTA curves of chitin beads obtained from the 0.5% solution of chitin in DMAc/LiCl solution mixed with (a) ethanol, (b) water and (c) acetone. Maximum decomposition temperatures can be observed to be
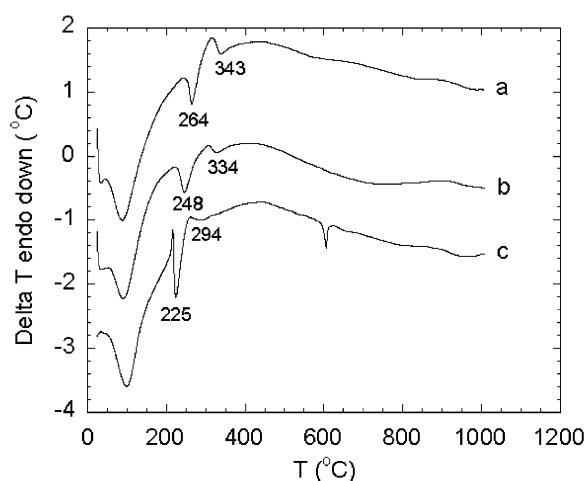

Fig. 4. DTA curve of chitin beads of (a) 0.5/DMAc/ethanol (b) 0.5/DMAc/water (c) 0.3/DMAc/acetone.
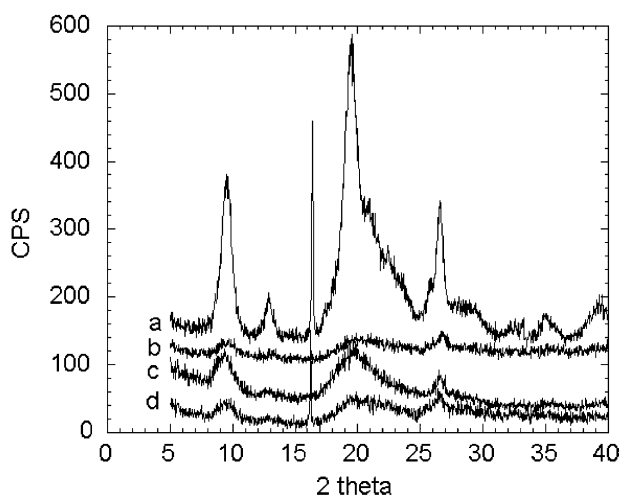

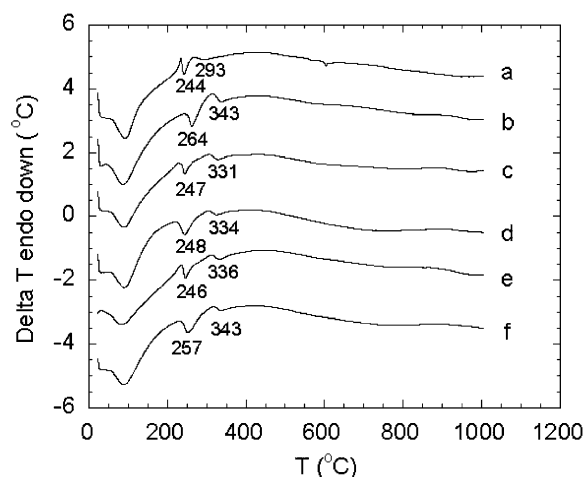Fig. 3. XRD spectrum of (a) chitin powder (b) 0.3/DMAc/acetone (c) 0.3/DMAc/water (d) 0.3/DMAc/ethanol.

Fig. 5. DTA curve of chitin beads (a) 0.5/NMP/ethanol (b) 0.5/DMAc/ethanol (c) 0.5/NMP/water (d) 0.5/DMAc/water (e) 1/NMP/water (f) 1/DMAc/water.

E. Yilmaz, M. Bengisu / Carbohydrate Polymers 54 (2003) 479–488
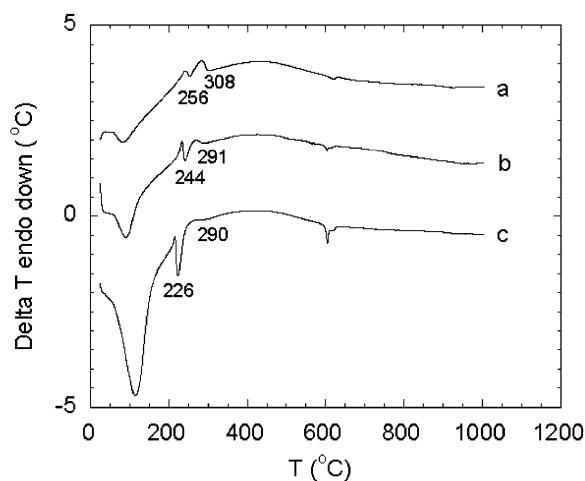

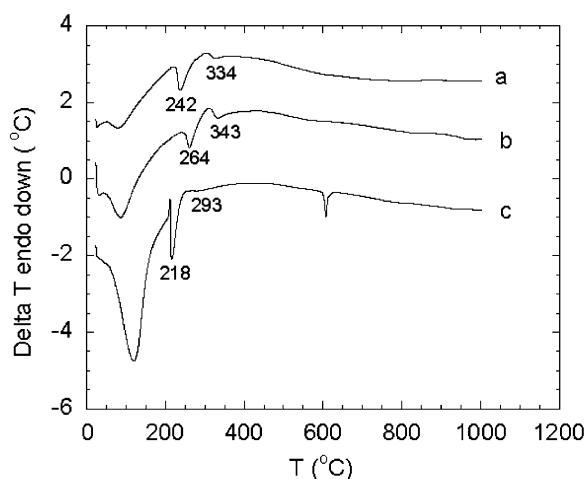Fig. 6. DTA curve of chitin beads (a) 1/NMP/ethanol (b) 0.5/NMP/ethanol (c) 0.3/NMP/ethanol.

Fig. 7. DTA curve chitin beads of (a) 1/DMAc/ethanol (b) 0.5/DMAc/ethanol (c) 0.3/DMAc/ethanol.

the highest for the beads generated in ethanol with 264 and 343 °C. Beads obtained in water result in the two main peaks at 248 and 334 °C, and those obtained from acetone at 225 and 294 °C. This suggests that a well-balanced associative network of chitin chains formed in ethanol gives the beads better thermal stability in addition to uniformity in bead size and shape.

DTA curves of beads obtained from 0.5% solutions of chitin in NMP/LiCl and DMAc/LiCl solutions mixed with ethanol are given in Fig. 5(a) and (b), respectively. DTA curves of the beads from the same solutions mixed with water are shown in Fig. 5(c) and (d), respectively. Finally, DTA curves of the beads generated from 1% chitin–NMP/LiCl and chitin–DMAc/LiCl solutions mixed with water are given in Fig. 5(e) and (f), respectively. The higher thermal stability of DMAc-based beads is a clue in favor of a stronger network structure formed.

The thermal behavior of the beads is affected by the solution concentration as well. The maximum temperatures of thermal decomposition decrease with decreasing concentration of NMP/LiCl solutions of chitin. Smaller polymer concentration causes a looser network to form. This behavior can be followed in DTA curves given in Fig. 6. While beads generated in ethanol from 1% NMP solution have decomposition temperatures of 256 and 308 °C Fig. 6(a), those from 0.5 and 0.3% solutions have peaks at 244, 291 °C, and at 226, 290 °C, respectively, Fig. 6(b) and (c). A similar trend can be observed with chitin beads obtained from the DMAc/LiCl/ethanol system with the exception that the most uniform beads obtained from 0.5% chitin–DMAc/LiCl solution gelled in ethanol still have higher decomposition temperatures than those from the 1% solution, Fig. 7. Decomposition temperatures of the beads from 1, 0.5 and 0.3% solutions of chitin in DMAc/LiCl are 242 and 334; 264 and 343; and 218 and 293, respectively, Fig. 7(a)–(c). It can be observed in Fig. 5–7 that first decomposition temperatures of the beads from DMAc solutions are higher in general. In addition, regardless of concentration or
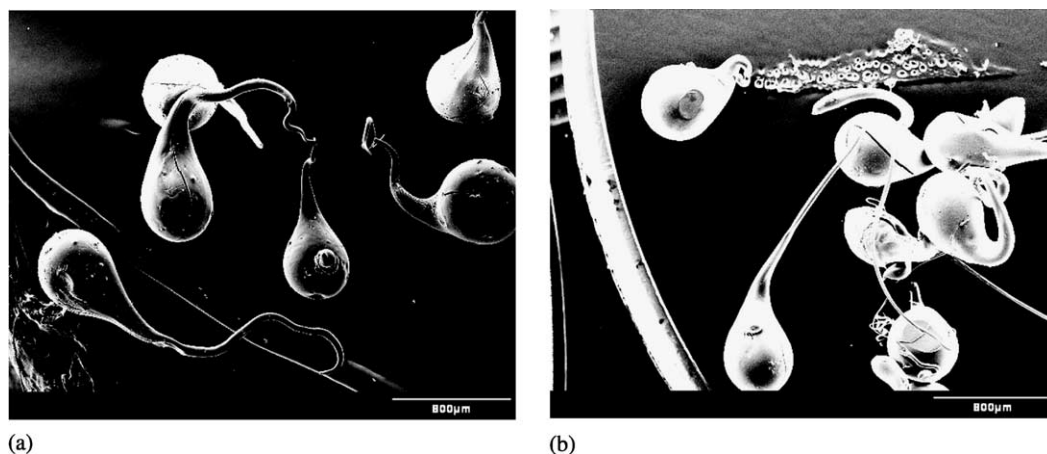

Fig. 8. SEM micrograph of chitin beads from (a) 1/NMP/ethanol and (b) 1/DMAc/water after DTA analysis.
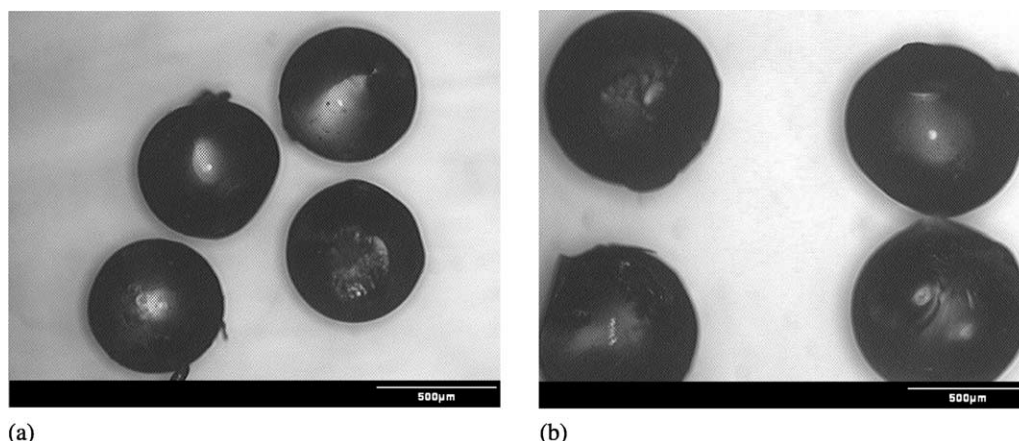
Fig. 9. Optical micrograph of beads from 0.5/DMAc/ethanol (a) before and (b) after swelling.

the type of nonsolvent used, beads formed from DMAc solutions have higher second maximum decomposition temperatures than their analogues formed from NMP solutions. Thermal behavior of chitin gels followed by DTA analysis has been confirmed by DSC analysis as well.

It was observed after DTA analysis that all the chitin beads retained a comparable morphology to their original shapes at temperatures as high as 1000 °C. SEM micrographs indicated that some size reduction occurred (Fig. 2(d) and 8(b), which is due to pyrolysis of chitin into carbonaceous forms (Bengisu & Yilmaz, 2002). In some cases, spherical beads were formed from initially irregular-shaped beads (Fig. 1(f) and 8(a)). More detailed analysis is required to understand the nature of the physical and chemical changes that the beads undergo during thermal treatment.

3.5. Swelling behavior

Optical micrographs of the original, Fig. 9(a), and 24 h-water soaked, Fig. 9(b), chitin beads from 0.5% chitin/DMAc/LiCl-water system show that the beads grow uniformly during water uptake.

Swelling ratios of chitin gel obtained in acidic and basic aqueous media are given in Table 3. The swelling ratio value decreases both with increasing acidity of the medium and with time in acidic medium. This observation may be attributed to the destruction of the physical crosslinks in the acidic medium. Table 4 shows swelling ratios of chitin beads in distilled water. While the bulk gel has a swelling ratio value around 4.0 in distilled water, chitin beads swell less than the bulk gel with swelling ratios changing between 2.1 and 2.4. Beads obtained from 0.3% solutions have somewhat smaller swelling ratio values than those generated from the more concentrated ones, namely 0.5 and 1%. Network elasticity of these beads should be less than the others due to the smaller amount of polymer involved in bead formation, causing the formation of weaker gels.

4. Conclusions

The effect of various factors on bulk gel and bead formation from chitin solutions was determined. Physical gel formation occurs instantaneously upon contact of chitin solutions of DMAc/LiCl or NMP/LiCl with nonsolvents such as water, ethanol or acetone. A pre-requisite for gel formation is the ability of the solvents to efficiently dissolve chitin. Microstructural and thermal studies indicated that DMAc/LiCl is preferable over NMP/LiCl solvent systems for more uniform beads and a stronger gel network. Another important factor is the type of the nonsolvent. Among the nonsolvents studied, ethanol provided optimal results in terms bead morphology while acetone yielded inferior results. Chitin gels formed by nonsolvent coagulation

Table 3
Swelling ratio values of chitin gels

pH	Swelling ratio, (Q)		
	0.25 h	1 h	19 h
1	3.6	3.3	3.3
3	3.6	3.4	3.2
6	4.1	4.0	3.8
9	3.7	3.8	3.8
11	4.5	4.3	4.3

Gels prepared from 1/DMAc/W system.

Table 4
Swelling ratio values of chitin beads in distilled water

Bead[a]	Swelling ratio, (Q)			
	1 h	2 h	4 h	6 h
1/DMAc/Et	2.3	2.3	2.4	2.3
0.5/DMAc/Et	2.2	2.2	2.3	2.3
0.3/DMAc/Et	2.3	2.2	2.1	2.1
1/NMP/Et	2.6	2.4	2.4	2.4
0.5/NMP/Et	2.4	2.4	2.4	2.4
0.3/NMP/Et	2.2	2.1	2.1	2.1

[a] Concentration of chitin solution %(w/v)/solvent/nonsolvent.

method described here can swell up to a swelling ratio of 4 in water. The swelling ratio decreases with increasing acidity of the surrounding medium and with time in acidic media. The possibility of recycling chitin from purified and dried gels was demonstrated. Additionally, chitin beads were shown to retain their original shapes up to relatively high temperatures (1000 °C). Overall, beads and bulk gels formed by the nonsolvent coagulation method have favorable properties that may have technological advantages such as simplicity and versatility of the process, high temperature retention, and recyling capability due to nonchemical (physical) gel formation. Further study is recommended for specific applications. For example, the potential hazard of LiCl, DMAc or NMP remaining in the gel in biotechnological applications is of concern and the possibility of its removal by chemical and thermal treatment deserves detailed investigation. A comparative study on the solution properties of chitin in DMAc/LiCl and NMP/LiCl may provide additional insight on the gelation mechanisms.

References

Bengisu, M., & Yilmaz, E. (2002). Oxidation and pyrolysis of chitosan as a route for carbon fiber derivation. *Carbohydrate Polymers*, *50*(2), 165–175.

Bianchi, E., Marsano, E., & Tacchino, A. (1997). Termoreversible gels of chitin. *Carbohydrate Polymers*, *32*, 23–26.

Clark, A. H. (1996). Properties of biopolymer gels. In C. A. Finch (Ed.), *Industrial water soluble polymers* (pp. 106–130). Cambridge: RSC.

Gedde, U. W. (1995). *Polymer physics*. London: Chapman and Hall.

Hermans, P. H., & Weidinger, A. (1949). Estimation of crystallinity of some polymers from X-ray intensity measurements. *Journal of Polymer Science*, *4*, 709–723.

Hirano, S., & Horiuchi, K. (1989). Chitin gels. *International Journal of Biology and Macromolecules*, *11*, 253–254.

Khor, E., Wan, A. C. A., Tee, C. F., & Hastings, G. W. (1997). Reversible water swellable chitin gel. *Journal of Polymer Science, Part A: Polymer Chemistry*, *35*(10), 2049–2053.

Kumar, M. N. V. R. (2000). A review of chitin and chitosan applications. *Reactive and Functional Polymers*, *46*, 1–27.

Mima, S., Miya, M., Iwamoto, R., & Yoshikawa, S. (1983). Highly deacetylated chitosan and its properties. *Journal of Applied Polymer Science*, *28*, 1909–1917.

Muzzarelli, R. A. A. (1977). *Chitin*. Oxford: Pergamon Press.

Muzzarelli, R. A. A., Rochetti, R., Stanic, V., & Weckx, M. (1997). Methods for the determination of the degree of acetylation of chitin and chitosan. In R. A. A. Muzzarelli, & M. G. Peter (Eds.), *Chitin handbook* (pp. 109–119). Italy: Atec.

Striegel, A. M. (1997). Theory and applications of DMAc/LiCl in the analysis of polysachharides. *Carbohydrate Polymer*, *34*, 267–274.

Terbojevich, M., & Cosani, A. (1997). Molecular weight determination of chitin and chitosan. In R. A. A. Muzzarelli, & M. G. Peter (Eds.), *Chitin handbook* (pp. 87–101). Italy: Atec.

Urbanczyk, G., Lipp-Symonowicz, B., Szosland, I., Jeziorny, A., Urbaniak-Domagala, W., Dorau, K., Wrzosek, H., Sztajnowski, S., Kowalska, S., & Sztajnert, E. (1997). Chitin filaments from dibutyrylchitin precursor: Fine structure and physical and physicochemical properties. *Journal of Applied Polymer Science*, *65*(4), 807–819.

Vachoud, L., Zydowich, N., & Domard, A. (1997). Formation and characterization of a physical chitin gel. *Carbohydrate Research*, *302*, 169–177.

Yusof, N. L. B. M., Lim, L. Y., & Khor, E. (2001). Preparation and characterization of chitin beads as a wound dressing precursor. *Journal of Biomedical Material Research*, *54*, 59–68.