JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE **12** (2001) 861–864

Chitosan-based hydrogels: Synthesis and characterization

A. BORZACCHIELLO[1], L. AMBROSIO[1], P. A. NETTI[1], L. NICOLAIS[1], C. PENICHE[2], A. GALLARDO[2], J. SAN ROMAN[2]
[1]*Institute of Composite Materials Technology-CNR and CRIB, University of Naples ''Federico II'', P.le Tecchio 80, 80125, Naples, Italy*
[2]*Ist. di Cienca y Tecnologia de Polimeros CSIC, Juan de la Cierva 3, 28006, Madrid, Spain*

Chitosan (CHI) is a polysaccharide of β-1,4-linked 2-amino-2-deoxy-D-glucopyranose derived by N-deacetylation of chitin in aqueous alkaline medium. The shells of crustaceans such as crabs, shrimp, and lobster are the current source of chitosan. It is known to be non-toxic, odourless, biocompatible in animal tissues and enzymatically biodegradable. For these reasons much research interest has been paid to its biomedical, ecological, and industrial applications over the past decade. However, its rigid crystalline structure, poor solubility in organic solvents and poor processability have limited its use. To broadening its range of applications, a growth research effort has been devoted to explore ways of modifying Chitosan. Here it has been reported on the synthesis of new hydrogels, obtained by self-curing chitosan with acrylic acid (AA) and methyl acrylate (MA). The hydrogels were characterized by FTIR, swelling and rheological analysis. The results of this study showed that the swelling and mechanical properties of chitosan are highly improved by the presence of poly acrylate. The swelling degree of these materials does not depend upon the ratio MA/AA. It is possible to improve and modulate the mechanical properties of the hydrogels by changing the relative MA/AA ratio.

Introduction

Chitosan (CHI) is a polymer of β-1,4-linked 2-amino-2-deoxy-D-glucopyranose derived by N-deacetylation of chitin in aqueous alkaline medium. The shells of crustaceans such as crabs, shrimp, and lobster are the current source of chitosan. It is known to be non-toxic, odorless, biocompatible in animal tissues and enzymatically biodegradable. For these reasons much research interest has been paid to its biomedical, ecological, and industrial applications over the past decade [1]. Chitosan was first used industrially as a cationic natural flocculent for waste-water treatment [2]. In the biomedical field it is known for its anti-hyperlipidemic and anti hypercholesterolemic properties, assisting in weight control and wound healing. Under physiological conditions, chitosan can immobilise at least 4–6 times its weight in lipids [3].

However, its rigid crystalline structure, poor solubility in organic solvents and poor processability have limited its use. To broadening its range of applications, a growing research effort has been devoted to explore ways of modifying Chitosan [1, 2]. To this end CHI has been alkylated, acylated, carboxyalkylated [4–8]. Interpenetrating polymer network membranes obtained by crosslinking chitosan with synthetic polymers, such as poly-(acrylic acid) [9], or biopolymers, such as gelatin [10, 11], have been widely studied . Blend membranes of chitosan and acrylic- and vinyl-polymers, forming inter-polymer complexes, have been produced [12]. The interpolymer complexation of chitosan and poly-(acrylic acid) (PAA) is widely described in literature [9, 12, 13]. One step forward has been done by polymerizing acrylic acid (AA) in the presence of chitosan and obtaining interpenetrating networks of chitosan and PAA with improved absorbing properties [14].

Following the lines of improving chitosan-based materials properties the synthesis of new hydrogels, obtained by self-curing chitosan with AA and methyl acrylate (MA) is reported. The hydrogels were characterized from a physico-chemical and mechanical point of view.

Experimental

Materials

Chitosan (D.D. $= 79.9\%$ determined by ^{1}H-nmr, $M_v = 2 \times 10^5$) was obtained from shells of lobsters (*Panulirus argus*) as described elsewhere [15]. Chitosan flakes were dissolved in aqueous acetic acid, filtered and precipitated with aqueous NaOH. The precipitated gel was washed several times with water and vacuum dried in a desiccator. The white chitosan foam thus obtained was stored in a closed flask until used.

Acrylic acid (MERCK) and methyl acrylate (ALDRICH) were distilled before use. Ammonium persulfate (ALDRICH) was used without further purification.

TABLE I Relative composition of chitosan/acrylic acid/methyl acrylate reaction mixtures

Sample	CHI (wt %)	AA (wt %)	MA (wt %)	MA/AA
M0	15.0	85.0	0	0/100
M10	15.0	76.5	8.5	10/90
M20	15.0	68.0	17.0	20/80
M30	15.0	59.5	25.5	30/70

Preparation of membranes

Chitosan powder was dispersed in water containing 1.0 wt % ammonium persulfate. The amount of water was always equal to the weight of monomer mixture to be added. Acrylic acid containing the desired amount of methyl acrylate was added with agitation. The paste-like mixture was placed in a closed flask and maintained at 37 °C for 15 h, then it was placed at 50 °C for another 3 h. The resulting material was swollen in distilled water at 37 °C for another 3 h to remove any soluble material. During this time water was changed periodically. Finally it was washed for 48 h with 5 wt % aqueous NaOH solution. During this time the solution was changed at various times.

The solution was filtered off using a small mesh polyester fabric and the polymer washed thoroughly with distilled water. During this stage the polymer swells considerably and the washings were continued until the pH of the filtered water became neutral. Afterwards the sample was lyophilized within a week.

The relative composition of chitosan (CHI), acrylic acid (AA) and methyl acrylate (MA) in the reacting mixture is shown in Table I.

Methods

IR spectra

FTIR spectra were recorded on a Nicolet 520 spectrometer. Spectra were taken with a resolution of $2\,\mathrm{cm}^{-1}$ and were averaged over 120 scans. Samples were thoroughly grounded with exhaustively dried KBr and discs were prepared by compression under vacuum.

Swelling tests

Swelling behavior of chitosan-based hydrogels was studied in double distilled water at 25 °C. The degree of swelling (SW) for each sample was calculated from the following expression:

$$SW = (W_s - W_d)/W_d$$

where W_s and W_d are the weight of the samples at the equilibrium swelling state and dry state, respectively. The dry state was obtained by placing the lyophylizated samples under vacuum overnight.

In practice, an established amount of dry polymers was closed in small bags constituted of an hydrophobic water permeable material and immersed in double distilled water. The bags containing the swollen gels were weighed at different time intervals until equilibrium swelling was reached; the exact values of W_s were calculated as difference with the weight of the bags.

Rheological tests

The rheological characterization was performed on the hydrogels, prepared by adding double distilled water to the different lyophilized product in order to obtain concentrations of 10, 15 and $20\,\mathrm{mg\,ml}^{-1}$, and using a strain controlled rheometer (Bohlin VOR Rheometer, Bohlin Reologi A B, Lund, Sweden) at controlled temperature of 25 °C. The geometry used were plate and plate (PP 30 cell). Small-amplitude oscillatory shear experiments were performed to measure the unsteady response of the samples and hence the determination of their linear viscoelastic properties.

In dynamic experiment the material is subjected to a sinusoidal shear strain:

$$\gamma = \gamma_0 \sin(\omega t)$$

where γ_0 is the shear strain amplitude, ω is the oscillation frequency (which can be also expressed as $2\pi f$ where f is the frequency in Hz) and t the time. The mechanical response, expressed as shear stress τ of viscoelastic materials, is intermediate between an ideal pure elastic solid (obeying to the Hooke's law) and an ideal pure viscous fluid (obeying to the Newton's law) and therefore is out of phase respect to the imposed deformation as expressed by:

$$\tau = G'(\omega)\gamma_0 \sin(\omega t) + G''(\omega)\gamma_0 \cos(\omega t)$$

where $G'(\omega)$ is the shear storage modulus and $G''(\omega)$ is the shear loss modulus. G' gives information about the elasticity or the energy stored in the material during deformation, whereas G'' describes the viscous character or the energy dissipated as heat [16].

Strain sweep tests at a fixed oscillation frequency (consisting in monitoring the viscoelastic properties while logarithmically varying the strain amplitude γ_0) were performed on the materials to determine the strain amplitude at which linear viscoelasticity is valid.

Results and discussion

IR spectra

The IR spectra of the samples are shown in Fig. 1. The strong absorption bands at 1560, 1481, 1408, 1325 and $514\,\mathrm{cm}^{-1}$ are typical of sodium polyacrylate. Absorption bands at $1660\,\mathrm{cm}^{-1}$ (Amide I) and at the 1200–$1000\,\mathrm{cm}^{-1}$ region are assigned to polysaccharide. Chitosan also contributes to the absorption at $1595\,\mathrm{cm}^{-1}$ ($-NH_2$ bending) and $1314\,\mathrm{cm}^{-1}$ (Amide III). Polymethylacrylate contributes to the absorption bands at 1449, 1379 and $1333\,\mathrm{cm}^{-1}$.

Since the absorption bands at $1560\,\mathrm{cm}^{-1}$ (A_{1560}) and $514\,\mathrm{cm}^{-1}$ (A_{514}) are mostly due to polyacrylic acid and the absorption band at $1073\,\mathrm{cm}^{-1}$ is originated by the presence of chitosan, the absorbance ratios A_{1560}/A_{1073} and A_{514}/A_{1073} should be indicative of the acrylate/chitosan content of the polymers. It can be observed in Fig. 2 that there is an almost linear correspondence between both absorbance ratios and the initial AA content in the reacting mixture, indicating that the composition of the polymers follow the same trend as the composition of the starting monomer/chitosan mixture.

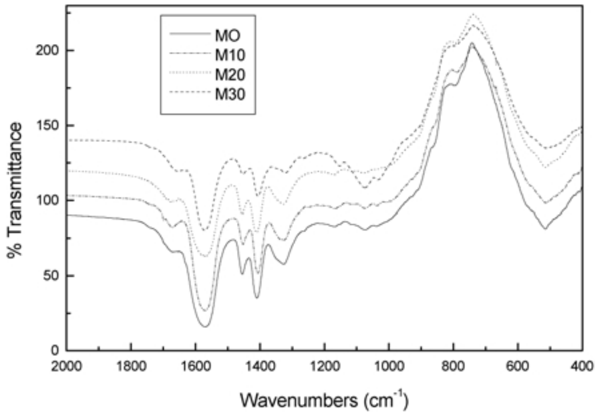

Figure 1 FTIR spectra of the chitosan based materials.

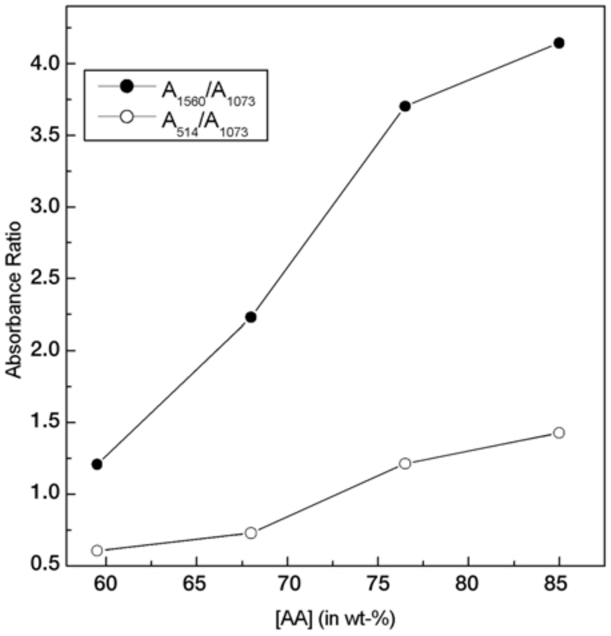

Figure 2 A_{1560}/A_{1073} and A_{514}/A_{1073} dependence on acrylic acid feed-weight composition.

Swelling tests

The degrees of swelling of the chitosan-based materials in double distilled water at 25 °C are reported in Table II. These materials absorb up to 500 times of their dry weight and can be classified as super-absorbents. The absorbing capability of chitosan [13, 17] is highly improved by the presence of polyacrylate. It is worth to pointing out, that the swelling degree of these materials does not depend upon the MA/AA ratio.

Rheological tests

The small-amplitude oscillatory shear experiments were performed to evaluate the viscous (G'') and elastic (G') responses of chitosan based materials and their dependence upon the concentration and upon the materials composition.

TABLE II Degree of swelling of chitosan-based materials in double distilled water ($T = 25$ °C)

Sample	SW
M0	490
M10	550
M20	550
M30	560

Fig. 3 shows the mechanical spectrum of the sample without methyl acrylate, sample M30 (CHI/AA/MA, 15/85/0) at 20 mg ml^{-1}. The dynamic elastic modulus G' is higher than the viscous modulus G'' within the frequency range investigated (0.1–10 Hz) and both moduli show some slight increase at higher frequency. These results indicate these materials behave as solid-like materials and their rheological behavior is typical of ''weak gels'' [18, 19]. The rheological response is due to the contributions of crosslinks, mainly due to entanglements formed by free polymer chains, physical interactions such as hydrogen and electrostatic bonding. The existence of specific interactions, salt bridge NH_3^+ ^-OOC, between the amino group of the amino group of the chitosan and the carboxylic group of the PAA has been reported in literature [9, 12, 13]. The intermolecular ionic bonds also can be converted in NH–CO amide bonds by dehydration of the PAA–chitosan complex membrane. The presence of crosslinks bring about a reduction of intrinsic mobility of the polymer chains, then an increase in the relaxation times and consequently at short times, the materials show an elastic behavior. Both moduli increase as the concentration increases, doubling the concentration, the elastic modulus at 1 Hz changes from 16 to 43 Pa.

The introduction of methyl acrylate in the materials leads to a change of the mechanical properties. From the mechanical spectrum of M30 at 20 mg ml^{-1} (feed-weight composition: CHI/AA/MA, 15/59.5/25.5), reported in Fig. 4, is evident that the elastic modulus G' shows almost no dependence with frequency, and is about one order of magnitude greater than the viscous modulus. Those features are characteristic of a ''strong'' rather than a ''weak'' gel [20]. Fig. 5, in which is reported the elastic modulus frequency dependence of all the samples at 20 mg ml^{-1}, shows that the elastic modulus MA containing samples is one order of magnitude higher than that of M30. Contrary on what reported in literature [21], here the increase in mechanical modulus (Table III) is not associated to a

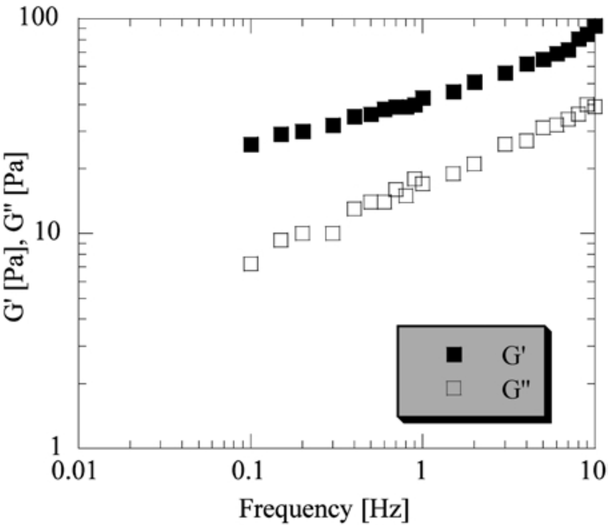

Figure 3 Mechanical spectrum of M0 (feed-weight composition: CHI/AA/MA, 15/85/0) at 20 mg ml^{-1}, $T = 25$ °C.

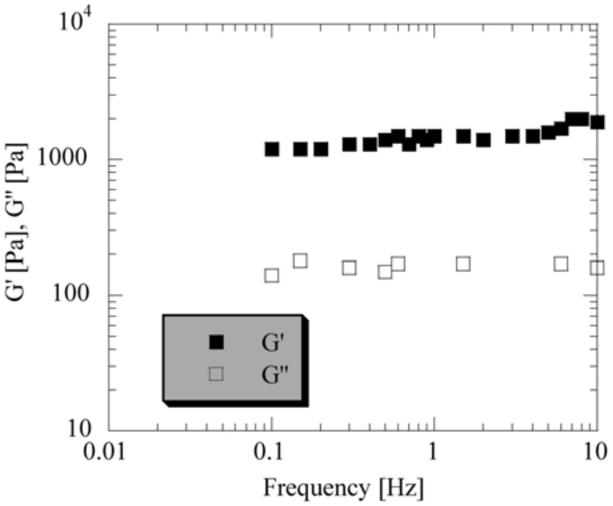

Figure 4 Mechanical spectrum of M30 (feed-weight composition: CHI/AA/MA, 15/59.5/25.5) at 20 mg ml^{-1}, $T = 25\,°C$.

reduction of water content as all samples swelling degree does not vary significantly (Table II). Therefore the enhancement in mechanical stiffness of the gel richer in MA may be due to an increase of chain stiffness brought about more hydrophobic intra-chain interactions promoted by methyl acrylate groups. Further studies are ongoing to test this hypothesis. The results obtained so far, show that by modulating the inter and intra-molecular interaction, it is potentially possible to enhance the mechanical properties while retaining the swelling properties. The dynamic moduli increase as the concentration increase (Table III).

TABLE III Elastic modulus of chitosan-based materials at $f = 1$ Hz and $T = 25\,°C$

Sample	10 mg/ml	15 mg/ml	20 mg/ml
M0	16	28	43
M10	360	430	590
M20	490	620	980
M30	670	880	1500

Conclusions

New hydrogels based on chitosan have been synthesized. They were obtained by self-curing chitosan with acrylic acid (AA) and methyl acrylate (MA). The hydrogels were characterized by FTIR, swelling and rheological analysis. The results of this study showed that the swelling and mechanical properties of chitosan are highly improved by the presence of polyacrylate. The swelling degree of these materials does not depend upon the ratio MA/AA. It is possible to improve and modulate the mechanical properties of the hydrogels by changing the MA/AA ratio.

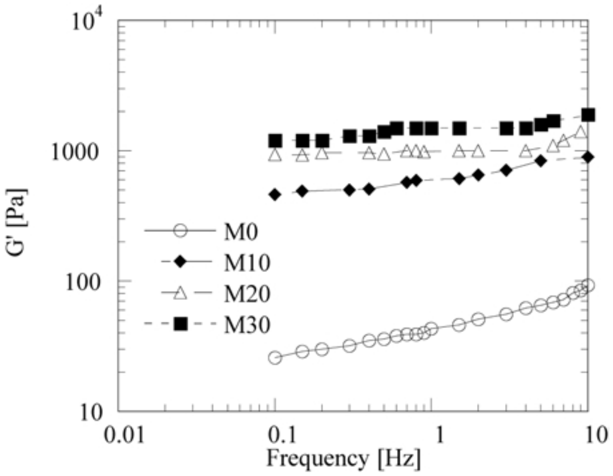

Figure 5 Elastic modulus frequency dependence of chitosan-based materials at 20 mg ml^{-1}, $T = 25\,°C$.

References

1. Z. ZONG, Y. KIMURA, M. TAKAHASHI and H. YAMANE, *Polymer* **41** (2000) 899.
2. S. HIRANO, *Polym. Int.* **48** (199) 732.
3. K. DEUCHI, *Biosci., Biotechnol. Biochem.* **58** (1994) 1613.
4. L. D. HALL and M. YALPANI, *J. Chem. Soc. Chem. Commun.* (1980) 1153.
5. M. YALPANI and L. D. HALL, *Macromolecules* **17** (1984) 272.
6. S. HIRANO, Y. OHE and H. ONO, *Carbohydr. Res.* **47** (1976) 315.
7. G. K. MOORE and A. F. GOERGE, *Int. J. Biol. Macromol.* **3** (1981) 292.
8. R. A. A. MUZZARELLI and F. TANFANI, *Pure Appl. Chem.* **54** (1982) 2141.
9. H. WANG, W. LI, Y. LU and Z. WANG, *J. Appl. Polym. Sci.* **65** (1997) 1445.
10. F. SHEN, Y. L. CUI, L. F. YANG, K. D. YAO, X. H. DONG, W. Y. JIA and H. D. SHI, *Polym. Int.* **49** (2000) 1596.
11. Y. J. YIN, K. D. YAO, G. X. CHENG and M. JB, *ibid.* **48** (1999) 429.
12. H. WANG, W. LI, Y. LU and Z. WANG, *J. Appl. Polym. Sci.* **61** (1996) 2221.
13. C. PENICHE, C. ELVIRA and J. SAN ROMAN, *Polymer* **39** (1998) 6549.
14. C. PENICHE, W. ARGÜELLES-MONAL, N. DAVIDENKO, R. SASTRE, A. GALLARDO and J. SAN ROMAN, *Biomaterials* **20** (1999) 1869.
15. W. ARGÜELLES and C. PENICHE, *Macromol. Chem. Rapid Commun.* **9** (1988) 693.
16. J. D. FERRY, ''Viscoelastic Properties of Polymers'' (Wiley, New York, 1970).
17. Y. M. LEE, S. S. KIM and S. H. KIM, *J. Mater. Sci: Mater. in Med.* **8** (1997) 537.
18. S. B. ROSS-MURPHY in ''Polymer Gels. Fundamentals and Biomedical Applications'', edited by D. De Rossi, K. Kajiwara, Y. Osada and A. Yamauchi (Plenum Press, New York, 1991) p. 21.
19. A. H. CLARK and S. B. ROSS-MURPHY, *Adv. Polym. Sci.* **83** (1987) 61.
20. S. B. ROSS-MURPHY and K. P. SHATWELL, *Biorheology* **30** (1983) 217.
21. H. JIANG, W. SU, P. T. MATHER and T. J. BUNNING, *Polymer* **40** (1999) 4593.

Received 14 May
and accepted 24 May 2001