Conference Paper No: PF-02

Effect of chitosan concentration on gelation of chitosan/ nanohydroxyapatite/ Na_2CO_3 composite

P. S. D. Perera and S. U. Adikary*

Department of Materials Science and Engineering,
Faculty of Engineering, University of Moratuwa, Sri Lanka
suadi@uom.lk*

Abstract

An injectable in situ forming chitosan/nanohydroxyapatite/Na_2CO_3 (chitosan/nHA/Na_2CO_3) composite gel was investigated to study its gelation time. This study used locally synthesized chitosan as the polymer material to synthesis chitosan thermosensitive gel. The sol-gel phase transition was primarily examined. We found that chitosan/nHA/Na_2CO_3 sol with 1.6% of chitosan has required coagulant performance. The lower concentration of chitosan had reported longer gelation behavior while the higher concentration of chitosan exhibited unstable behavior. The surface morphology of the chitosan/nHA/Na_2CO_3 composite depicts the structure of polymer gel composite. Scanning electron microscope confirmed the presence of hydroxyapatite on the chitosan matrix, indicating chitosan/nHA polymer gel composite was a suitable polymer substrate for injectable gel systems. EDX spectra shows the Ca/P value of the synthesized nano-hydroxyapatite to be 1.67. It was confirming the presence of nanohydroxyapatite on the composite. The kinetic study of this gelation process will make it possible to adjust the properties of the chitosan/nHA/Na_2CO_3 composite gel. The gelation temperature was carried out at body temperature (37 ^{0}C).

Keywords: Chitosan, Chitosan/nHA/Na_2CO_3 composite, nanohydroxyapatite.

Introduction

There has been a noticeable increment towards the improvement and use of polymer hydrogel biomaterials for the repairing and re-establishment of damaged bone tissue due to their biocompatibility, osteoconductivity, biodegradability, injectability and enhanced bone regeneration ability[1]. These polymer hydrogels can be used as growth ingredients to target tissues through injection. These hydrogels are liquid state at the time of injection and turn into a gel at the body temperature and pH. Therefore these hydrogels have the ability to restore bone defects and form the required shape[2]. The formulations based on chitosan can be held liquid at room temperature and turn into a hydrogel in situ at body temperature in a short period.

Natural bone is a complex with hydroxyapatite while hydroxyapatite salts are well-known biological agents. Hydroxyapatite (HA) is a mineral form of calcium apatite with the formula $Ca_5(PO4)_3(OH)$, present in bone and teeth. Bone is made mostly of HA crystals deposited in a collagen matrix. Hence 65 to 70% of the mass of bone is HA[13]. Many researchers have reported that chitosan hydroxyapatite composites possess a good bonding ability with surrounding tissues. The present approach is to synthesize chitosan/hydroxyapatite composite materials using mechanical mixing. The arrangement

of polymer solutions with hydroxyapatite can improve the mechanical properties of the gel system.

Chitosan ((1-4)-2-amino-β-D-glucose) was synthesized by the deacetylation of chitin, which is naturally found in the exoskeleton of shellfish, including crab, lobster, and shrimp [3]. The non-polar behaviour of chitosan is a major disadvantage in the industrial approach [7]. It is slightly soluble in organic acids [8] and only dissolves in acidic conditions because the positively charged amino groups available in the chitosan have been increased. Therefore, the polysaccharide chains repel each other to accomplish stability by disbanding [8].

This study aims to develop an injectable biopolymer using chitosan/nanohydroxyapatite composite coagulated by Na_2CO_3. The nano-hydroxyapatite and chitosan are known as the primary aggregates of the system. Therefore, the objective of this work is making new formulations combining calcium phosphate compounds with chitosan, to avoid the longer gelation time, frequent migration of bioceramic particles(hydroxyapatite) from the implant site, reducing potential damage to soft tissue in the vicinity of the implant and improving biodegradability, biocompatibility, curing properties and injectability. Hence, we started to evaluate the effect of chitosan concentration as our first step and the ability of using chitosan/nHA/Na_2CO_3 composite for biomedical applications. This proposed gel is expecting to work under physiological pH and it is remaining as a solution during injection and it turns into gel at body temperature for short time.

Methodology/materials and methods

Chitosan and nanohydroxyapatite was synthesized by the method developed by Adikary et al. The gel mixtures were made mixing Na_2CO_3 with chitosan and nano-hydroxyapatite with several concentrations.

The chitosan–hydroxyapatite solution with a concentration of 1.2% to 2.0% were prepared dissolving chitosan (1.8g) into 6% acetic acid (50ml) and hydroxyapatite(1.5g) into 6% acetic acid (50 ml) with stirring for 5 minutes to get a perfectly transparent solution at the temperature of 37^0C and prepared Na_2CO_3 (2.4g) in 6% acetic acid solution(50ml) will be mixing at 37^0C. Afterwards, the experimental mixtures will be stirring gently to yield an elastic, ideal, and thermosensitive chitosan gel. Repeating the above process by changing the initial concentrations of aggregates as followed by Table 1. To investigate the time-dependent mechanical properties of the gel, chitosan/nanohydroxyapatite samples were placed in a beaker together with Na_2CO_3 solution. When the reagent was added, the viscosity was immediately determined with a commercial viscometer (Brookfield LVDV-II digital viscometer with the LV-4 spindle, at 12 rpm, Viscosity data were logged every other second for at least 15 min.

Table 1. *Chitosan/nHA/Na_2CO_3 process parameters.*

Reference number	% of chitosan	Chitosan/g	HA/g	Na_2CO_3/g	Acetic acid/ml
1	1.2	1.8	1.5	2.4	150
2	1.4	2.1	1.5	2.4	150
3	1.6	2.4	1.5	2.4	150
4	1.8	2.7	1.5	2.4	150
5	2.0	3.0	1.5	2.4	150

Gel morphology

The morphology of the chitosan/nHA/Na_2CO_3 gel was examined by scanning electron microscope(SEM). The gel was placed in sterilized petri dish and placed vacuum oven at 37 ^{0}C for 48 h for complete dry. The dried gels were cut with a sharp blade to expose the internal microstructure, placed on double sided tape, and sputter-coated with gold for SEM at 5.0 kV by using a Carl Zeiss ECO D8 scanning electron microscope. The surface and longitudinal sections were observed.

FTIR Spectroscopic analysis

The structural changes in chitosan (A) and the complex of chitosan/nanohydroxyapatite/sodium carbonate(B) were characterized by Fourier transformed infrared (FTIR) spectroscopy (Bruker Alpha-T) in the range of 1000 to 4000 per cm.

Results and Discussion

I. Gelation time

The gelation time (t_{gel}) was determined by the rheological measurements of the final mixture. The t_{gel} has been determined from dynamic viscosity slope experiments. The time associated with the t_{gel} is depicted by a dramatic increase in the complex viscosity. The chemical reaction influences the molecular structure of polymers. Furthermore, rheological properties of polymers depend on the molecular mobility.

In our work, the final concentration of chitosan was always above 1% (w/v), which is more than the critical concentration of chitosan that Boucard et al had proved. Figure 1 shows the average gelation time for chitosan/nHA/Na_2CO_3 hydrogels formed by using Na_2CO_3 as an initiator. The range of chitosan concentrations tested was 1.2–2.0%. Experimental observation proves that a concentration of 1.6% produced the best solid gel. The control sample with 0% Na_2CO_3 did not undergo a sol-gel phase change and remained as a solution until the end of the observation. The concentration and amount of acetic acid solutions in all groups were unchanged.

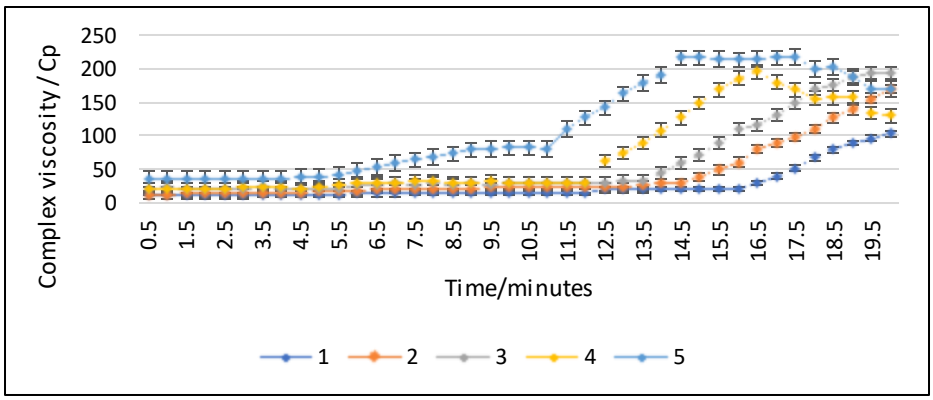

Figure 1. *The graphical represent of complex viscosity vs time in linear viscoelastic regime.*

To observe the sol-gel phase change behaviour of chitosan solutions at physiological body temperature, we measured the gelation time a 37 ^{0}C by the rheological method. Figure 1 proves the average gelation time of chitosan hydrogel at 1.6% of chitosan concentration and a hydrogel was formed in approximately 750 sec.

Table 2. *Chitosan/nHA/Na_2CO_3 gelation parameters.*

Ref. number.	Concentration of chitosan/ww	Average Gelation time/ seconds
1	1.2	930
2	1.4	835
3	1.6	750
4	1.8	645
5	2.0	510

Ref. number 1 and 2 describe the viscosity change of the resulting system versus time. This behavior depicts the degradation and cyclic behavior of polymer composite system across sol-gels. Samples 3,4 and 5 depict the viscosity nearly constant through the experiment.

II. Gel morphology

The examination of the morphology of chitosan/nHA/Na_2CO_3 composite by SEM to determine the typical structure of the gels also because of the gelation mechanism. Fig. 2 show a SEM micrograph of the chitosan/nHA/Na_2CO_3gel after removal of water.
It is evident that nanoparticles of hydroxyapatite, some of which were approximately spherical in shape. These may be nano-hydroxyapatite particles formed in situ. Tiny mineral particles such as hydroxyapatite-coated polymer gel substrate provide the initial matrix, which is useful in the development of injectable gels for bone tissue engineering applications.

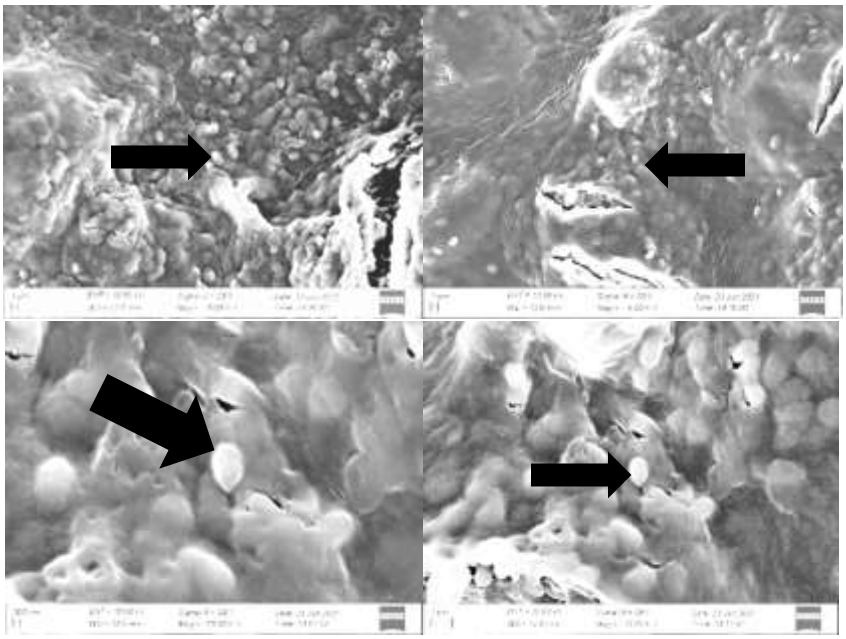

Figure 2. *SEM images of the cross-sectional morphologies of oven dried chitosan/nHA/Na_2CO_3 composite with 1.6% chitosan. Magnifications are 5.0 K x, 5.0 K x, 25.00 K x, 15.00 K x. The arrows indicate the possible hydroxyapatite nanoparticles.*

In Figure 3, the EDX spectra recorded on the examined chitosan/nHA/Na_2CO_3 are shown below. The presented spectrum proves the Ca/P value of the synthesized nano-hydroxyapatite to be 1.67 (16).

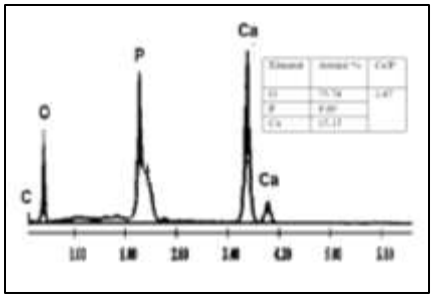

Figure 4. *EDX spectrum of nano-hydroxyapitate.*

III. FTIR spectra of chitosan and chitosan-sodium bicarbonate complex

The obtained infrared spectra is shown in Figure 4. The intermolecular and intramolecular hydrogen bonds create amino and hydroxyl groups, the OH stretching vibration and NH stretching vibration, which were located around 3438 cm^{-1} , fused into a broad intense band at 2922–3600 cm^{-1} in the FT-IR spectrum of chitosan (A). Whose characteristic absorption spectrum includes these peaks [3]. The spectrum of the complex (B) shows a completely different absorption peak at 3431 cm^{-1}, appearing as a broad peak, which indicates that the hydrogen bonds between the amino and hydroxyl groups were destroyed, thus showing obvious OH or NH stretching vibrations. The two absorption peaks at 1628 cm^{-1} and 1564 cm^{-1}, which are represent amide bonds, are also characteristic peaks of chitosan (A) [14]. In the complex (B), these two characteristic peaks still exist but with a slight shift (1568 cm^{-1} and 1416 cm^{-1}), indicating the existence of a –NH–CO– structure in the complex.

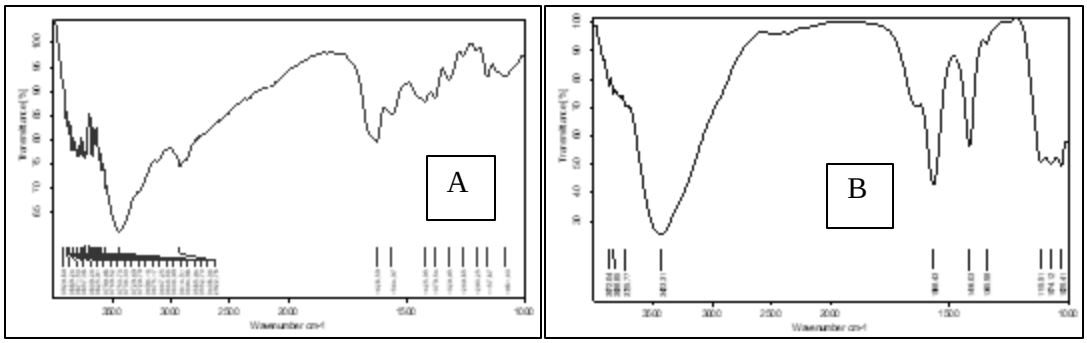

Figure 4. *(FT-IR) spectra of chitosan (A) and complex of chitosan/nHA/sodium carbonate (B).*

Conclusion

These preliminary studies indicate that the chitosan/nHA/Na_2CO_3 gel exhibit extended gelation time and with several modifications and improvements, this might have potential use in bone tissue engineering applications. In addition, rheological measurement indicated that the initial concentrations of chitosan have a significant effect on the gelation process and degradation of the resultant composite by unstable behaviors of composites which are above 1.6% of chitosan concentration.

Acknowledgment

This work was supported by Senate Research Committee, University of Moratuwa under the research grant No SRC/ST/2021/09.

References

Fangfang Li, Yang Liu, Yun Ding, Qiufei Xie (2014). A new injectable in situ forming hydroxyapatite and thermosensitive chitosan gel promoted by Na2CO3: Soft Matter, 10, 2292. https://pubs.rsc.org/en/content/articlelanding/2014/sm/c3sm52508b

Jianying, Q. Xiaomeng, W. Jie, S. Chang, S. Jinsong, G. Zhenghong ,X. Jian, J. Jinsong, S. A Novel Complex of Chitosan–Sodium Carbonate and Its Properties: Mar. Drugs 2018, 16, 416. https://www.science.gov/topicpages/j/jing+wang+wei.html

Kudryavtsev, P. (2020). Modelling of kinetics and structure formation at sol-gel transition: International Journal of Petrochemical Science & Engineering: 5(1), 49–54. https://medcraveonline.com/IPCSE/IPCSE-05-00121.pdf

Khaled R. Mohamed , Hanan H. Beherei, Zenab M. El-Rashidy, (2014). In vitro study of nano-hydroxyapatite/chitosan–gelatin composites for bio-applications: Journal of Advanced Research, Volume 5, Issue 2, Pages 201-208. https://www.sciencedirect.com/science/article/pii/S2090123213000325

Sewvandi, G.A. Adikary, S. U. (2012). Synthesizing and Characterization of Natural Biopolymer Chitosan Derived from Shrimp Type, Penaeus monodon: Tropical Agricultural Research Vol. 23 (3): 272 -276. https://www.researchgate.net/profile/Galhenage-Sewvandi

Yang, S.; Shao, D.; Wang, X.; Hou, G.; Nagatsu, M.; Tan, X.; Ren, X.; Yu, J. Design of Chitosan-Grafted Carbon Nanotubes: Evaluation of How the –OH Functional Group Affects Cs+ Adsorption. Mar. Drugs 2015, 13, 3116–3131. https://pubs.acs.org/doi/full/10.1021/es104047d

Younes, I.; Rinaudo, M. Chitin and Chitosan Preparation from Marine Sources. Structure, Properties and Applications. Mar. Drugs 2015, 13, 1133–1174. https://pubmed.ncbi.nlm.nih.gov/25738328

Jiang, Y.; Fu, C.; Wu, S.; Liu, G.; Guo, J.; Su, Z. Determination of the Deacetylation Degree of Chitooligosaccharides. Mar. Drugs 2017, 15, 332. https://europepmc.org/article/MED/26114886

Mourya, V.K.; Inamdar, N.N. Chitosan-modifications and applications: Opportunities galore. React. Funct. Polym. 2008, 68, 1013–1051. https://www.sciencedirect.com/science/article/pii/S1381514808000461

Riva, R.; Ragelle, H.; des Rieux, A.; Duhem, N.; Jérôme, C.; Véronique Préat, V. Chitosan and chitosan derivatives in drug delivery and tissue engineering, In Chitosan for Biomaterials II.; Jayakumar, R., Prabaharan, M., Muzzarelli, R.A.A., Eds.; Springer: Berlin, Germany, 2011; pp. 19–44. https://www.tandfonline.com/doi/full/10.3109/21691401.2013

Antonino D. Q.; R.S.C.M.; Lia Fook, B.R.P.; de Oliveira Lima, V.A.; de Farias Rached, R.Í.; Lima, E.P.N.; da Silva Lima, R.J.; Peniche Covas, C.A.; Lia Fook, M.V. Preparation and Characterization of Chitosan Obtained from Shells of Shrimp (Litopenaeus vannamei Boone), Mar. Drugs 2017, 15, 141. https://www.sciencedirect.com/science/article/pii/S2214785320402767

Habibah, TU; Salisbury, HG (January 2018). "Biomaterials, Hydroxyapatite". PMID 30020686. Archived from the original on 2020-03-28. Retrieved 2018-08-12. https://wikimili.com/en/Hydroxyapatite