Synthesis of pH-Sensitive CMC /Chitosan Hydrogels by Gamma Irradiation

N.F. Kassem*, A.S. Negm, N. Shukry and S. El-Kalyouby

Cellulose and Paper Department, National Research Center, Guiza, Egypt

Abstract: Gamma irradiation was used with different dosages to prepare hydrogels from carboxymethyl cellulose (CMC) and chitosan (CHI) with different ratios, in presence of different methylene bisacrylamide (MBA) concentrations as a crosslinking agent. The hydrogels were characterized by FT-IR spectroscopy which confirmed complexation between carboxylic group in CMC and amino group in CHI. The swelling behavior in different buffers of different pH values was also studied. The results indicated the formation of network structure of pH-sensitive hydrogels. The CMC/CHI hydrogels were evaluated for the possible use in drug delivery field, in which the release profile of aspirin, as a drug model, was investigated. Scanning electron microscopy was carried out before and after aspirin release proving the drug release.

Keywords: Carboxymethyl cellulose, Chitosan, Drug release, Gamma irradiation, pH sensitive hydrogels.

INTRODUCTION

The use of natural biopolymers for diversified applications in life sciences has several advantages, such as availability from replenishable agricultural or marine food resources, biocompatibility and biodegradability. They are more environmentally friendly than synthetic polymers. In recent years, there has been growing interest for preparation of new types of smart hydrogels for several applications using natural polymers such as polysaccharides. Hydrogels were first reported by Wichterle and Lím [1] in 1960. It is worth noting that the hydrogels have wide potential applications in the fields of food, biomaterials, agriculture, water purification, etc. Recently, scientists have devoted much energy to developing novel hydrogels for applications such as biodegradable materials for drug delivery [2,3], tissue engineering [4,5], sensors [6,7], contact lenses [8,9] and purification [10].

Among a wide variety of hydrogels we have polyelectrolyte complexes (PECs). They are mixtures of positively and negatively charged polymers blended at the molecular level [11, 12]. PECs are being developed in many fields, including tissue engineering, separation membranes and drug delivery [13]. Due to the specific pH range occurring at physiological, pathological, or subcellular sites such as stomach, intestine, endosome/lysosome, and tumor sites, pH sensitive hydrogels are the most widely used stimulus in environmentally responsive hydrogels. For this purpose, the most suitable polymers are those bearing weak polyelectrolyte (polyacid, polybase) or polyampholyte sequences. pH-sensitive polymers rely on the protonation/deprotonation equilibrium, which depends on the pKα of the acidic and/or basic moieties present in the polymer. Therefore, a pH sensitive polymer can be charged (yielding a swollen state) or uncharged (yielding a hydrophobic/collapsed state) depending on the pH of the environment [14, 15].

Chitosan (CHI) is a biocompatible and biodegradable cationic polysaccharide. As chitosan can form thin membranous films, it may be used in packaging [16], encapsulation and drug delivery systems [17, 18]. The potential use of CHI as a material for preparation of polyelectrolyte systems with a wide variety of water-soluble anionic polymers was studied for wide range of applications [13, 19-21]. Chitosan possesses many favorable biological properties, such as capability to enhance drug permeability and absorption at mucosal sites [22].

Carboxymethyl cellulose (CMC) is an anionic linear polymer in which an original H-atom of the cellulose hydroxyl group repeat unit is replaced by a carboxymethyl substituent, $–CH_2COOH$. CMC is used for preparing a wide range of biocompatible and pH sensitive hydrogels [23-26]. Additional researches have been reported in the literature on grafting some monomers onto CMC using variable initiation systems and for different purposes including medical and pharmaceutical applications [27-29]. CMC is classified as a good entrant for preparing polyelectrolyte complex hydrogel by mixing with another cationic polysaccharide like CHI. These polyelectrolytes display swelling behavior and pH response and have good potential to be applied for the drug delivery systems [30].

*Address correspondence to this author at the Cellulose and Paper Department, National Research Center, Guiza, Egypt; Tel: +2 02 33335926; Fax: +2 02 33370931; E-mail: nesrina00@yahoo.com

E-ISSN: 2311-8717/14

Chemical crosslinkers like glutaraldehyde and methylene bisacrylamide (MBA) are used to enforce the structure and the stability of polyelectrolyte hydrogels over a wide pH range [31-34]. This enforcement happens mainly by introducing new molecules between the polymeric chains to produce cross-linked chains.

The application of gamma radiation technique does not necessitate the inclusion of chemical initiators of any sort; however, it can be used to remove any residual initiator that is present after other conventional polymerization processes which act as an undesirable contaminant [35]. The gamma rays have very high penetrative power and the dose of radiation can be varied from 5 to 100 rad /sec. CMC hydrogels obtained by this method own typical anionic hydrogel swelling properties with high water absorption ability and good biodegradability. On the other hand, they have very poor mechanical strength in the hydrated state, which greatly limits many applications. Therefore, the addition of a crosslinker is favorable to restore the mechanical properties.

Lugao [36] reviewed the application of radiation in preparing hydrogels. It was shown that the radiation technique is a commanding tool for producing chemically pure hydrogels with enhanced properties. Moreover, the process is easy to control and flexible. So far, hydrogels obtained by radiation crosslinking have found applications in many fields, such as biomedical devices [37], wound dressing [38, 39] and controlled release of drug [40, 41]. In such applications, the biodegradable and biocompatible polymers are usually desired. Liu *et al.* [42] also studied the crosslinking of CMC using ^{60}Co γ - radiation technique to form hydrogel. Effect of preparation conditions on crosslinking of CMC and swelling behaviors of the hydrogel at different conditions were discussed.

In the last decade, increasing concentration has been given to the controlled drug delivery systems, which offer a probable benefit over conventional drug therapy. The hydrogels that are sensitive to external stimuli are often referred to as intelligent hydrogels. Among these, pH-sensitive hydrogels have been extensively investigated for the potential use in the delivery of drugs. Zhu *et al.* [43] entrapped drug-loaded microspheres (berberine hydrochloride in alginate microspheres) into carboxymethyl chitosan (CMC) hydrogel to form a new drug-delivery system.

The aim of this work is to evaluate CMC/CHI hydrogel as a drug delivery system. Herein, the hydrogel synthesis was carried out in presence of MBA as chemical crosslinker and gamma radiations with different doses. The processing factors affecting the swelling characteristics of CMC/CHI, pH sensitivity as well as the optimal conditions for their preparation was elucidated. The release profile of aspirin as a model for drug was studied in two consecutive buffer solutions of pH 2.1 and 7.4, similar to that of gastric and intestinal fluids, respectively.

MATERIALS AND METHODS

Chitosan, microcrystalline of molecular weight 100.000 - 300.000 and deacetylating grade 70 - 85% was purchased from Acros. Carboxymethyl cellulose sodium salt (high viscosity) and other chemicals reagents and solvents are of analytical grade and are used without purifications. N,N`-methylene bisacryl-amide, (MBA) $C_7H_{10}N_2O_2$, 98%, is obtained from Merck chemical Co. with the following specifications: M.wt=154.17 g/mol, mp=185^{o}C and d= 1.00 g/cm^3. Acetylsalicylic acid (Aspirin) is obtained from Winlab, with M.wt=180.16 g/mol.

Buffer Solution

A buffer solution was prepared from a mixture of phosphoric acid (54.0 mmol), boric acid (40.0 mmol) and acetic acid (42.0 mmol); then adjusted to the required pH value by drop wise addition of 0.2 N NaOH solution.

Preparation of CHI/CMC Polyampholytic Hydrogel

1 and 2% CHI solutions were prepared by dissolving chitosan in 2% aqueous acetic acid solution. Also, 1 and 2% CMC solutions were prepared by dissolving CMC in distilled water. CHI/CMC hydrogels were prepared by mixing the polymers in different weight ratios, namely 1:1, 1:2 and 2:1 in presence of different concentrations (0.1, 0.2 and 0.3%) of MBA as crosslinker. Each mixture was placed into small vial glass tubes and subjected to gamma irradiation at room temperature. The source of irradiation used for initiating the crosslinking was Cobalt-60 gamma cell 3500. This source is located at Middle Eastern Regional Radioisotopes Center for the Arab countries (Dokki, Cairo). The dose rate was 0.6 Gy/min. The formed hydrogel was filtered, washed by distilled water to remove unrestricted polymer chains and dried at 40^{o}C till constant weight.

Equilibrium Swelling

The equilibrium swelling of the hydrogel was measured in distilled water. A weighed sample of the

hydrogel was immersed in distilled water until equilibrium swelling was attained. The weight of the hydrogel was determined after removal of the surface liquid using tissue paper. The percent swelling was calculated by the following equation:

$$\%Swelling=100[(W_t-W_0)]/W_0$$

Where W_0 is the initial weight and W_t the final weight of the film at time *t*.

Aspirin Loading and *in Vitro* Release

The CHI:CMC (2:1) hydrogel prepared at 1% MBA and irradiated by 2.6 kGy was chosen for drug release study. Aspirin loading was carried out by impregnating the prepared CHI/CMC hydrogel in 5% aspirin solution. After swelling equilibrium, the gel was taken out, dried and reweighed to determine the amount of aspirin uptake. The *in Vitro* release studies were performed in the buffer solution at pH 2.1 and 7.4 for the different polymers concentrations (1 and 2%). Accurately weighed amounts of hydrogels were placed in covered glass vials containing 10 ml of buffer solution. Samples of 100 µl were withdrawn at specific time intervals for a total period of 24 h and replaced with the same amount of distilled water. Each sample was measured at λ_{max} 292 nm in a spectrophotometer. Aspirin concentration in the unknown samples was measured using a calibration curve created by known aspirin concentration solutions.

Infrared Spectrophotometer (IR)

FTIR analysis was carried out using Mattson 5000 FTIR spectrometer in the range of 4000-600 cm^{-1}. The samples were pressed as tablets with KBr.

Scanning Electron Microscopy (SEM)

The surface morphology of the hydrogels was examined with JEOL JXA-840 electron probe microanalyzer. Samples were coated with gold under vacuum and then examined.

RESULTS AND DISCUSSION

Carboxymethyl cellulose and chitosan are poly-saccharides that have a variety of functional groups. It is well known that -NH_2 groups in chitosan and -COOH groups in CMC work as cationic and anionic parts, respectively, to form inter-macromolecular complex through the strong electrostatic and hydrogen bonding interactions between these groups. γ radiation in presence of MBA was used to extend the interaction between the two polymers.

Being typically degradable polymer, chitosan is unable to crosslink itself under irradiation. On the other hand, it was found that crosslinking of CMC can be induced by ionizing radiation under highly concentrated, paste-like conditions [23, 24]. In the present work, the presence of chitosan is believed to play an important role in gel formation process. The role of chitosan in crosslinking can be considered in two cases. In the first, chitosan may act as a sort of additional crosslink points by ionic interaction or hydrogen bonds. In the second case, the presence of chitosan stimulates the formation of radicals or sustains the life of radicals longer during irradiation, which resulted in high efficiency of gel formation in CMC.

1. Effect of Gamma Irradiation Dosage on Swelling %

The equilibrium degrees of swelling of the CHI/CMC hydrogels with different CMC:CHI ratios and various concentrations of MBA as a function of gamma irradiation dose are presented in Figures **1-3**. The swelling curves indicated a typical swelling- dose relationship. All figures show that by increasing the gamma irradiation dose from 2.6 to 3.5 and further to 5.2 kGy, the swelling% decreased in all samples. The results can be explained on the basis that when the irradiation dose increases, the crosslinking density between the polymer chains increases. This restricts the extension of the chains of the prepared polymer and decreases the swelling of the hydrogel.

Therefore, the optimum irradiation dosage selected and fixed in further experiments was at 2.6 kGy.

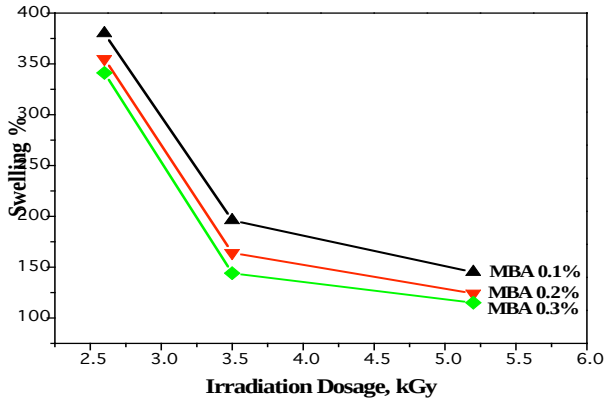

Figure 1: Effect of irradiation dosage on swelling % of hydrogel formed at MBA 0.1, 0.2 and 0.3% and CMC: CHI ratio 1:1

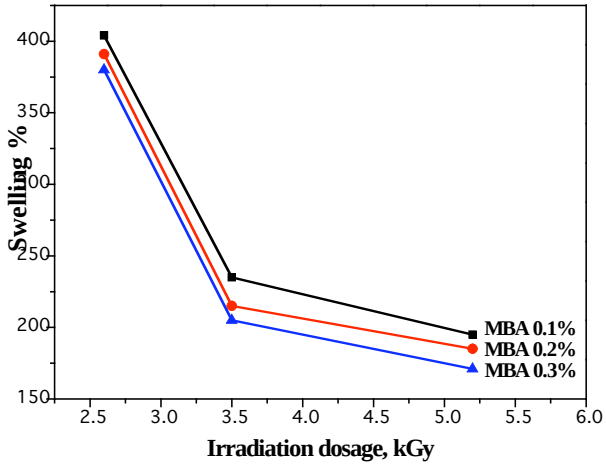

Figure 2: Effect of irradiation dosage on swelling % of hydrogel formed at MBA 0.1, 0.2 and 0.3% and CMC: CHI ratio 1:2

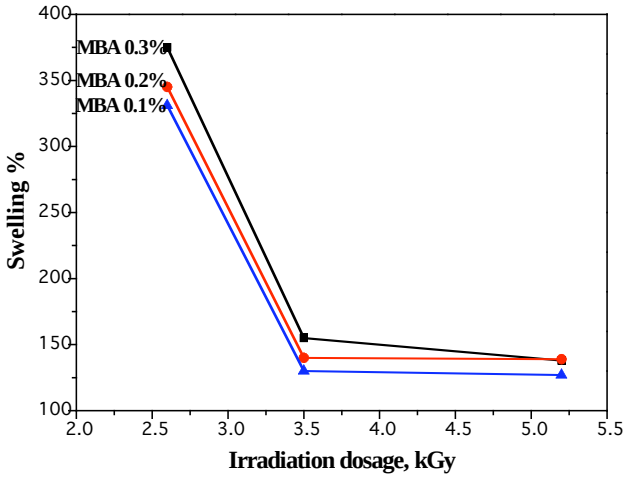

Figure 3: Effect of irradiation dosage on swelling % of hydrogel formed at MBA 0.1, 0.2 and 0.3% and CMC: CHI ratio 2:1.

2. Effect of CHI:CMC Ratio on Swelling %

Figure **4** shows the effect of chitosan content on the swelling % of the prepared hydrogels. The experiments revealed that by increasing chitosan content in the hydrogel, the swelling % gradually increased. The maximum swelling % (404) was attained at 66% chitosan content; i.e. 2:1 CHI:CMC ratio. This may be either because chitosan may act as a sort of additional crosslink points by ionic interaction or hydrogen bonds or because the presence of chitosan stimulates the formation of radicals or sustains the life of radicals longer during irradiation, which resulted in high efficiency of gel formation in CMC, as mentioned before.

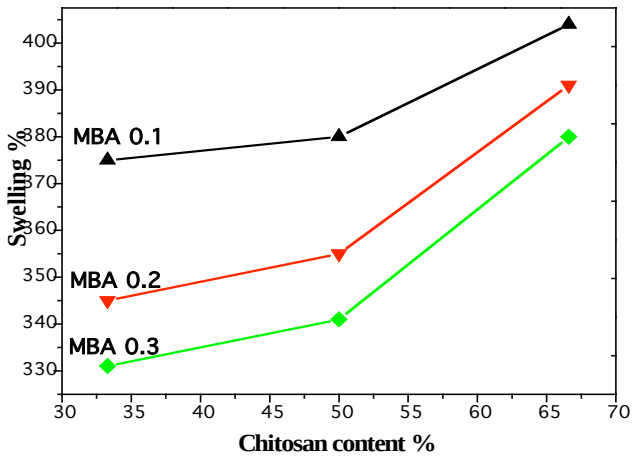

Figure 4: Effect of chitosan content on swelling % of hydrogel formed at Gamma ray dosage 2.6kGy and MBA 0.1, 0.2 and 0.3%.

3. Effect of MBA Concentration on Swelling %

The swelling% as a function of MBA concentration was investigated for crosslinked CHI /CMC hydrogel. As shown in Figure **5**, by increasing MBA concentration from 0.1 to 0.3%, the swelling % decreased from 380 to 341. The increase in percentage of MBA caused a decrease in the swelling capacity because it led to a higher crosslinking density and therefore decreased the spaces between the polymer chains and consequently, the resulting highly crosslinked rigid structure of hydrogel could not expand and hold a large quantity of water [26].

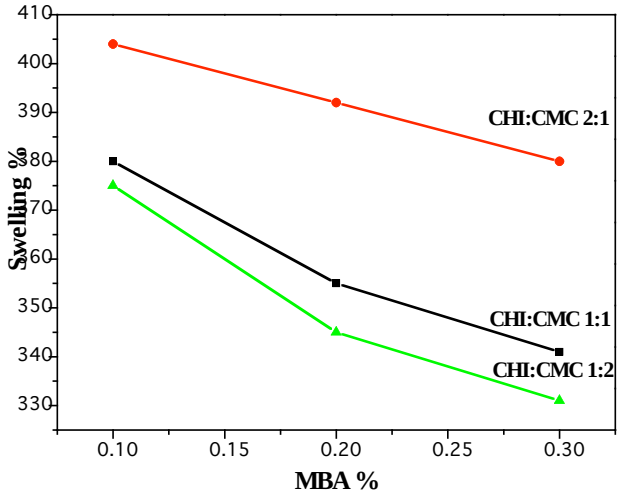

Figure 5: Effect of MBA concentration on swelling % of hydrogel formed at Gamma ray dosage 2.6kGy and different chitosan contents.

4. Effect of Polymers Concentration

The swelling property of the prepared polyampholytic hydrogel from CHI/CMC was investigated at 1% and 2% for both polymers. The reaction mixture was run at 2.6 kGy irradiation dose after adding 0.1% MBA. As seen in Table **1**, the increase in both chitosan and carboxymethyl cellulose concentration from 1 to 2% resulted in a high increase in the swelling % due to the increase of the number of functional groups and consequently increasing repulsion thus expansion of the network. By increasing the % of CMC and/or CHI than 2%, the high viscosity of the polymer's solutions hindered the ionic interaction between CHI and CMC chains.

Table 1: Effect of CMC and CHI Concentration on Swelling % of Hydrogels at CMC:CHI =1:2, Radiation Dosage 2.6 kGy and Different MBA Concentrations

% MBA	Swelling% of 1% CMC and 1% CHI	Swelling% of 2% CMC and 2% CHI
0.1	404	1851
0.2	391	1700
0.3	380	1569

5. Effect of pH

For the characterization of the response of chitosan/CMC hydrogel to the change in the external pH condition, hydrogel samples were allowed to swell to equilibrium in an aqueous swelling medium of pH 2, 3, 5, 6, 7 and 8 at 25 °C. The effect of the external pH on the swelling behavior of hydrogels is given in Figure **6**. From this figure, it is clear that at low pH, the sample that contained the highest amount of chitosan (CHI:CMC 2:1) showed maximum swelling value. Positively charged chitosan at a low pH showed a high swelling because of the repulsive force between the same positive charges of molecules causing long intermolecular distances and a greater hydrophilic state [27]. It is known that a high concentration of a charged ionic group in gel increases swelling because of charge repulsion and osmosis [28].

On the other hand, the highest swelling % value was attained for the sample containing the greatest amount of CMC (CHI:CMC 1:2) in the swelling medium of pH 8. That is mainly due to the presence of the carboxylic acid groups on carboxymethyl cellulose hydrogel which became progressively ionized (COO^-). In this case, the hydrogel swelled more significantly due to a large swelling force created by the electrostatic repulsion between the ionized acid groups [29].

The amphoteric hydrogel can act as a polyanionic or polycationic hydrogel depending on the pH value. In our case, the amino groups ($-NH_2$) on CHI in CHI/CMC hydrogel are protonated to become $-NH_3^+$ in acidic solution, while the carboxyl groups on CMC remain as -COOH, so it behaves as a polycationic hydrogel that swells in acidic medium. Conversely, the carboxyl groups in the CHI/CMC hydrogel are ionized to $–COO^-$ in basic solution, but the amino groups remain in their original uncharged form to give a polyanionic hydrogel, which swell in basic medium [34].

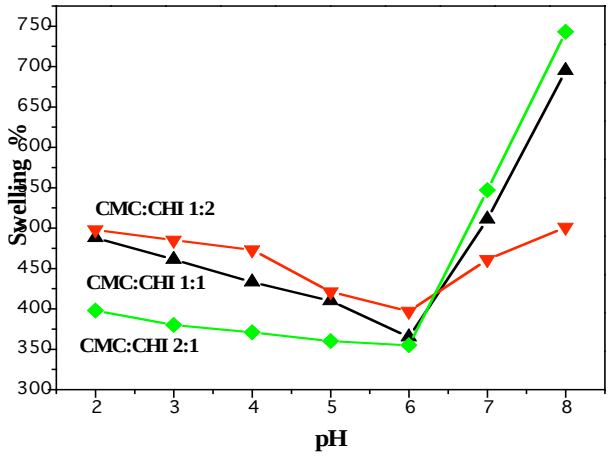

Figure 6: Effect of pH on swelling % of hydrogel formed at different weight ratios of chitosan and CMC.

6. FT-IR

FTIR spectra of chitosan, CMC and various chitosan /CMC hydrogels prepared under different conditions are shown in Figure **7**. The characteristic peaks of chitosan are located at 3450 cm^{-1} assigned to hydroxyl groups, 1636 and 1560 cm^{-1} for the amide groups. The amino groups have a characteristic band in the region 3500-3400 cm^{-1}, which is overlapped by the OH band. FTIR of CMC shows characteristic bands located at 3424, 1607 and 1059 cm^{-1} for hydroxyl, carboxyl and ether groups, respectively.

According to Wanchoo and Sharma (44), any interaction between chemical groups or unlike polymers should theoretically cause a shift in peak position of the participating groups. Our results indicate incidence of interactions between the functional groups in chitosan (amino and amide) and the functional groups in CMC (OH and COOH), as shifts in the corresponding bands are recognized.

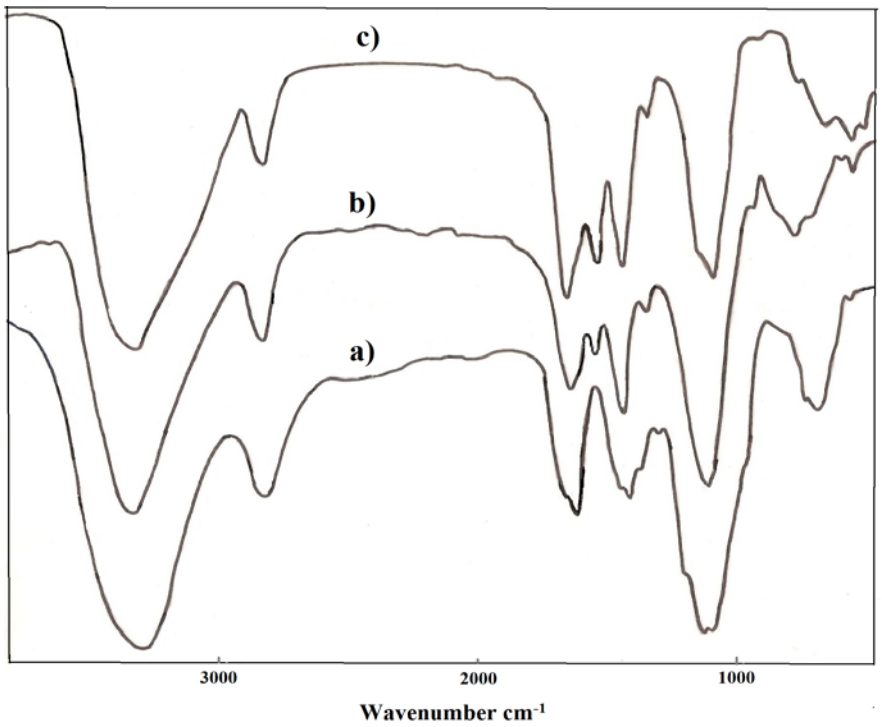

Figure 7: FTIR spectra of (a) chitosan and CHI/CMC formed at chitosan % 50, Gamma ray dosage 2.6 kGy, MBA 0.1%, and polymer initial concentrations 1% (b) and 2% (c).

7. Drug Release

Since hydrogels have high permeability for water soluble materials like drugs, the most common mechanism of drug release in the hydrogel systems is diffusion. Factors like polymer composition, water content, and crosslinking density are used to control the release rate and release mechanism from hydrogels. The release kinetics of a loaded hydrogel is closely related to its water sorption kinetics [31] as it has been already established that a highly swelled hydrogel should release a greater amount of solute entrapped within the gel. The release of solute from loaded gel involves the absorption of water into the matrix and simultaneous release of solute via diffusion. The aspirin release from CHI/CMC prepared hydrogel at 2:1 weight ratio was carried out by immersing the dried drug-loaded hydrogel in a phosphate buffer solution at room temperature.

7.1. Effect of pH on Aspirin Release

The release % of aspirin from CHI /CMC polyampholytic hydrogel was carried out in two different pHs of phosphate buffer solutions. From Figure **8** it is clear that, in both media (pH 3 and 8), the release percent from the aspirin loaded samples attained its equilibrium value after nearly 8 hours. The release% of aspirin was found to be higher in acidic medium than in alkaline one. This may be attributed to the dependence of the release rate on the degree of swelling of the gel, which mainly depends on solution pH. At lower pH, an expulsive force within the tested hydrogel is created due to the protonation of primary amino groups ($–NH_3^+$) on chitosan.

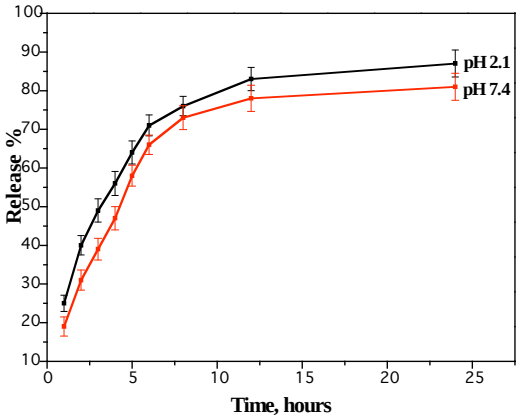

Figure 8: Effect of pH on *in Vitro* release profile of aspirin at 2% polymer conc.

7.2. Effect of Polymer Concentration

Figure **9** shows the effect of varying CMC and chitosan concentration of the prepared hydrogel on the

release rate of the loaded aspirin. From this figure, it can be seen that the effect of polymer concentration has a strong influence on release rate. Samples prepared from 2% chitosan and 2% CMC were more efficient for the aspirin release than those prepared by using 1% polymer solutions. The results can be explained that, in the acidic medium, increasing polymer concentration in the hydrogel may increase the repulsive force between positive amino groups on the polymer chain and hence increasing the swelling % and diffusion of the loaded aspirin from the hydrogels. On the other hand, in alkaline medium, the role of carboxylate ions dominates in the same way. After a 24-h release, the cumulative aspirin released maintained at approximately 78 and 87 % for 1% and 2% polymer concentration, respectively. This is because some aspirin molecules may be entangled within the hydrogel network, and those cannot be released unless polymer matrixes are degraded.

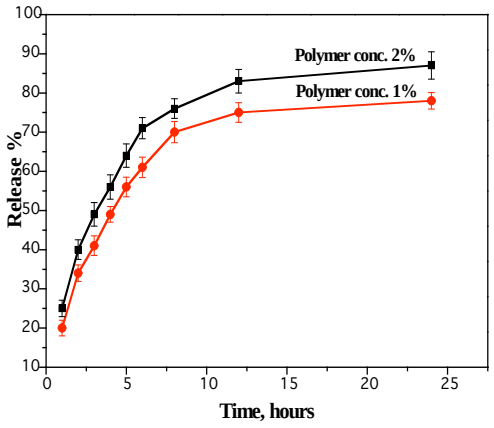

Figure 9: Effect of polymer conc. on *in Vitro* release profile of aspirin at pH 2.1.

7.3. Scanning Electron Microscopy (SEM)

The morphology of CHI/CMC hydrogel before and after aspirin release is shown in Figure **10**. It can be seen that the surface of the aspirin loaded hydrogel is very smooth, but turned to rough surface after aspirin release.

CONCLUSION

pH sensitive hydrogels were prepared successfully from CMC and CHI under different gamma irradiation dosages in presence of MBA as a crosslinker. The results of FTIR indicate the occurence of interactions between the functional groups in chitosan (amino and amide) and the functional groups in CMC (OH and COOH). The effect of changing CMC: CHI ratio, polymers concentration, MBA concentration and gamma irradiation dosage on the swellability of the hydrogels was studied. The experimental results proved that the CMC/CHI hydrogels exhibited superabsorbent capacity and high % swelling equilibrium contributed to the presence of highly hydrophilic CMC.

The hydrogels possess good release behavior of aspirin at different pHs. Based on the current results, the pH sensitivity of such hydrogels prepared in this work could play an important role in drug release application. Considering the biodegradable nature of both components, the CMC/CHI prepared by gamma irradiation could be viable biodegradable materials. In fact, these new materials can potentially be applied in biomedical fields.

ACKNOWLEDGEMENTS

Financial support for this work was funded by the National Research Center throughout the project number 9140106. The authors express their deep gratitude to the National Research Center for this support.

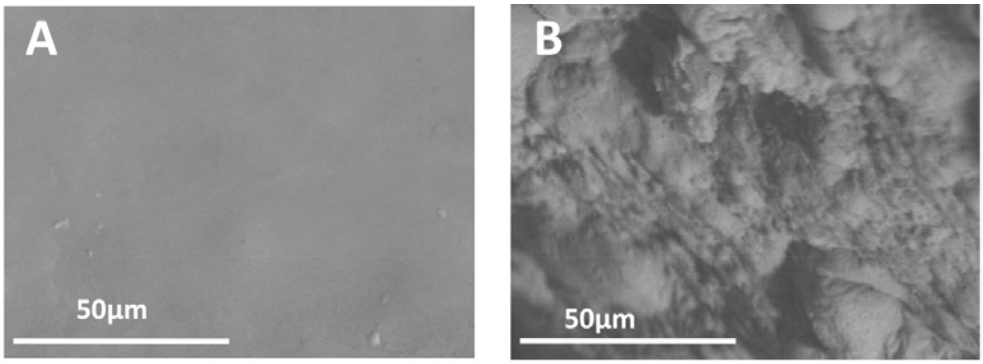

Figure 10: SEMs showing the surface of Asprin-loaded CMC/CHI hydrogel formed at polymer concentration 1%, chitosan % 50, Gamma ray dosage 2.6 kGy , MBA 0.1%, before (a) and after (b) Asprin release from hydrogel.

REFERENCES

[1] Wichterle O and Lím D. Hydrophilic gels for biological use. Nature 1960, 185: 117-8. http://dx.doi.org/10.1038/185117a0

[2] Bajpai AK, Shukla SK, Bhanu S and Kankane S. Responsive polymer in controlled drug delivery. Prog Polym Sci 2008, 33(11): 1013-8. http://dx.doi.org/10.1016/j.progpolymsci.2008.07.005

[3] Wu D et al. Fabrication of supramolecular hydrogels for drug delivery and stem cell encapsulation. Langmuir 2008, 24 (18):10306-12. http://dx.doi.org/10.1021/la8006876

[4] Khan F, Tare R, Richard O, Oreffo R and Bradley M. Versatile biocompatible polymer hydrogels: scaffolds for cell growth. Angew Chem Int Ed 2009, 48(5): 978-82. http://dx.doi.org/10.1002/anie.200804096

[5] Lee KY and Mooney DJ. Hydrogel for tissue engineering. Chem Rev 2001, 101(7): 1869-79. http://dx.doi.org/10.1021/cr000108x

[6] Lee YJ and Braun PV. Tunable inverse opal hydrogel pH sensors. Adv Mat 2003, 15(7-8): 563-6. http://dx.doi.org/10.1002/adma.200304588

[7] Sorber J et al. Hydrogel-based piezoresistive pH sensors: Investigations using FT-IR attenuated total reflection spectroscopic imaging. Anal Chem 2008, 80(8): 2957-62. http://dx.doi.org/10.1021/ac702598n

[8] Katsoulos C, Karageorgiadis L, Vasileiou N, Mousafeiropoulos T and Asimellis G. Customized hydrogel contact lenses for keratoconus incorporating correction for vertical coma aberration. Ophthalmic Physiol Opt 2009, 29(3): 321-9. http://dx.doi.org/10.1111/j.1475-1313.2009.00645.x

[9] Yasuda H. Biocompatibility of nanofilm-encapsulated silicone-hydrogel contact lenses. Macromol Biosci 2006, 6(2): 121-38. http://dx.doi.org/10.1002/mabi.200500153

[10] Ha EJ et al. Purification of his-tagged protein using Ni^{2+}-poly(2-acetamidoacrylic acid) hydrogel. J Chromat B2008, 876(1): 8-12.

[11] Sun Z, An Q, Zhao Q, Shangguan Y and Zheng Q. Study of Polyelectrolyte Complex Nanoparticles as Novel Templates for Biomimetic Mineralization. Cryst Growth Des 2012, 12(5): 2382–8. http://dx.doi.org/10.1021/cg3000476

[12] Fukuda H and Kikuchi Y. Polyelectrolyte Complexes of Sodium Carboxymethylcellulose with Chitosan. Makromol Chem 1979, 180(6): 1631–3. http://dx.doi.org/10.1002/macp.1979.021800629

[13] Y. Hu, T. Yang, X. Hu, Novel polysaccharides-based nanoparticle carriers prepared by polyelectrolyte complexation for protein drug delivery. Polym. Bull. 2011, 68(4), 1183–99. http://dx.doi.org/10.1007/s00289-011-0683-9

[14] Yao D E, Peng T, Feng HB and He YY. Swelling Kinetics and Release Characteristic of Crosslinked Chitosan: Polyether Polymer Network (Semi-IPN) Hydrogels. J PolymSci A Polym Chem1994, 32(7), 1213–23. http://dx.doi.org/10.1002/pola.1994.080320702

[15] Vermonden T, Censi R and Hennink W E. Hydrogels for Protein Delivery. Chem Rev 2012, 112(5): 2853–88. http://dx.doi.org/10.1021/cr200157d

[16] Srinivasa PC, Ramesh MN, Kumar KR and Tharanathan RN. Properties of chitosan films prepared under different drying conditions. J Food Eng 2004, 63(1): 79–85. http://dx.doi.org/10.1016/S0260-8774(03)00285-1

[17] Noel SP, Courtney HS, Bumgardner JD and Haggard WO. Chitosan sponges to locally deliver amikacin and vancomycin: a pilot *in Vitro* evaluation. Clin Orthop Relat Res 2010, 468(8): 2074–80. http://dx.doi.org/10.1007/s11999-010-1324-6

[18] Chen H and Fan M. Chitosan/Carboxymethyl Cellulose Polyelectrolyte Complex Scaffolds for Pulp Cells Regeneration. J Bioact Compat Polym 2007, 22(5): 475–91. http://dx.doi.org/10.1177/0883911507081329

[19] Torelli-souza RR, Bastos LAC, Nunes HGL, Camara CA, Val R and Amorim S. Sustained Release of an Antitumoral Drug from Alginate-Chitosan Hydrogel Beads and Its Potential Use as Colonic Drug Delivery. J Appl Polym Sci 2012, 126(S1): E408–17. http://dx.doi.org/10.1002/app.36928

[20] Yu L, Gong J, Zeng C and Zhang L. Synthesis of Monodisperse Zeolite A / Chitosan Hybrid Microspheres. Ind Eng Chem Res 2012, 51(5): 2299–308. http://dx.doi.org/10.1021/ie202242e

[21] Watthanaphanit A, Supaphol P, Furuike T and Tokura S. Novel Chitosan-Spotted Alginate Fibers from Wet-Spinning of Alginate Solutions Containing Emulsified Chitosan - Citrate Complex and their Characterization. Biomacromol 2009, 10(2): 320–7. http://dx.doi.org/10.1021/bm801043d

[22] van der Lubben IM, Kersten G, Fretz MM, Beuvery C, Verhoef JC and Junginger HE. Chitosan microparticles for mucosal vaccination against diphtheria: oral and nasal efficacy studies in mice.Vaccine 2003, 21(13-14): 1400–8. http://dx.doi.org/10.1016/S0264-410X(02)00686-2

[23] Wang Q, Wang W, Wu J and Wang A. Effect of Attapulgite Contents on Release Behaviors of a pH Sensitive Carboxymethyl Cellulose- g -Poly (acrylic acid)/ Attapulgite / Sodium Alginate Composite Hydrogel Bead Containing Diclofenac. J Appl Polym Sci 2012, 124(6): 4424–32.

[24] Barbucci R, Leone G and Vecchiullo A. Novel carboxymethylcellulose-based microporous hydrogels suitable for drug delivery. J Biomater Sci Polym Ed 2004, 15(5): 607–19. http://dx.doi.org/10.1163/156856204323046870

[25] Barbucci R, Magnani A and Consumi M. Swelling Behavior of Carboxymethylcellulose Hydrogels in Relation to Cross-Linking , pH , and Charge Density. Macromolecules 2000, 33(20): 7475–80. http://dx.doi.org/10.1021/ma0007029

[26] Sannino A et al. Cellulose derivative-hyaluronic acid-based microporous hydrogels cross-linked through divinyl sulfone (DVS) to modulate equilibrium sorption capacity and network stability. Biomacromol 2004, 5(1): 92–6. http://dx.doi.org/10.1021/bm0341881

[27] El-sherbiny IM, Salama A and Sarhan AA. Ionotropically cross-linked pH-sensitive IPN hydrogel matrices as potential carriers for intestine-specific oral delivery of protein drugs. Drug Dev Ind Pharm 2011, 37(2):121-30. http://dx.doi.org/10.3109/03639045.2010.495754

[28] Bokias G, Mylonas Y, Staikos G, Bumbu GG and Vasile C. Synthesis and Aqueous Solution Properties of Novel Thermoresponsive Graft Copolymers Based on a Carboxymethylcellulose Backbone. Macromol 2001, 34(14): 4958-64. http://dx.doi.org/10.1021/ma010154e

[29] Karakasyan C, Lack S, Brunel F, Maingault P, Hourdet D and Polyme P. Synthesis and rheological properties of responsive thickeners based on polysaccharide architectures. Biomacromol 2008, 9(9): 2419–29. http://dx.doi.org/10.1021/bm800393s

[30] Mitsumata T, Suemitsu Y, Fujii K, Fujii T and Taniguchi T. Carboxymethyl Cellulose Sodium Salt Complex Hydrogels. Polymer 2003, 44(23): 7103–11. http://dx.doi.org/10.1016/j.polymer.2003.09.001

[31] Cai X, Tong H, Shen X, Chen W, Yan J and Hu J. Preparation and characterization of homogeneous chitosan-polylactic acid/hydroxyapatite nanocomposite for bone tissue engineering and evaluation of its mechanical properties. Acta Biomater 2009, 5(7): 2693–703. http://dx.doi.org/10.1016/j.actbio.2009.03.005

[32] Hoffmann B., Seitz D., Mencke A, Kokott A and Ziegler G. Glutaraldehyde and oxidised dextran as crosslinker reagents for chitosan-based scaffolds for cartilage tissue engineering. J Mater Sci Mater Med 2009, 20(7): 1495–503. http://dx.doi.org/10.1007/s10856-009-3707-3

[33] El-Sherbiny IM, Lins RJ, Abdel-Bary EM and Harding DRK. Preparation, characterization, swelling kinetics and in-vitro evaluation of new pH-sensitive gel beads based on chemically modified chitosan for oral delivery of protein drugs. Eur Polym J 2005, 41: 2584–91. http://dx.doi.org/10.1016/j.eurpolymj.2005.05.035

[34] Sheng J, Shao Z and Chen X. Electrical Behavior of a Natural Polyelectrolyte Hydrogel. Biomacromol 2008, 9(4): 1208–13. http://dx.doi.org/10.1021/bm701204j

[35] Hoffman AS. Glutaraldehyde and oxidised dextran as crosslinker reagents for chitosan-based scaffolds for cartilage tissue engineering. Radiat Phy. Chem 1977, 9: 207–19.

[36] Lugao AB and Malmonge SM. Use of radiation in the production of hydrogels. Nucl Instrum Methods B 2001, 185(1-4): 37–42. http://dx.doi.org/10.1016/S0168-583X(01)00807-2

[37] Rosiak JM and Olejniczak J. Medical application of radiation formed hydrogels. Radiat Phys Chem 1993, 42(4-6): 903–6. http://dx.doi.org/10.1016/0969-806X(93)90398-E

[38] Wu NH, Bao BR, Yoshii F and Makuuchi K. Irradiation of crosslinked, poly(vinyl alcohol) blended hydrogel for wound dressing. J Radioanal Nucl Chem 2001, 250(2): 391–5. http://dx.doi.org/10.1023/A:1017988822121

[39] Park KR and Nho YC. Synthesis of PVA/PVP hydrogels having two-layer by radiation and their physical properties. Radiat Phys Chem 2003, 67(3-4): 361–5. http://dx.doi.org/10.1016/S0969-806X(03)00067-7

[40] Zhai ML, Liu N, Li J, Yi M, Li JQ and Ha HF. Radiation preparation of PVA-g-NIPAAm in a homogeneous system and its application in controlled release. Radiat Phys Chem 2000, 57(3-6): 481–484. http://dx.doi.org/10.1016/S0969-806X(99)00476-4

[41] Savas H and Güven O. Investigation of active substance release from poly(ethylene oxide) hydrogels. Int J Pharm 2001, 224(1-2): 151–8. http://dx.doi.org/10.1016/S0378-5173(01)00745-1

[42] Liu P, Zhai M, Li J, Peng J, Wu J. Radiation preparation and swelling behavior of sodium carboxymethyl cellulose hydrogels. Rad Phys Chem 2002, 63(3-6): 626-62. http://dx.doi.org/10.1016/S0969-806X(01)00649-1

[43] Zhu AM, Chen JH, Liu QL, Jiang YL. Controlled release of berberine hydrochloride from alginate microspheres embedded within carboxymethyl chitosan hydrogels. J Appl Polym Sci 2011,120(4): 2374–80. http://dx.doi.org/10.1002/app.33433

[44] Wanchoo RK and Sharma PK. Viscometry study on the compatibility of some water- soluble polymer- polymer mixtures. Eur Polym J 2003, 39(7): 1481-90. http://dx.doi.org/10.1016/S0014-3057(02)00386-5

Received on 23-03-2014 Accepted on 05-04-2014 Published on 24-09-2014

DOI: http://dx.doi.org/10.12974/2311-8717.2014.02.01.3