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Development and Characterization of Chitosan based Polymeric Hydrogel Membranes

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Abstract

The current study deals with the development of glutaraldehyde crosslinked chitosan-based delivery system with improved mechanical properties utilizing reactive solvent technique. The prepared systems were characterized by Fourier Transform Infrared (FTIR) spectroscopy, x-ray diffraction (XRD), thermo gravimetric analysis (TG) and differential scanning calorimetry (DSC). The systems were also analysed for mechanical and pH-dependent swelling properties and they were also analysed for their hemocompatibility and cytocompatibility. FTIR studies indicated formation of imine bonds during the crosslinking reaction of chitosan and glutaraldehyde. The thermal, XRD and mechanical studies indicated optimal properties for the system prepared with 5% methacrylic acid as reactive solvent. The developed systems indicated pH-dependent swelling behaviour with increased swelling at lower pH. The prepared delivery systems were found to be highly hemocompatible in the presence of human blood and cytocompatible in the presence of L929 fibroblast cells. The release of the model drug (salicylic acid) from the delivery systems in 0.01 N hydrochloric acid indicated Fickian kinetics. The developed delivery systems could be used to deliver drug selectively in the stomach.

Key-words: cationic polymer, membrane, polyelectrolyte, cytocompatibility.

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1. Introduction

In recent years, extensive efforts have been devoted to the use of potential pharmaceutical devices such as novel drug delivery systems (DDS) which proposes a suitable means of site-specific and/or time-controlled delivery of therapeutic agents[1,2]. Among various kinds of drug delivery systems, hydrogel-based drug delivery devices have become a major area of research interest.

Hydrogels are cross-linked, three dimensional hydrophilic polymeric networks that swell but not dissolve when brought into contact with water. Hydrogels can be formulated in a variety of physical forms, including slabs, microparticles, nanoparticles, coatings, and films. As a result, they are commonly used in clinical practice and experimental medicine for a wide range of applications, including biosensors, tissue engineering and regenerative medicine, separation of biomolecules or cells and barrier materials to regulate biological adhesions. Hydrogels can protect drugs from hostile environments, e.g. the presence of enzymes and low pH within the body fluids. Their porosity permits loading of drugs into the gel matrix and subsequent drug release at a pre-designed rate. Hydrogels can also control drug release by changing the gel structure in response to environmental stimuli, such as pH [3], temperature [4], ionic strength [5], and electric field [6].A number of natural and synthetic polymers have been investigated for the development of in situ gel-forming systems, due to the ability of these hydrogels to release an entrapped drug into aqueous medium and to regulate the drug release depending on their swelling behaviour and crosslink density. Taking these factors in mind we have chosen “chitosan”, a natural polymer, to prepare a different kind of hydrogel.

Chitosan, a cationic biopolymer, consists of β-(1-4)-2-acetamido-2-deoxy-D-glucose unit which is main alkaline deacetylation product of chitin. Chitosan has advantage over other polysaccharides due to its non-toxicity,biocompatibility and biodegradability. Chitosan and its derivatives have become useful polysaccharides in the biomedical area. Chitosan is highly abundant and renewable sources for valuable polymeric starting blocks. It also helps in the development of new routes to produce valuable products, which is an important research task. Only chitosan or other ionic molecules combined with chitosan can be used in the formation of many polyionic hydrogels due to their sustainable drug release properties and good biocompatibility. The presence of hydroxyl and amine groups which are good crosslinkable sites for various crosslinking agents to prepare inter-penetrating network (IPN) structures. The

IPN structure enhances mechanical strength and thermal stability in chitosan based desired final matrix. Glutaraldehyde (GA), Mo (IV) polyoxyanions, genipin and blocked diisocyanate [7] are few useful crosslinkers for chitosan.

The present study has been designed to develop chitosan based delivery system crosslinked with GA. Attempts have been made to characterize the developed systems to ascertain its drug-delivery capability in different pH medium. Moreover, the effect of increasing active solvent concentration within the systems has also been monitored.

2. Experimental

2.1 Materials

Chitin (M.W. ~ 400,000), methacrylic acid (MA), GA (25 % solution in water) and salicylic acid (SA) were obtained from Loba chemie Pvt. Ltd. (Mumbai, India). Hydrochloric acid (35%) was obtained from Merk Limited (Mumbai, India) while sodium hydroxide flakes were supplied by Ranbaxy Laboratories Ltd (SAS Nagar, Punjab). Double distilled water was used throughout the study.

Methyl tetrazolium (MTT) dye, Dubelcco's minimum essential medium (DMEM) and dimethyl sulphoxide (DMSO) were received as gift from Department of Biotechnology, Indian Institute of Technology, Kharagpur, India.

Different buffer solutions of pH 1.4, 1.6, 3.6, 4.6, 5.4 and 7.6 were prepared as per Indian Pharmacopoeia, 1996.

2.2 Preparation of Chitosan

Four grams of pure chitin was deacetylated in presence of 100 ml of 50% (w/v) sodium hydroxide (NaOH) solution at 120^{o}C for 1 hr. The residual mass was filtered and washed thoroughly with distilled water. The washing was continued until the residual mass was free from sodium hydroxide which was ascertained by checking the pH of the distilled water after washing to show a pH of 7.0. The solubility profile of the final mass showed that it was readily soluble at pH 1.4 and was insoluble at pH's above 6.6, indicating the formation of chitosan which was further confirmed by the FTIR spectroscopy.

2.3 Preparation of hydrogel membranes

Chitosan solution was prepared by dissolving 0.25 g of chitosan (degree of deacetylation: 87.44 %) in 25 ml of MA- water solutions (1%, 2%, 5%, 7% and 9%

(v/v)) at 70^{o}C with continuous stirring for 30 minutes. To the above solutions, 0.5 ml of 5% (v/v) GA was added with constant stirring and subsequently converted into membranes by conventional solution casting technique. The membranes were dried at room-temperature (25^{o}C) for 48 hours. The membranes, so obtained were abbreviated as 1G, 2G, 5G, 7G and 9G, respectively.

SA loaded films were developed by two methods. In the first method (Patch-A), specified amount (0.8 g) of SA was dispersed in 25 ml of MA solutions of 5 %, 7 % and 9 % (v/v) concentrations. To the above dispersion, 0.25 g of prepared chitosan was added with constant stirring and was subsequently heated at 70^{o}C for 30 min. This was followed by the addition of 0.5 ml of 5% (v/v) GA solution to the above dispersion and was subsequently converted into membranes by conventional solution casting method. The membranes, so obtained, were abbreviated as 5G+SA(A), 7G+SA(A) and 9G+SA(A), respectively.

In the second method (Patch-B), 0.25 g of the prepared chitosan was dissolved in 25 ml of MA solutions of 5%, 7% and 9% of (v/v) concentrations followed by the addition of 0.5 ml of 5% (v/v) GA solution. The above solution was heated at 70^{o}C with the subsequent addition of 0.8 g of SA to it. Films were obtained by solution casting method and were abbreviated as 5G+SA(B), 7G+SA(B) and 9G+SA(B), respectively.

2.4 Characterization

The raw materials, both liquid and powder samples, were characterized by FTIR spectroscopy in the range of 4000-400 cm^{-1} by KBr pellet method, while the crosslinked membranes were subjected to attenuated total reflectance (ATR) spectroscopy in the range of 4000-400 cm^{-1}. FTIR spectrophotometer (NEXUS—870, Thermo Nicolet Corporation) was used for the characterization.

The films were subjected to X-ray diffraction (XRD-PW 1700, Philips, USA) in the diffraction angle range between 5.00 and 60.00, 2θ, using Cu Kα radiation generated at 40 KV and 40 mA.

The DSC unit (Netzsch DSC-200 PC Phox, Germany) was used to analyse the thermal properties of the films and chitosan in the temperature range of 30-250 ^{o}C at a heating rate 10 ^{o}C/min in nitrogen atmosphere. The DTA-TG analysis of the prepared films were performed in closed alumina crucible in the temperature range of 25-650 ^{o}C at a heating rate of 10 ^{o}C/min in nitrogen environment.

The tensile strength of the films was determined using Hounsfield H10KS tensile testing machine (Horsham, PA) with a crosshead speed of 5 mm/min at 16 ^{o}C temperature and 60 % relative humidity.

2.5 Swelling Study

The prepared films were immersed directly into the buffer solutions of pH 1.4, 1.6, 3.6, 4.6, 5.4 and 7.6 (prepared as per Indian Pharmacopoeia 1996, Ministry of Health and Social Welfare, New Delhi, India) at room temperature (25 °C) for 72 hr and were weighed accurately (W_s). Thereafter, the swollen products were dried at 37 °C under vacuum to a constant weight. The equilibrium percentage of swelling (% swelling) of the product was calculated as per eq. 1.

$$\% \text{ Swelling} = [(Ws-W_d)/W_d] \times 100 \quad (1)$$

Where W_s = the weight of the swollen test sample after 72h

W_d = the weight of the dried test sample

2.6 Drug release Study

The SA-loaded film of dimension 2 cm x 2 cm was put in a beaker containing 100 ml of 0.01 N HCl continuously stirred at 100 rpm using a magnetic stirrer. Samples of 2 ml were periodically withdrawn from the beaker and simultaneously replaced with an equal volume of fresh dissolution media. The SA release study was conducted for a period of 90 minutes and the collected samples were analyzed spectrophotometrically at 294 nm wavelength using a UV-Vis spectrophotometer for determining the concentration of SA.

2.7 Hemocompatibility Test

The samples were analysed for hemocompatibility as described by Pal et al. [8]. % hemolysis was calculated as per the following formula:

$$\% \text{ Hemolysis} = \{(OD_{test} - OD_{negative}) / (OD_{positive} - OD_{negative})\} \text{ X } 100 \quad (3)$$

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2.8 MTT assay test

The patches were cut into 7 mm x 7 mm dimension and were treated with 0.1 M glycine solution for 4 hr to neutralize un-reacted GA, if any. The samples were placed on polystyrene petri plates and were sterilized by ethanol-UV treatment. This was followed by seeding with 20 µl of L929 fibroblast cell suspension (4 X 10^5cell/ml) and incubation at 37^{o}C for 1 hr to allow cell adherence on the surface of the samples. Thereafter, the samples were incubated for another 3 days after addition of 2 ml of DMEM medium. After the incubation period, 200 µl of MTT dye solution in DMSO (4 mg/ml) was added to the system and incubated for another 3 hr. Finally, the media was drained out from petriplate followed by the addition of 400 µl of DMSO for the development of the characteristic colour. The coloured solution, hence produced, were analysed spectrophotometrically at 570nm [9,10].

Relative cell proliferation was measured by using the following formula:

$$R_p = (A_{test} / A_{control}) \quad (4)$$

Where, R_p = Relative cell proliferation.

A_{test} = Absorbance of the hydrogel membrane containing sample.

$A_{control}$ = Absorbance of the control.

3. Results and discussion

3.1 FTIR Characterization

The FTIR spectra of CS, MA, CS-MA solution and crosslinked films are shown in **Fig. 1** & **Fig 2**. FTIR spectra of CS indicated the presence of saturated hydrocarbon chromophore. The peak at 2919 cm^{-1} indicated alkane C-H stretching vibration while the other peaks at 1381 cm^{-1} and 1423 cm^{-1} indicated alkane C-H bendings. The broad stretching peak at 3442 cm^{-1} can be attributed to either the hydrogen bonded O-H or N-H (primary amines) bonds or both. The peak at 1598 cm^{-1} is due to the bending vibration of the N-H bonds of the primary amine group. The peak at 1154 cm^{-1} corresponds to the anti-symmetric stretching of the C-O-C bridge while the peak at 1078 cm^{-1} is due to the skeletal vibrations involving the C-O stretching and are generally regarded as the fingerprint peak for the saccharide structure of chitosan. The FTIR spectra of MA indicated a broad peak at 2930 cm^{-1} which can be attributed to the O-H stretching of carboxylic acid groups present in MA. Peaks at 1697 cm^{-1} and

1636 cm^{-1} confirm the presence of carbonyl group in –COOH functional group and –C=C- stretching respectively within its molecular structure.

The broad peak at 3435 cm^{-1} present in the CS-MA solution spectra, indicates the presence of strong intermolecular hydrogen bonding among the CS and MA molecules. The crosslinked CS films shows peaks at 1572 cm^{-1} indicating the formation of imine bonds due to cross-linking reaction of free amino groups of CS with the aldehydic groups of GA. The presence of peaks at 1756 cm^{-1} and 1761cm^{-1} indicated the presence of free COO^- stretching while the peaks at 1637 cm^{-1}, 1635 cm^{-1} and 1636 cm^{-1} can be attributed to the presence of NH_3^+ absorption in xero-gels. The crosslinking of the CS with GA resulted in a products showing reduced absorption for the O-H and N-H stretching vibration peaks. The absence of peak in the 1740-1720 cm^{-1} region indicated the absence of aldehydic group in the crosslinked product and hence any residual unreacted GA.

The above discussion confirms that the water insoluble pure CS is completely solubilised in MA due to the protonation of the amine groups within the chitosan polymeric network thereby forming NH_3^+ ions.

3.2 XRD Characterization

XRD pattern of pure CS and crosslinked CS films have been studied in the 2θ range of 5^o to 60^o. The X-ray diffraction profile of CS shows peaks at ~23^o and ~50^o 2θ (**Fig. 3**). The crystallinity of the CS (~ 3.02 %) increases with the increase in degree of deacetylation and is attributed to increase in intermolecular hydrogen bonding due to the presence of more free NH_2 groups (higher degree of deacetylation) within the molecular structure, which in turn results the better packing of the macromolecular polymeric chains and consequent increase in the crystallinity [11].

The XRD patterns of the membranes are shown in **Fig. 3**. The 1G membrane showed only one broad hump at ~ 21^o 2θ while the other films showed three more peaks at ~29°, ~42° and ~50° 2θ in addition to the peak at 21^o 2θ. The % crystallinity of the films, calculated from the XRD patterns, were found to be 6.13, 16.58, 17.78, 15.9, and 12.99 for 1G, 2G, 5G, 7G and 9G films respectively. The crystallinity of the membranes increased with increase in MA content of the membranes upto 5G and thereafter decrease in crystallinity was observed with further increase in MA content of the membranes. This result can be attributed to the increase in intermolecular hydrogen bonding with increasing MA concentration up to 5G and then decrease in

intermolecular hydrogen bonding due to the steric hindrances induced at higher MA concentration.

3.3 Tensile Study

The tensile strengths of the crosslinked chitosan films are shown in **Fig. 4**. The results indicate improvement in mechanical properties with the increase in MA concentration up to 5G where the tensile strength at failure was 24 ± 2 MPa. Thereafter, there was a decrease in the mechanical properties with the subsequent increase in the MA concentration. The results can be explained with the results of XRD experimentation where it was found that with the increase in MA concentration, there was a subsequent increase in intermolecular hydrogen bonding up to 5 G and thereafter there was a decrease in intermolecular hydrogen bonding.

3.4 Thermo-gravimetric Study

Pure Chitosan and the prepared membranes showed 2 major weight loss steps during the thermal scanning from the temperature of 50 ^{o}C to 650 ^{o}C. The first weight loss transition may be accounted to the release of the absorbed moisture by chitosan (polysaccharide skeleton) having hydrophilic groups, viz. hydroxyl and amino. This absorbed moisture or bound water is slowly released when the polymer is heated to ~100 ^{o}C. Similar responses were also obtained when the crosslinked gels were subjected to similar treatment. But with the increase in reactive solvent in the samples 1G to 5G, there was a subsequent decrease in the weight loss from 10 % (approx.) to 4 % (approx.) due to thermal treatment and can be accounted to the increased stability of the polymer matrix. Thereafter, with the increase in the reactive solvent concentration the thermal stability of the polymer matrix decreased and the % weight loss again increased to 10 % (approx.).

The second weight transition showed a weight loss of 40 % (approx.) for chitosan and the prepared films. The weight loss during this phase followed similar pattern. With the increase of the concentration of reactive solvent (MA) medium the resulting films showed higher thermal stability from 2G to 5G which is mainly due to the presence of better macromolecular chain alignment, packing and the higher number of H-bondings within the film and can be easily correlated with their DSC & XRD results.

3.5 DSC Study

Fig. 6 shows the differential scanning calorimetric scans of the prepared hydrogels. Small endothermic humps in DSC curves for each of the membranes are due to their glass transition (Tg) of molecules at the particular temperature [12]. Glass transition temperature of prepared chitosan was found to be at ~130^{o}C. The glass transition temperature for the 2G, 5G, 7G and 9G membranes are ~153 ^{o}C, ~155 ^{o}C, ~152 ^{o}C and ~146 ^{o}C, respectively. Higher Tg value of 5G film can be attributed to the higher intermolecular hydrogen bonding resulting in restricted chain mobility, also supported well by results from other characterization techniques.

3.6 Swelling Study

Fig. 7 shows the pH dependent swelling behavior of cross-linked chitosan hydrogels under equilibrium swelling conditions which can be attributed to the presence of ionisable groups within the gel structure [13]. Swelling behaviour of the hydrogels was investigated in buffer solutions of pH 1.4, 1.6, 3.6, 4.6, 5.4 and 7.6 at room temperature. The hydrogel films indicated highest swelling properties at a pH of 1.4 and 1.6. Thereafter, with the increase in the pH there was a decrease in the swelling properties with lowest swelling properties at the pH of 7.6. The observed results can be attributed to the presence of free amino groups which undergo protonation at lower pHs. As the pH of the external medium is increased there is a subsequent decrease in the ionization of the free amino groups thereby resulting in the reduced water uptake of the membranes [13, 14].

3.7 Hemocompatibility Study

The hemocompatibility test results for crossed linked chitosan films have been summarized in **Table 2.** The hemolysis of the blood in the presence of crosslinked chitosan films was found to be below 5 % indicating that the prepared hydrogel films were highly hemocompatible [8].

3.8 MTT assay test

The MTT assay helps in the determination of the viability of the cells when incubated in the presence of samples. The test helps in the estimation of the mitochondrial impairment, which can be generally correlated with cell proliferation and has been used for the preliminary *in vitro* cytotoxic screening of the developed products. The

relative proliferation of the L929 murine fibroblast cells in the presence of 5G, 7G and 9G samples were found to be 1.1, 1.3 and 1.0, respectively. The results indicated that the developed hydrogel films were cytocompatibile in nature and hence could be tried for the development of products for biomedical applications.

3.9 Drug Release study

In general, the release of the drug is maximum when the polymer matrices are fully swollen. Hence in the current study, the release studies were carried out in 0.01 N HCl media so as to simulate gastric conditions. The SA release from Patch A and Patch B films in 0.01 N HCl media (**Figure 8**) indicated a quick release of the drug in Patch-B when compared to Patch-A. This can be attributed to the fact that in Patch-A, SA has been dispersed prior to the addition of the chitosan which resulted in the uniform dispersion of the SA deep within the chitosan polymeric network. While in Patch-B, SA was added after the dissolution of the chitosan thereby preventing the uniform dispersion within the polymeric network due to the viscous nature of the chitosan solutions. The dispersion of SA within the polymeric network in Patch-A resulted in the increased resistance to the diffusion of SA when compared to Patch-B.

The drug release from the diffusion controlled release system follows either Higuchian or Fickian kinetics. The matrix type release system, where the drug molecules are uniformly dispersed within polymer matrices, generally follow Higuchian kinetics [15-17]. The model assumes that the drug present in the outer layer is depleted followed the depletion of the drug from the next available layer until the polymer matrix is devoid of any drug. The polymer matrix devoid of any drug is known as polymer ghost. The release kinetics of SA (**Fig. 9**) from all the patches indicated Higuchian kinetics indicating that the delivery system to be a matrix type release system.

Drug release patterns from Patch-A and Patch-B indicated that these patches can be used as controlled drug delivery system.

4. Conclusions

The current work describes the successful development and characterization of GA crosslinked chitosan films with different reactive solvent composition. FTIR studies confirmed the formation of imine bonds during the crosslinking reaction with GA in addition to the presence of ammonium ions. The properties, *viz.* crystallinity, tensile

strength and thermal stability, of the films showed improvement were were found to be optimum for the membrane abbreviated 5G. The prepared membranes showed pH-dependent swelling behaviour with a higher swelling at lower pHs. The prepared membranes showed excellent hemocompatiblity to human blood and were also found to be highly cytocompatible to L929 fibroblast cells. The films loaded with SA showed Higuchian kinetics and quicker release of SA from the Patch-B as compared with patch-A. The above preliminary results indicate that the developed films could be tried for the development of delivery systems in the gastric environment.

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Captions to Tables and Figures

Table 1: Composition of the samples

Table 2: Hemocompatibility test results of chitosan cross-linked hydrogels.

Figure 1: Stacked FTIR spectra of CS and MA.

Figure2: Stacked FTIR spectra of CS-MA solution and 1G hydrogel.

Figure 3: XRD patterns of chitosan and developed membranes.

Figure 4: Tensile Strength pattern of chitosan cross-linked hydrogels (1G, 2G, 5G, 7G&9G)

Figure 5: TG plot of pure chitosan and chitosan crossed linked hydrogels (2G& 9G)

Figure 6: DSC thermogram of chitosan cross-linked hydrogels (2G, 5G, 7G & 9G)

Figure 7: Swelling nature of chitosan cross-linked hydrogels (1G, 2G, 5G, 7G&9G).

Figure 8: Drug release profile of chitosan cross-linked SA loaded hydrogels of Patch-A and Patch-B

Figure 9: Drug release kinetics from chitosan cross-linked films of Patch-A and Patch-B

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Table1:

Designation	Description
CS	Chitosan
MA	Methacrylic acid
1G	Chitosan+ glutaraldehyde+ 1% methacrylic acid
2G	Chitosan+ glutaraldehyde+ 2% methacrylic acid
5G	Chitosan+ glutaraldehyde+ 5% methacrylic acid
7G	Chitosan+ glutaraldehyde+ 7% methacrylic acid
9G	Chitosan+ glutaraldehyde+ 9% methacrylic acid
5G+SA (A)	Chitosan cross-linked (5G) SA loaded hydrogels of Patch-A
5G+SA (B)	Chitosan cross-linked (5G) SA loaded hydrogels of Patch-B
7G+SA (A)	Chitosan cross-linked (7G) SA loaded hydrogels of Patch-A
7G+SA (B)	Chitosan cross-linked (7G) SA loaded hydrogels of Patch-B
9G+SA (A)	Chitosan cross-linked (9G) SA loaded hydrogels of Patch-A
9G+SA (B)	Chitosan cross-linked (9G) SA loaded hydrogels of Patch-B

Table 2:

	OD at 545nm	**% Hemolysis**	**Remarks**
Positive control	0.597	-	-
Negative control	0.031	-	-
1G	0.041	1.76	Highly hemocompatible
5G	0.040	1.59	Highly hemocompatible
7G	0.043	2.12	Highly hemocompatible
9G	0.038	1.23	Highly hemocompatible

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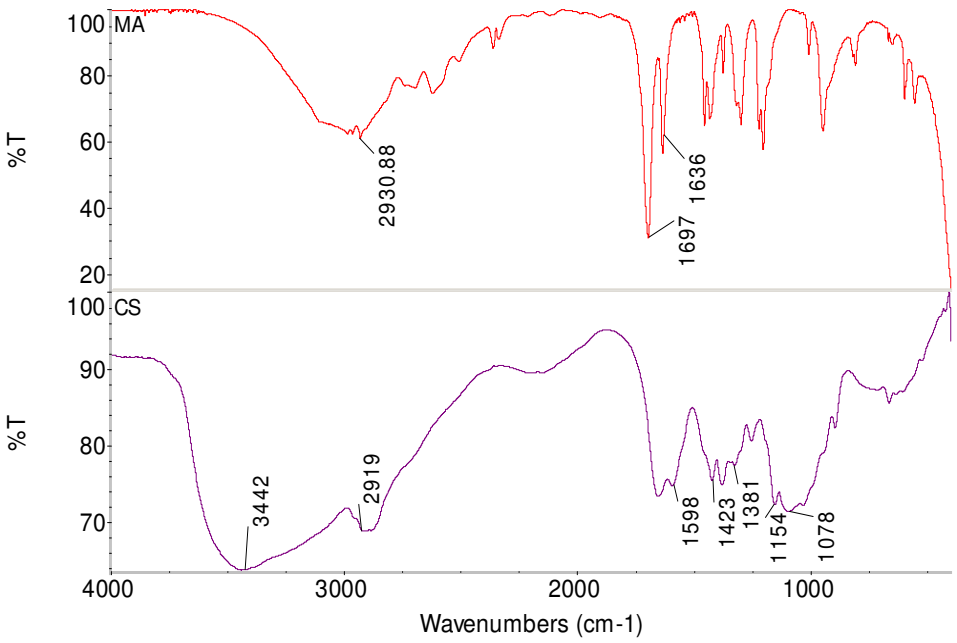

Figure 1:

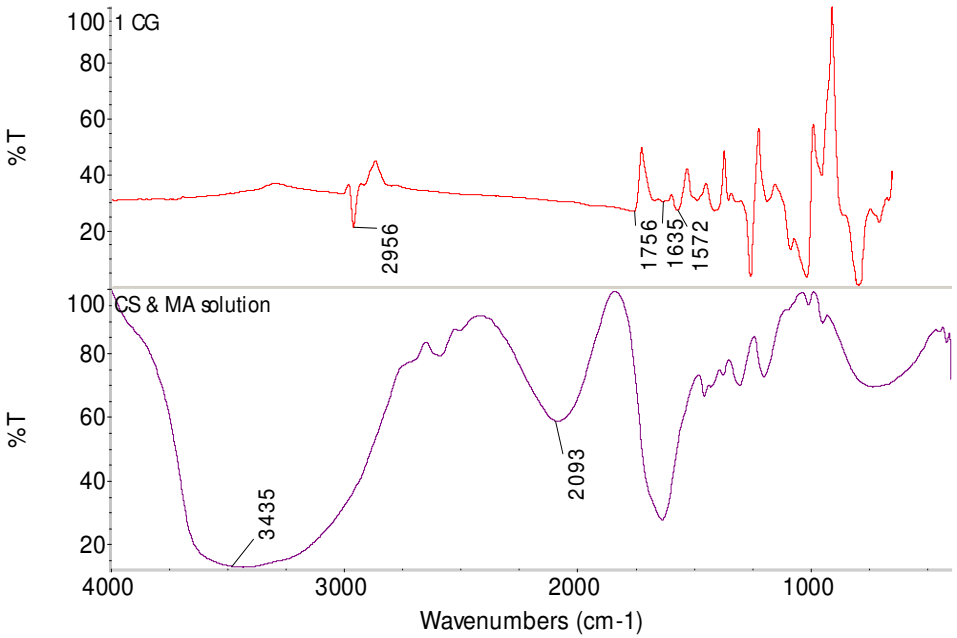

Figure2:

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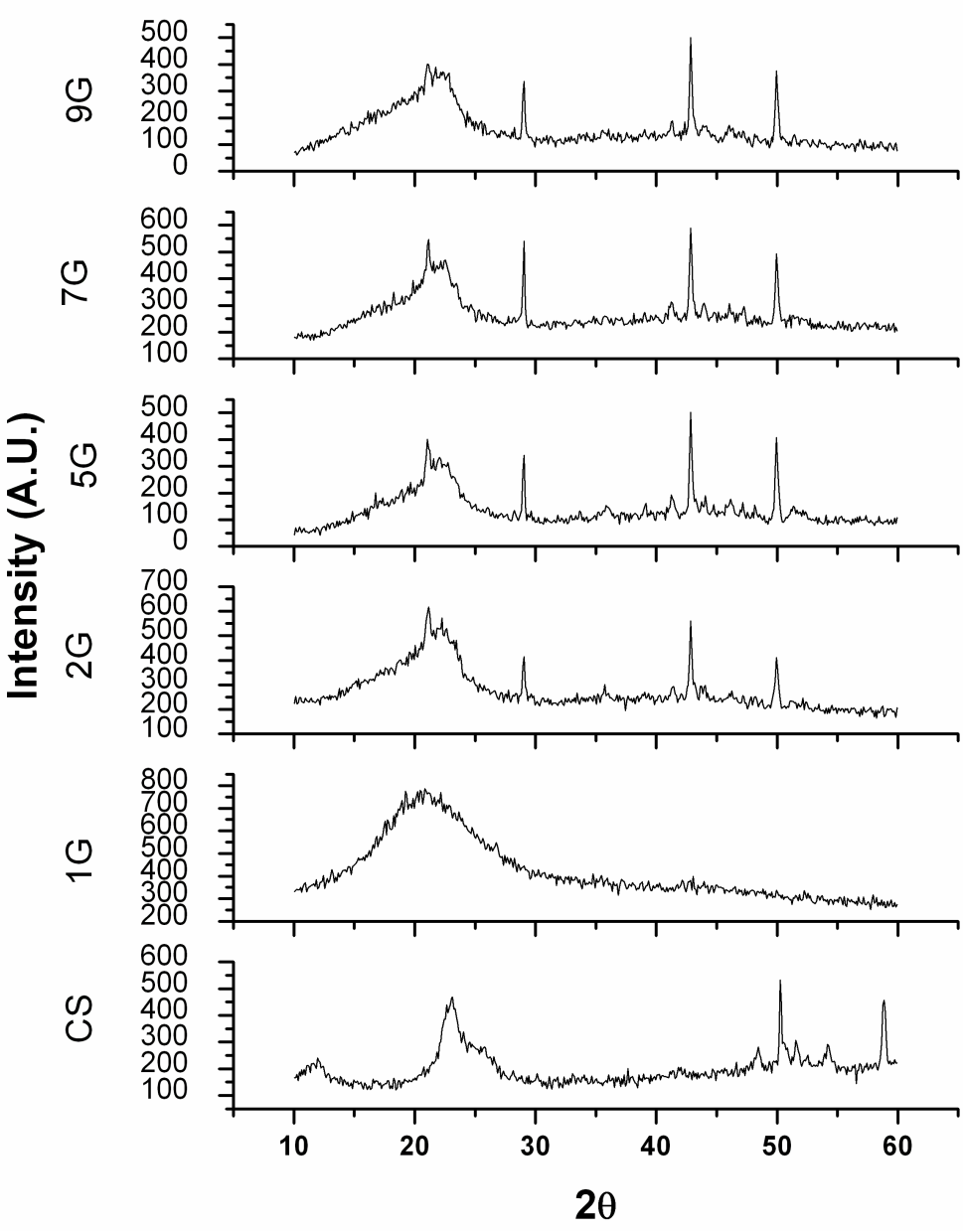

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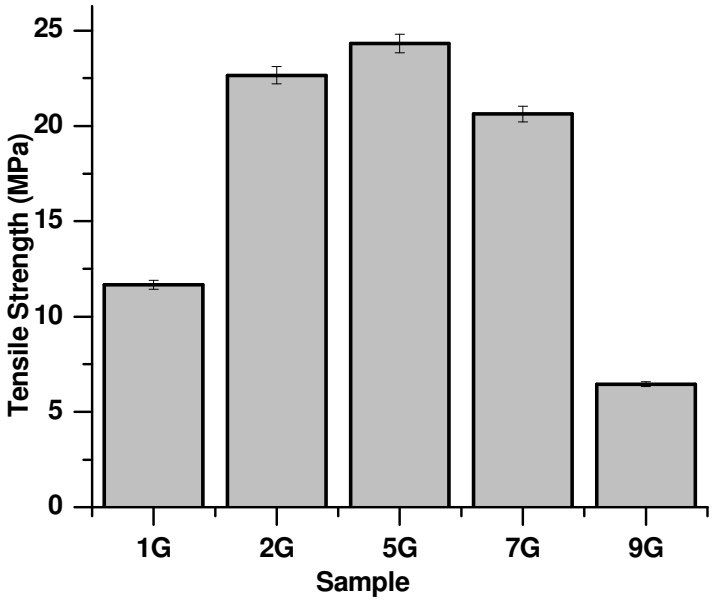

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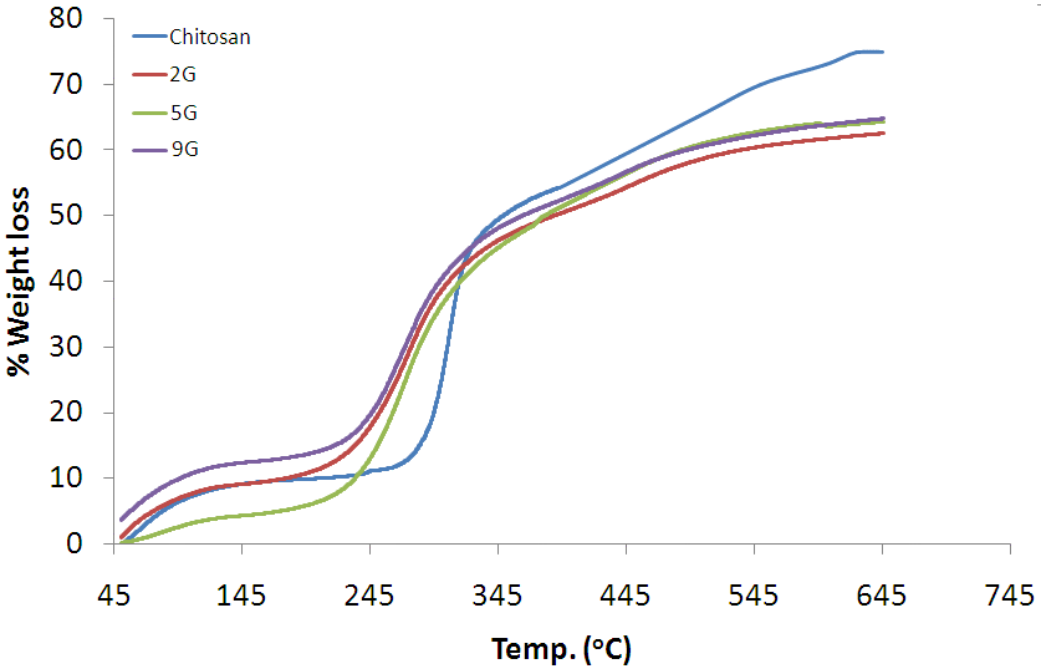

Figure 5:

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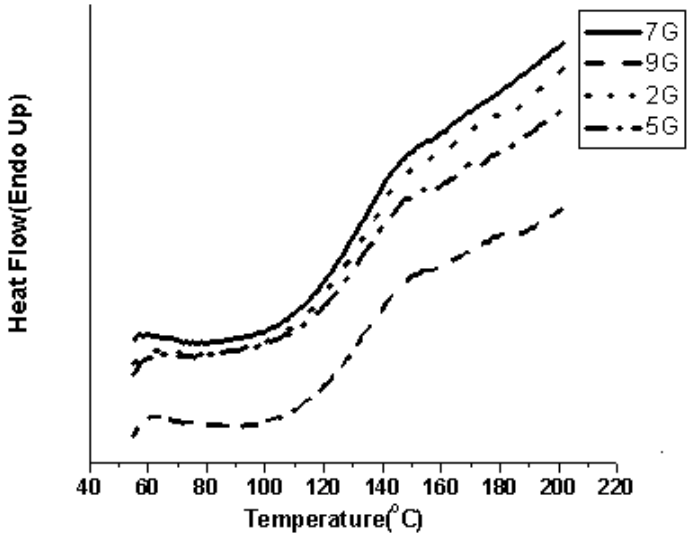

Figure 6:

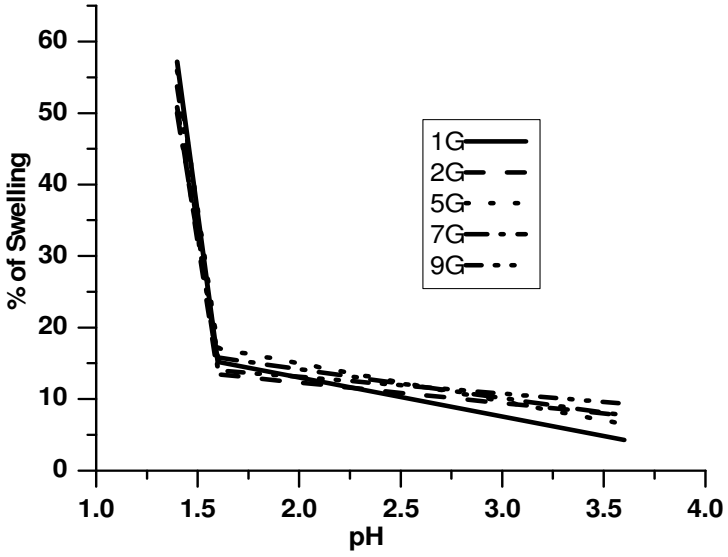

Figure 7:

Source: Designed Monomers & Polymers, Volume 13, Number 3, 2010, pp. 193-206(14)

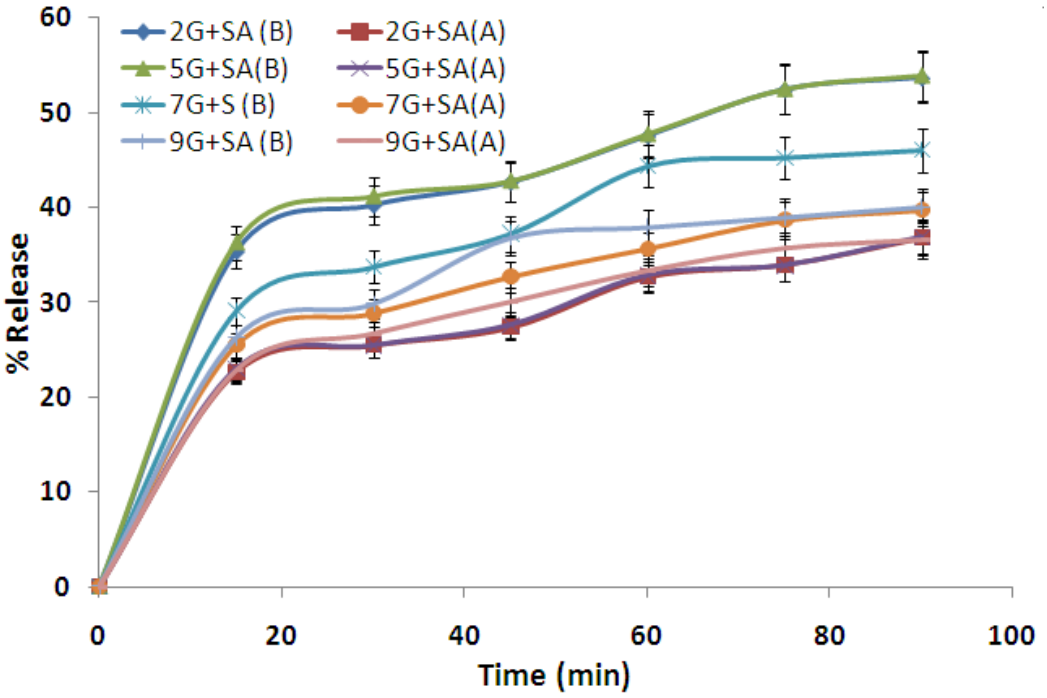

Figure 8:

Source: Designed Monomers & Polymers, Volume 13, Number 3, 2010, pp. 193-206(14)

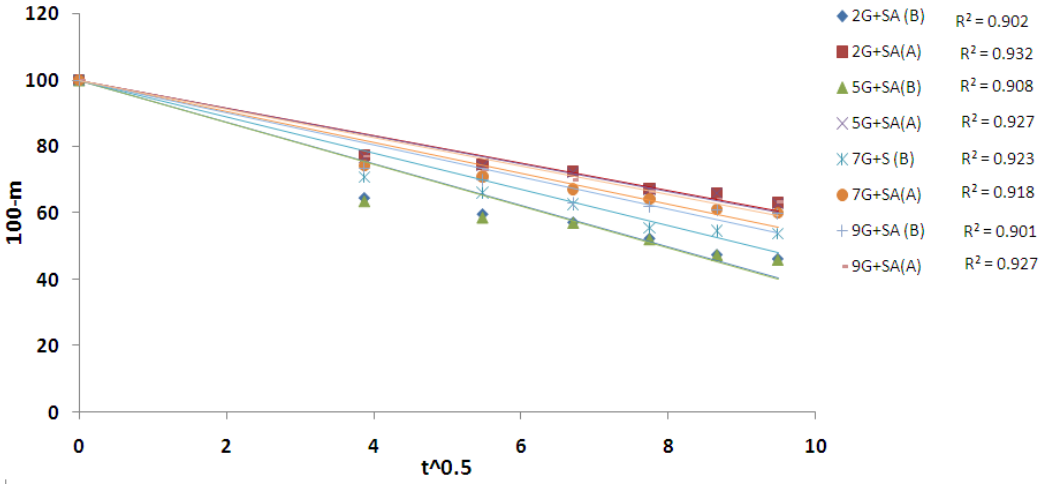

Figure 9: