

Synthesis, Characterization, Swelling Behavior and Metal Uptake Studies of Dichloroglyoxime Crosslinked Chitosan Derivative

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ABSTRACT

New crosslinked derivative of chitosan was prepared by the condensation reaction of chitosan (CS) and dichloro glyoxime. Structural analysis of chitosan derivative (CSL) was performed by elemental analysis (C, H, N), SEM measurements, FT-IR, solid-state ^{13}C CP/MAS NMR, XRD-powder and TGA. Crosslinking ratio was determined from the elemental analysis by using the C/N and found to be 70%. The swelling behavior of derivative was investigated on pH 3-7 and 10 at 25 and 37°C. Metal ion uptake capacity was studied towards of selected transition metals Cu(II), Ni(II), Co(II), Fe(II) and (Cd(II) cations in aqueous medium. According to the results of the analyses; water retention capacity and metal uptake capacity of new cross-linked chitosan derivative is higher than chitosan and metal uptake sequence are Cu(II) > Fe(II) > Cd(II) > Co(II) > Ni(II) respectively.

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INTRODUCTION

Hydrogels are physically or chemically cross-linked hydrophilic polymeric structures. They absorb a large amount of water (or fluids) and swell [1]. The hydrogels are separated into two groups, natural (biopolymer based) and synthetically [2]. Today, there is growing interest to hydrogels due to their, biocompatibility, biodegradability and tissue mimicking properties. [3].

Biopolymers are derived from natural materials such as crustaceans, fungi and wood. Among the biopolymers, Chitosan has great potential in hydrogel preparation. [4].

Chitosan biopolymer is an amino polysaccharide which is biocompatible, biodegradable, non-toxic and

also antimicrobial. It is open to chemical and mechanical modification in order to gain new features and functions [5,6].

Chitin and CS are important biopolymers because of the presence of the amino groups, they can be easily derivatized to desired reactive properties, biological compounds and soluble polymers [7,8].

The derivatization of chitosan can increase the complexing capacity of chitosan with metals, which enhances the adsorption properties of chitosan. [9,10].

Cross-linked polymers with the ability to swell by holding more solvent than 20% of their mass are termed xerogel. When the solvent is water, these crosslinked

structures take the name of hydrogel [11].

Hydrogels in the aqueous medium swell by holding water. Due to this ability they are used in adsorbing on areas such as water purification, heavy metal removal, dewatering, controlled release of fertilizers and medicines, ion exchange applications, chromatographic applications, solvent extraction, removal of water from industrial wastes containing petroleum and oil, and prevention of corrosion in telecommunication [12,13].

It is important to examine swelling properties in the characterization of cross-linked polymers that exhibit swelling behavior. For this purpose, it must first be created in the swelling curves. Swelling curves are formed by monitoring changes in the mass or volume of the polymer containing the appropriate solvent over time [14,15].

At acidic pH (3.5-5.5), the complex formation between the metal ion and the chitosan derivative reduce as the free amine groups of the chitosan are protonated [16]. Amino groups are present as free amines at pH 6.5-7.0 and the metal retention capacity of the chitosan derivative increases as the phenolic OH groups which may be present in the chitosan derivative at this pH value are deprotonated [16,17].

The aim of this work is crosslink of chitosan with dichloroglyoxime and characterization of this new crosslinked chitosan derivative. In addition, we aimed to investigate their metal ions uptake capacities and examination of swelling behaviors as application parts of this study.

MATERIALS AND METHODS

Materials

Dichloro glyoxime was synthesized and purified according to reported literature procedure [18].

Crosslinked chitosan derivative was prepared from dichloroglyoxime and chitosan. All reagents and solvents

were reagent-grade quality and they were obtained from commercial suppliers and were purified before use.

Stock solutions of Cu(II), Ni(II), Co(II), Fe(II) and Cd(II) were prepared by dissolving the analytical grade metal salt in deionized water.

C, H and N elemental analyses of the compounds were made with LECO-CHNS-932. FTIR analysis was carried out with Perkin Elmer spectrum two FTIR spectrophotometer, equipped with a diamond tipped ATR accessory. The cell was cleaned with acetone after each spectrum. ^{13}C NMR spectrum was obtained by a Bruker Superconducting FT/NMR Spectrometer Avance TM 300 MHz WB with CP/MAS technique (cross-polarization, magic-angle-spinning). The X-ray diffraction patterns of sample were recorded at room temperature on a using a Rigaku System RadB X-Ray Diffractometer, using monochromated Cu K α radiation in the range 2–40° (2 θ), at 25 °C. Thermogravimetric analysis (TGA) of the sample was performed with DuPont 951 thermal analyzer under air atmosphere, heated from 20 °C to 800 °C at a heating rate of 10 °C min⁻¹. Morphological analysis of images obtained by the scanning electron microscopy JEOL 5500 / OXFORD Inca-X (SEM). Metal analyses were carried out by MP-AES: Agilent 4100.

Methods

Synthesis of Crosslinked Chitosan Derivative (CSL)

1 g Chitosan was stirred in a 1% solution of 100 ml of acetic acid at 60 °C for 1 hour to obtain a homogeneous mixture. To the resulting chitosan solution, solution of dichloroglyoxime (5.83 mmol, 0.91 g) in 50 mL of ethanol was added and boiled under reflux for 12 hours. The temperature of the solution was reduced to room temperature by stirring and at the end the mixture was precipitated in the form of Chitosan derivative gel by adding 1 M NaHCO₃. The gel was filtered and dried at 70 °C in vacuum oven. The gel was purified by soxhlet extraction using ethanol as solvent.

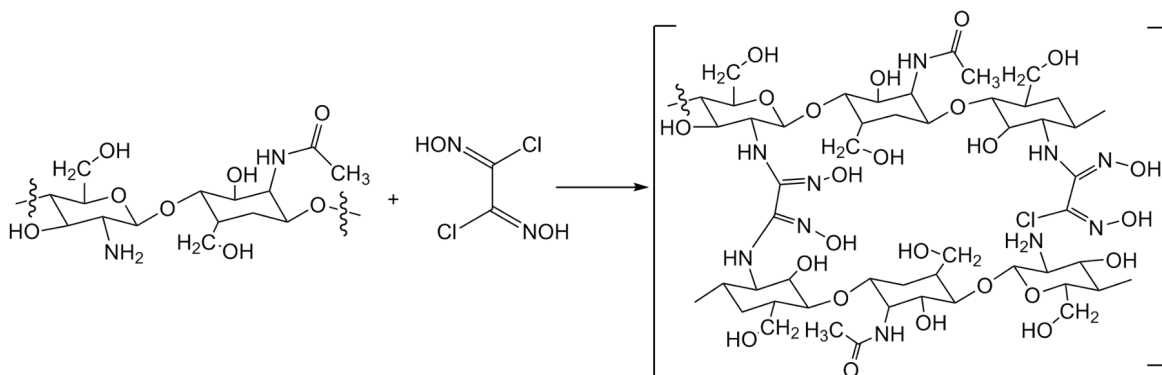


Figure 1. Synthesis of crosslinked chitosan derivative

The synthetic route is shown in Fig. 1. chitosan amine group displaced the chloride of dichloro glyoxime and gave the desired crosslinked product.

Metal Ion Uptake Studies

Study of metal ion uptake was performed at 25 °C by batch technique in aqueous metal solutions. The CSL powder was added to a known volume and concentration of the metal solution and the heterogeneous mixture was agitated two hours over orbital shaker. pH of the solution was adjusted using acetic acid/sodium acetate buffer for pH 6.

The studies were carried out by shaking 50 mg of CSL in 50 ml of 100 ppm Cu(II), Ni(II), Cd(II), Co(II) and Fe(II) solutions on an orbital shaker in buffered medium for three hours at 25 °C. After that, the suspension was filtered and diluted (1:20) and amounts of metal ions in the filtrate were determined by MP-AES.

As a result of MP-AES analysis the contents of Cu(II), Co(II), Ni(II), Cd(II) and Fe(II) in the diluted filtrate were found as 0.26; 2.86; 3.05; 1.65 and 1.10 ppm respectively.

According to the calculations, percent of the metal uptake of Cu(II), Co(II), Ni(II), Cd(II) and Fe(II) were found as: 94.80%; 42.80%; 39.00%; 67.00% and 78.00% and the amount of metal for per gram CSL was found as: 94.8; 42.8; 39.00; 67.00 and 78.00 mg/g respectively.

Swelling Studies

The swelling experiments of the derivative were carried out in the water medium at 25 and 37 °C and pH 3.0, 7.0 and 10.0. 0.1 M NaOH and 0.1 M HCl were used to prepare the pH solutions. The samples were weighed at a sensitivity of 0.0001 g and placed in a water environment sensitive to ± 0.1 °C. When the hydrogel was left in the solution, t=0 was taken and the hydrogel removed from the water at certain time intervals was dried and weighed. After 180 minutes, the experiment was terminated because of the swelling slowed.

RESULTS AND DISCUSSION

Solubility

Chitosan is insoluble in water, organic solvents and aqueous bases, but soluble in acids such as acetic, nitric, hydrochloric, perchloric and phosphoric acid. [8,19].

As a result of the derivatization, the solubility of the chitosan derivative decrease due to the decrease of free amine groups by cross-linking of chitosan. Consequently, crosslinked chitosan derivative lesser soluble in acids than chitosan.

Elemental Analyses

The C, H and N elemental analysis results of CS and CSL were given in Table 1 and the C/N ratio was calculated and added to the chart. In the chitosan derivative, C% and H% content were remained constant and N% was increased due to the dioxime compound (include nitrogen atom) entering the reaction. This change is more easily detected by calculating C/N ratios. Therefore, it can be easily understood the reason of the decrease in the carbon to nitrogen ratio (C/N).

When the C/N ratios were examined, it was seen that the C/N ratio of CSL is lower than chitosan. This shows that the new chitosan derivative was obtained.

We can determine the degree of cross-linking and the rate of realization by substitution degree. The number of carbonyls attached to each NH₂ group in the chitosan was calculated as the binding degree (DS) by the following equation [20].

$$DS = \frac{a(C/N)_m - (C/N)_0}{n} \quad (1)$$

(C/N)_m is the derivatized chitosan C/N ratio, (C/N)₀ is the initial chitosan C/N ratio, 'a' and 'n' are the number of nitrogen and carbon contained after the chitosan has been modified, respectively. The substitution degree (DS) of CSL was calculated as 72.11%. Table 1.

Table 1. Operating conditions of the gas turbine power plant at -1 °C

	%C	%H	%N	C/N	DS	a	n
CS	40.95	6.96	7.41	5.53			
CSL	39.67	6.63	7.46	5.31	72.11	2	7

FT-IR spectra

In order to characterize the CSL, a spectrum of chitosan was also recorded. The main bands appearing in the IR spectra of the oxime compounds, the C=N stretching vibrations in the range of 1600-1665 cm⁻¹, the N-O vibration in the range of 940-885 cm⁻¹ and the O-H vibration in the range of 3500-3200 cm⁻¹ which were overlapped to the stretching vibration of N-H [21,22]. The vibrations in the range of 1680-1480 cm⁻¹ are attributed to amide (C=O), the vibrations in the range from 1160 cm⁻¹ to 1000 cm⁻¹, the bands near 1080-1025 cm⁻¹ are attributed to etheric CO and the peak at ~890 cm⁻¹ corresponds to wagging of the saccharide structure of chitosan appear [23,24].

It was observed that the FTIR spectra of chitosan (CS) and CSL were different from each other when they were compared. Despite the overlapping the peaks that the secondary amide band (1645 cm⁻¹), stretching vibrations of

OH groups and primary amine ($3500 - 3000 \text{ cm}^{-1}$) and CH deformation of the β -glycosidic bond ($\sim 890 \text{ cm}^{-1}$) of chitosan with the C=N band ($1600-1665 \text{ cm}^{-1}$), the N-O vibration bands ($940-885 \text{ cm}^{-1}$) and the O-H vibration bands ($3500-3200 \text{ cm}^{-1}$) of the glyoxime respectively.

The FT-IR spectra of CS and CSL are shown in Fig. 2. In the spectrum of the CSL, the NH stretching vibrations of chitosan disappeared and OH stretching vibrations were observed as a broad band in the range from 3000 to 3600 cm^{-1} , and the 1651 band shifted to 1658 cm^{-1} which can be attributed to the C=N of azomethine group respectively. These changes in the peaks show that the synthesis takes place.

On the other hand, similar spectra are obtained for the CSL-metals.

Morphology

The scanning electron micrographs (SEM) of chitosan (a) and CSL (b) were given in Fig. 3.

Chitosan film has a smooth surface. The SEM micrographs were indicating to significant differences among morphologies of chitosan and CSL. Therefore, these differences can be attributed to the formation of CSL.

X-Ray diffraction (XRD) study

The XRD spectra of pure chitosan (CS) and CSL were given in Fig. 4. It was previously reported that, CS exhibits

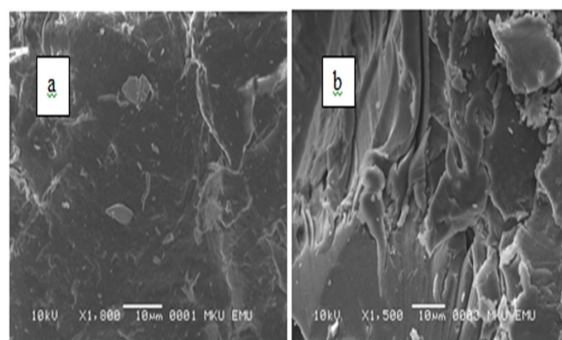


Figure 3. The scanning electron micrographs (SEM) of chitosan (a) and CSL (b)

two sharp crystalline peaks at $2\theta = 10^\circ$ and $2\theta = 20^\circ$ [25]. In the XRD patterns of CSL, the intensity of peaks decreased and broadening of the peaks was observed when compared with the free chitosan. These results suggest that CSL has less crystalline and more amorphous structure than the pure chitosan.

It is known that intermolecular and intramolecular hydrogen bondings which form between amino group and a hydroxyl group stabilize the crystalline structure of chitosan.

Crystallinity decreases by the deformation of some of inter and intramolecular hydrogen bonds, because of both the decreasing free amino group and steric hindrance after crosslinking [26-28].

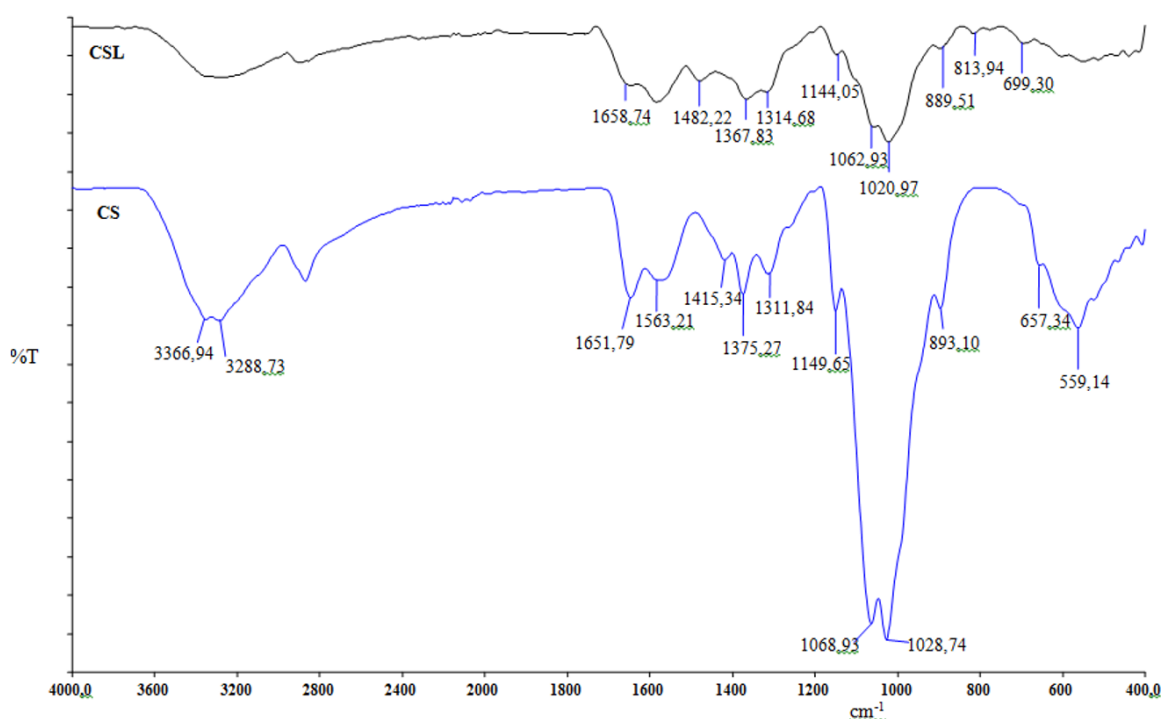


Figure 2. FT-IR spectra of CS and CSL

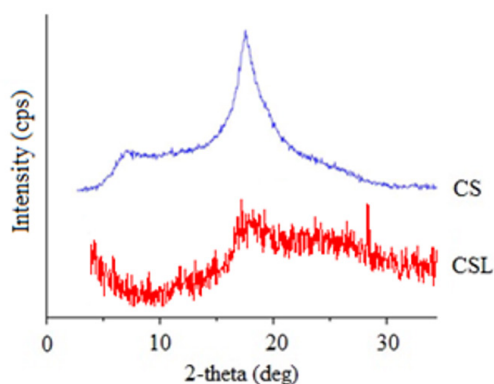


Figure 4. XRD Diffractograms of chitosan and its derivative

¹³C NMR analysis

¹³C CP-MAS solid-state NMR chemical shift values of chitosan were observed at 23 ppm ($-\text{CH}_3$), 58 ppm (C2), 61 ppm (C6), 76 ppm (C5,C3), 85 ppm (C4), 106 ppm (C1), 174 ppm (C=O) [29] and dichloroglyoxime azomethine (C=N) carbon were observed in the range 150-160 ppm [30,31].

When compared CSL with chitosan, in addition to the peaks of chitosan, observation of the azomethine peak of glyoxime at 160 ppm (azomethine C=N), confirming the formation of crosslinked chitosan derivative (Fig. 5).

TGA analysis

The thermal property of the CSL was investigated by TGA techniques under air atmosphere. The TGA curve of CSL was given in Fig. 6. It is well known that chitosan have two mass loss stages as water elimination and the decomposition. These stages were observed at 40–100 °C range and 280–400 °C range, respectively [32]. Therefore, in the thermogram of the CSL a weight loss of about 7-8% in the range of 50-150 °C was observed by means of the loss of water. In addition to the loss of water, CSL also displays other significant weight losses (40.75%) in the region 250-350 °C, attributable to the decomposition of chitosan chains. On the other hand, another weight loss (39.12%) was observed at 450-600 °C range, which attributed to the degradation and interchain crosslink of the chitosan as an exothermic process [33].

When the results of CSL compared with the results of the chitosan in terms of the thermal behaviors; there are many important differences can be observed might be due to the interaction between chitosan and dichloroglyoxime.

As a result, CSL exhibited a better final degradation temperature than chitosan.

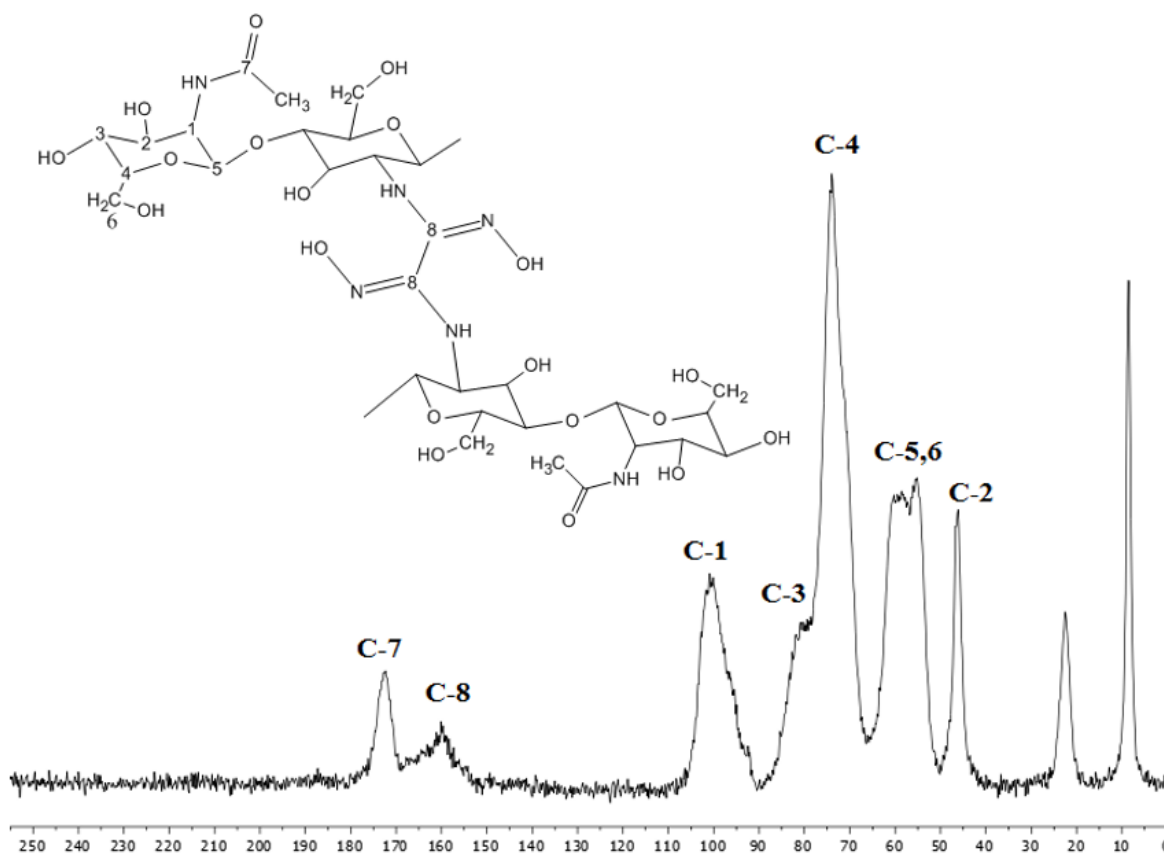


Figure 5. ¹³C CP-MAS NMR Spectrum of CSL

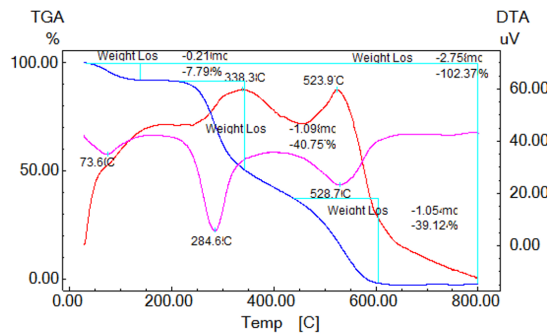


Figure 6. TGA curves of CSL

Metal Ion Uptake Results

Metal retention capacity of CSL (mg/g) was calculated by the following equation.

$$C = Wa/Wp \quad (2)$$

Where C is the metal holding capacity in mg/g, the amount of metal retained as *Wa* (mg), and *Wp* is the mass of the derivative in grams.

The results of metal ion uptake studies were given in mg/g for metal ion bound on the CSL. CSL exhibit higher uptake capacity for Cu(II) ion (94.8 mg/g) than the other metal ions used in the study. The capacities for the other metal ions were found as Ni(II) (39.00 mg/g), Fe(II) (78.00 mg/g), Cd(II) (67.00 mg/g) and Co(II) (42.8 mg/g). Thus, the obtained data showed that the order of the overall uptake capacity of the CSL as Cu(II) > Fe(II) > Cd(II) > Co(II) > Ni(II). As a result of this study CSL shows selectivity toward Cu(II), Fe(II) and Cd(II) ions.

Swelling Behaviors

Percent Swelling

Swelling % (S %); is calculated by the following equation.

$$S\% = \frac{W_t - W_o}{W_o} \times 100 \quad (3)$$

Where S%, *W_t* and *W_o* are percent of swelling, weight at different times and initial weight of the sample, respectively.

At equilibrium, hydrogel has the greatest swelling value.

With the help of the obtained data, swelling kinetics of network polymers can be determined by calculating values such as equilibrium percent swelling, swelling rate, constant initial swelling rate and theoretical equilibrium percent swelling [34].

Percent swelling rate (S%) was calculated according to equation 3. The values of percent swelling of CSL are given in Table 2.

Table 2. The values of percent swelling of CSL at different temperature and pH.

CSL (S%)	25°C			37°C		
	pH 3	pH 7	pH 10	pH 3	pH 7	pH 10
	86.4	99.6	128.3	99.2	101.0	158.8

In chitosan films, as the cross-linker concentration increased, the water retention capacity and the release of active substance from the film decrease [35].

The swelling curves at different temperatures and pH of CSL are given in Fig. 7 and 8.

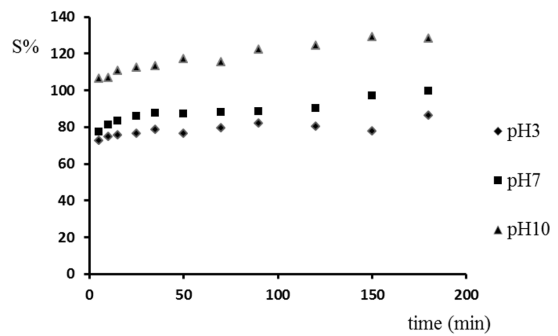


Figure 7. % Swelling values, at 25 °C and different pH values

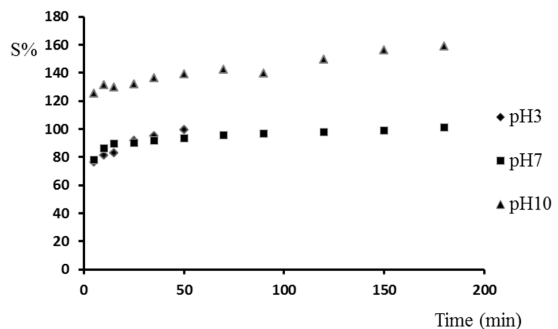


Figure 8. % Swelling values, at 37 °C and different pH values

Equilibrium Water Content (EWC)

Cross-linked polymers, after a certain time after having been exposed to the solvent environment, the rate at which the solvent enters and leaves the structure becomes equal and the balance is reached. At this point, hydrogel has the greatest swelling value.

For hydrogels in equilibrium with solvent, the equilibrium water content is calculated by equation 4. [36]

$$EWC = \frac{W_e - W_o}{W_e} \quad (4)$$

In equation, *W_e*; At equilibrium (swollen) polymer mass, *W_o*; shows the mass of dry polymer

The equilibrium liquid content is a parameter which is calculated for the hydrogel in this case and is very important for biocompatibility. The EWC values of hydrogel was given in Table 3.

Table 2. The EWC values of CSL.

	25°C			37°C		
	pH 3	pH 7	pH 10	pH 3	pH 7	pH 10
CSL	0.46	0.50	0.56	0.50	0.50	0.61

Swelling Kinetics Studies

For calculation swelling of polymers, the following second order kinetics equation can be used [37].

$$\frac{t}{s} = A + Bt \quad (5)$$

Where t is time, S is swelling at t, $B = 1/S_{max}$ is the inverse of the maximum or equilibrium swelling, $A = 1/kS S_{max2}$ is the reciprocal of the initial swelling rate $[(dS/dt)_0]$ of the hydrogel, and kS is swelling rate constant.

The initial swelling rate, the swelling rate constant and the values of theoretical equilibrium swelling of the hydrogel was calculated from the slope and the intersection of the lines, respectively. (see the Supporting Information) (Fig. S9-14) The results are presented in Table 4.

Table 2. The EWC values of CSL.

	25°C			37°C		
CSL	pH 3	pH 7	pH 10	pH 3	pH 7	pH 10
r_o	35.97	20.87	32.57	36.90	33.90	31.25
S_{max}	83.33	99.01	129.87	104.12	101.01	101.01
$k_s \times 10^{-3}$	5.18	2.13	1.93	3.40	3.32	1.24

CONCLUSIONS

In this work, a crosslinked chitosan derivative (CSL) was synthesized by using chitosan and dichloroglyoxime. The all data support that the chitosan is crosslinked with dichloro glyoxime.

Also, we have investigated swelling behaviors and Cu(II), Ni(II), Co(II), Cd(II), and Fe(II) metal uptake properties of the new derivative.

Highly crosslinked hydrogels have a tighter structure, and swell less than lower crosslinked hydrogels. Crosslinking hinders the mobility of polymer chain, hence lowering the swelling ratio.

It can be said that Chitosan was cross-linked too much when considering the swelling ratios. This is supported by

the DS. Increasing the degree of crosslinking of the system will result in a stronger gel and a higher degree of crosslinking creates a more brittle structure.

EWC value (0.61) of CSL at 37 °C and pH 10 is close to the percent water content values of the body 0.60 (or 60%). Accordingly, CSL exhibits liquid contents similar to living tissues and it can be use as hydrogel.

The CSL metal uptake order is Cu(II)> Fe(II)> Cd(II)> Co(II)> Ni(II). As a result of this study CSL shows selectivity toward Cu(II), Fe(II) and Cd(II) ions.

References

1. Peppas NA, Bures P, Leobandung W, Ichikawa H, Modular Hydrogels for Drug Delivery, *European Journal of Pharmaceutics and Biopharmaceutics* 50 (2000) 27–46.
2. Mogosanu GD, Grumezescu AM, Natural and synthetic polymers for wounds and burns dressing, *International Journal of Pharmaceutics* 463 (2014) 127–136.
3. Jayakumar R, Prabakaran M, Kumar PTS, Nair SV, Tamura H, Biomaterials based on chitin and chitosan in wound dressing applications, *Biotechnology Advances* 29 (2011) 322–337.
4. Boonsongrit Y, Mitrevej A, Mueller BW, Chitosan Drug Binding by Ionic Interaction, *European Journal of Pharmaceutics and Biopharmaceutics* 62 (2006) 267–274.
5. Pillai CKS, Paul W, Sharma CP, Chitin and chitosan polymers: Chemistry, solubility and fiber formation, *Progress in Polymer Science* 34 (2009) 641–678.
6. Jayakumar R, Prabakaran M, Nair SV, Tokura S, Tamura H, Selvmurugan N, Novel carboxymethyl derivatives of chitin and chitosan materials and their biomedical applications, *Progress in Materials Science* 55 (2010) 675–709.
7. Muzzarelli RAA, "Chitin", Pergamon Press, Oxford. 309, 1977.
8. Rinaudo M, Chitin and chitosan: Properties and application, *Progress in Polymer Science* 31 (2006) 603–632.
9. Rodrigues CA, Laranjeira MCM, De-Fávere VT, Stadler E, Interaction of Cu(II) on N-(2-pyridylmethyl) and N-(4-pyridylmethyl) chitosan, *Polymer* 39 (1998) 5121–5126.
10. Wan MW, Kan CC, Rogel BD, Dalida MLP, Adsorption of copper(II) and lead(II) ions from aqueous solution on chitosan-coated sand, *Carbohydrate Polymers* 80 (2010) 891–899.
11. Laftah WA, Hashim S, Ibrahim AN, Polymer hydrogels: A review, *Polymer Plastics Technology and Engineering* 50 (2011) 1475.
12. Rivas BL, Geckeler KE, Synthesis and metal complexation of poly(ethylenimine) and derivatives, *Advances in Polymer Science* 102 (1992) 173–183.
13. Rosso F, Barbarissi A, Barbarissi M, Petillo O, Margarucci S, Calarco A, Peluso G, New polyelectrolyte hydrogels for biomedical applications, *Materials Science and Engineering* 23 (2003) 371–376.
14. Evmenenko G, Alexev V, Budtova T, Buyanov A, Frenkel S, Swelling-induced changes of polyelectrolyte gels, *Polymer* 40 (1999) 2975–2979.

15. Dolbow J, Eliot F, Ji H, Chemically induced swelling of hydrogels. *Journal of the Mechanics and Physics of Solids* 52 (2004) 51–84.
16. Yang Z, Shu J, Zhang L, Wang Y, Preparation and adsorption behavior for metal ions of cyclic polyamine derivative of chitosan, *Journal of Applied Polymer Science* 100 (2006) 3018–3023.
17. Trimukhe KD, Varma AJ, A morphological study of heavy metal complexes of chitosan and crosslinked chitosans by SEM and WAXRD, *Carbohydrate Polymer* 71 (2008) 698–702.
18. Lance KA, Goldsby KA, Busch DH, Effective new cobalt(II) dioxygen carriers derived from dimethylglyoxime by the replacement of the linking protons with difluoroboron(1+), *Inorganic Chemistry* 29 (1990) 4537–4544.
19. Sankararamakrishnan N, Sanghi R, Preparation and characterization of a novel xanthated chitosan, *Carbohydrate Polymers* 66 (2006) 2, 160–167.
20. Sheldrick GM, SHELX 97 Programs for the refinement of Crystal Structures, University of Göttingen: Germany 1997.
21. Taner B, Deveci P, Bereket S, Solak AO, Ozcan E, The first example of calix[4]pyrrole functionalized vic-dioximeligand: synthesis, characterization, spectroscopic studies and redox properties of the mononuclear transition metal complexes, *Inorg Chimica Acta* 363 (2010) 4017–4023.
22. Coşkun A, Yılmaz F, Akgemici EG, Synthesis, characterization and electrochemical investigation of a novel vic-dioximeligand and its some transition metal complexes, *Journal of Inclusion Phenomena and Macrocyclic Chemistry* 60 (2008) 393–400.
23. Brugnerotto J, Lizardi J, Goycoolea FM, Arguëlles-Monal W, Desbrieres J, Rinaudo M, An infrared investigation in relation with chitin and chitosan characterization, *Polymer* 42 (2001) 3569–3580.
24. Kasaai MR, A review of several reported procedures to determine the degree of N-acetylation for chitin and chitosan using infrared spectroscopy, *Carbohydrate Polymers* 71(2008) 497–508.
25. Baran T, Açıksoz E, Menteş A, Highly efficient, quick and green synthesis of biaryls with chitosan supported catalyst using microwave irradiation in the absence of solvent, *Carbohydrate Polymers* 142 (2016) 189–198.
26. Wang X, Du Y, Fan L, Liu H, Hu Y, Chitosan–metal complexes as antimicrobial agent: synthesis, characterization and Structure–activity study, *Polymer Bulletin* 55 (2005) 105–113.
27. Franca EF, Lins RD, Freitas LCG, Straatsma TP, Characterization of chitin and chitosan molecular structure in aqueous solution, *Journal of Chemical Theory and Computation* 4(12) (2008) 2141–2149.
28. Wan Y, Creber KAM, Peppley B, Bui VT, Ionic conductivity and tensile properties of hydroxyethyl and hydroxypropyl chitosan membranes, *Polymer Physics* 42(8) (2004) 1379–1397.
29. Jothamani B, Sureshkumar S, Venkatachalapathy B, Hydrophobic structural modification of chitosan and its impact on nanoparticle synthesis-A physicochemical study, *Carbohydrate Polymers* 173 (2017) 714–720.
30. Serin S, New vic-dioxime transition metal complexes, *Transition Metal Chemistry* 26(3) (2001) 300–306.
31. Voloshin YZ, Kostromina NN, Roland K, Clathrochelates synthesis, structure and properties, Elsevier, Amsterdam, Netherlands, 2002.
32. Demetgül C, Serin S, Synthesis and Characterization of a New vic-dioxime of Chitosan Derivative and Its Transition Metal Complexes, *Carbohydrate Polymers* 72(3) (2008) 506–512.
33. Sharma AL, Saxena V, Annapoorni S, Malhotra BD, Synthesis and characterization of a copolymer: Poly(aniline-co-fluoroaniline), *Journal of Applied Polymer Science*, 81(6) (2001) 1460–1466.
34. Üzümlü ÖB, Karadağ E, Dye sorption and water uptake properties of crosslinked acrylamide/sodium methacrylate copolymers and semi-interpenetrating polymer networks composed of PEG, *Separation Science and Technology*, 46(2011) 489–499.
35. Duman SS, Şenel S, Kitosan ve Veteriner Alandaki Uygulamaları, *Veteriner Cerrahi Dergisi* 10(3–4) (2004) 62–72.
36. Saraydın D, Karadağ E, Isıkver Y, Sahiner N, Güven O, The influence of preparation methods on the swelling and network properties of acrylamide hydrogels with crosslinkers, *Journal of Macromolecular Science Part A: Pure and Applied Chemistry* A41(4) (2004) 421–433.
37. Karadağ E, Saraydın D, Swelling of Superabsorbent Acrylamide/Sodium Acrylate Hydrogels Prepared by Using Multi-Functional Cross-Linkers, *Turkish Journal Of Chemistry* 26 (2002) 863–875.