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Research Article

Synthesis and Physicochemical Characterization of New Amidic Derivative of Sodium Alginate

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ABSTRACT

In this study, our objective was to synthesis a new derivative of alginate, a natural biocompatible, biodegradable and non-toxic biopolymer to improve the gelling mechanism. For this purpose, ethylenediamine (EDA) was coupled to sodium alginate (NaA) in an aqueous-phase reaction using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl) and N-hydroxy succinimide (NHS) as coupling reagents to synthesis ethylenediamine-grafted amphiphilic sodium alginate-amide derivative (NaA-g-EDA). Synthesized derivative showed no environmental sensitivity but the swelling percentage of hydrogels shows that hydrogels obtained from NaA-g-EDA had higher water absorption compared to non-functionalized sodium alginate. It seems that hydrogels can uptake water more than 100% of their weight but in a slow manner. This character is a perfect property for wound dressing. Biodegradable synthesized hydrogel can be decomposed into non-toxic by-products. The purity and grafting of copolymers were characterized using fourier transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (^{1}HNMR), X-ray diffraction (XRD), and elemental analysis (CHNX). These analytical methods confirmed the structure of NaA-g-EDA.

Keywords: Density Functional Theory, High Spin, Low Spin, enthalpy, SrMnO3, CaMnO3

1. Introduction

Alginates are calcium, magnesium, and sodium salts of alginic acid. These biopolymers form by random copolymerization of β-1,4-d-mannuronic acid and α-1,4-l-guluronic acid. Linear alginate polymer chains contain multiple carboxyl groups that can bind to divalent cations (Ca^{2+}, Ba^{2+}) to promote the formation of cross-linked structures and are extracted from algae sources [1-3]. Alginic acid and alginate are used as emulsifiers, gelling agents, coating and thickening agents in the food industry, medicine, and biotechnology, in paper sizing, textile printing, as well as other gel-like pigment preparations and aqueous printing inks. Commercial alginates are mainly produced from Laminaria hyperborea, Macrocystis pyrifera, Laminaria digitata, Ascophyllum nodosum, Laminaria japonica, Ecklonia maxima, Lessonia nigrescens, Durvillaea antarctica, and Sargassum spp [4].

The association behavior and gelation of most polysaccharides are temperature dependent in many cases, while alginate gels are cold setting and more or less independent of temperature. Alginates have the capacity to absorb a large amount of water quickly. They are capable of taking up 200–300 times their weight in water forming a kind of viscous gum. Chemical modification of alginate by grafting some monomers such as vinyl monomers and then cross-linking the material is a way to improve the properties of the ionic alginate hydrogel [5].

Alginate has several hydroxyl (−OH) and carboxyl (−COOH) groups in its structure and the large number of lone pair electrons in each of these groups is an ideal candidate for chemical functionalization [6]. By forming alginate derivatives through functionalizing available hydroxyl and carboxyl groups, properties such as solubility, hydrophobicity, physicochemical and biological characteristics may be modified using techniques such as oxidation, sulfation, esterification, amidation, or grafting methods [7-9].

In this research, commercial alginate is functionalized with ethylenediamine which is a linear alkyl diamine using EDC-HCl/NHS as coupling agents to form amide linkages on the alginate backbone [10,11]. All products are characterized by elemental analysis, FTIR, ^{1}HNMR, and XRD.

2. Materials and methods

2.1. Materials

Sodium alginate (NaA) (14000 cps Sigma, Germany), Ethylenediamine (EDA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl), and N-Hydroxy succinimide (NHS), were purchased from Merck (Germany). Other reagents with an analytical grade were used as received without modification or purification.

2.2. Instrument

The structures of all samples were determined by Bruker Vertex 70 FTIR (Germany) and ^{1}HNMR by 500 MHz, Varian–INOVA (England). Elemental analysis was obtained on Costech ECS 4010 (Italy). XRD measurement of the product structure was investigated using X-ray, Seifert XRD 3003(Germany) electronic balance (Sartorius, BSA224S-CW, China).

2.3. Synthesis of the NaA-g-EDA polymers

Amination of alginate was carried out by Elsayed 2016 method with slight modification [12]. Briefly, 0.5g of sodium alginate was dissolved in 50 ml distilled water. Then 0.14 g NHS and 1.16 g EDC-HCl were added to the reaction mixture at room temperature and stirred until a homogeneous solution was obtained. Then, the solution of ethylenediamine was added to the reaction mixture, and stirring continued for 24 h in the dark at room temperature. The modified NaA-g-EDA was precipitated by adding an excess amount of acetone. The precipitates were filtered and washed several times with ethanol solution (ethanol: water (80:20) % v/v) to remove unreacted components. The grafted polymers were dried at 40 °C to

get a constant weight. The structure of the product was characterized by ^{1}HNMR, FTIR, XRD, and elemental analysis.

2.4. Hydrogel manufacture

Hydrogel was prepared after the reaction of ethylenediamine with sodium alginate. The hydrogel was prepared by dissolving (0.03, 0.05, and 0.07 g) of NaA powder and NaA-g-EDA in 2 ml of distilled water, followed by stirring until gel solutions were obtained. Finally, the hydrogel of NaA-g-EDA and NaA with three different concentrations 1.5, 2.5, and 3.5% (W/V) were prepared.

2.4.1. Phase transition temperature

The phase transition behavior of NaA and NaA-g-EDA were investigated at a temperature range of 5 to 65 ˚C in three different concentrations (1.5, 2.5, and 3.5%). 2 ml of each concentration of the samples was placed in a suitable test tube in a water bath in the temperature range of 5-65 ˚C, and the state of the hydrogel was examined for each degree of temperature change.

2.4.2. pH-induced phase transition

The swelling behavior and phase transition of NaA and NaA-g-EDA were studied at three different concentrations and two different pH (4.5, 6.8) at room temperature. Three different concentrations (1.5, 2.5, and 3.5%) of NaA, and its derivative were prepared by dissolving the powder of these substances in two buffer solutions with pH 4.5 and 6.8, and the gel state of each substance was examined at each pH.

2.4.3. Swelling behavior

The swelling studies were performed in distilled water at 25 °C. The amount of fluid imbibed as a function of time was calculated gravimetrically. The known amounts of dry gels were put in a petri dish containing 20 mL of swelling medium. Then swollen hydrogels were taken out from the swelling medium at regular time intervals and the surface adhered solution was blotted with a tissue paper and

the weights of hydrogels were taken. The percentage of swelling ratio (% DS) was calculated from the following formulae (Eq. 1).

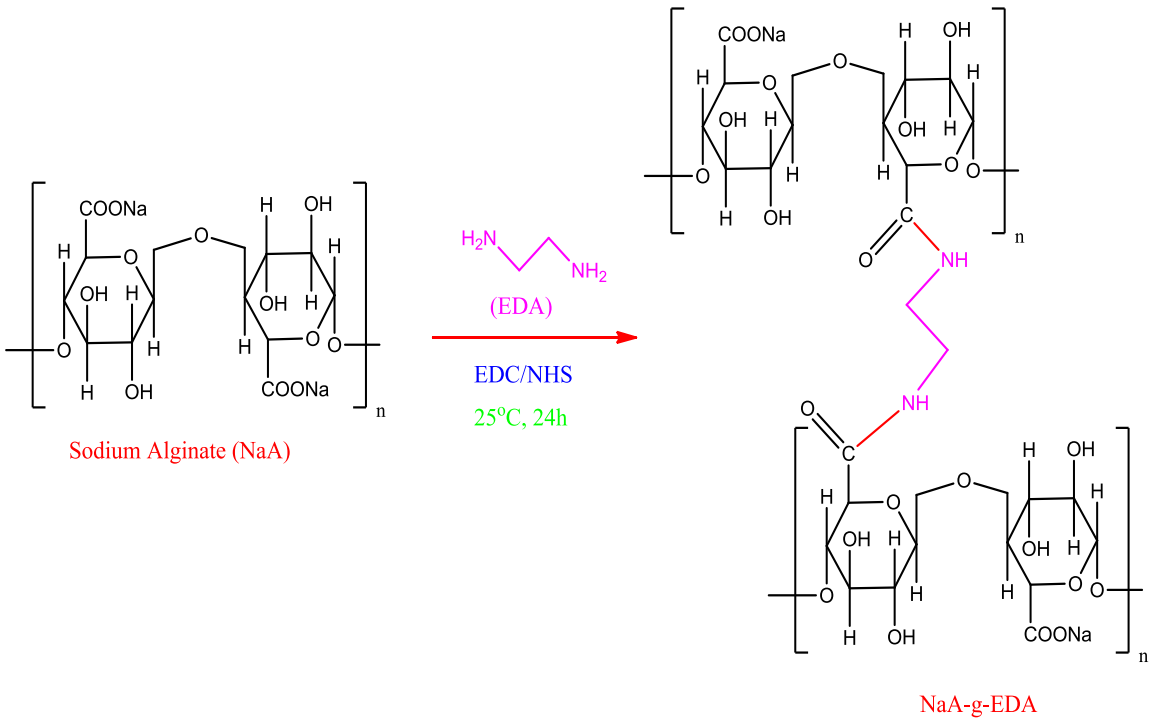
$$\% DS = \frac{Mt-Md}{Md} \times 100 \qquad \text{(Eq. 1)}$$

Where M_d is the weight of dry gel, and M_t is the weight of swollen hydrogel at time t [13, 14].

3. Result and discussion

3.1. Chemical reaction

Sodium alginate was chemically reacted with ethylenediamine (EDA), to prepare alginate derivative with clickable groups for hydrogel cross-linking application. The co-reactant N-hydroxysuccinimide stabilizes the reactive EDC-HCl intermediate against a competing hydrolysis reaction, raising the efficiency of amide bond formation [12]. Ethylenediamine was conjugated to the sodium alginate backbone through amide bond linkages as shown in Scheme 1.



Scheme 1. Schematic for the synthesis of the NaA-g-EDA in the presence of EDC-HCl and NHS as coupling agents.

3.2. Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) studies were performed to confirm the grafting, and cross-linking and examine the chemical stability of NaA-g-EDA. FTIR spectra of pure NaA and NaA-g-EDA were studied and shown in Fig. 1. As shown in Table 1, the FTIR spectra of NaA show the stretching vibrations of O–H at 3419.63 cm^{-1}, C–H at 2330.58 $cm^{-1,}$ and carboxylate group at 1620.13 cm^{-1}. Vibrational bands at 1411.82 cm^{-1} are assigned to bending C–H. The band at 668.18 cm-1 was assigned to the mannuronic acid. The NaA-g-EDA spectra show a band at 1046.19 cm^{-1} may be because of C–N stretching vibrations, and two bands at 1605.44, 1401.04 cm^{-1} were assigned to C=O and N-H bending in amides [15, 16].

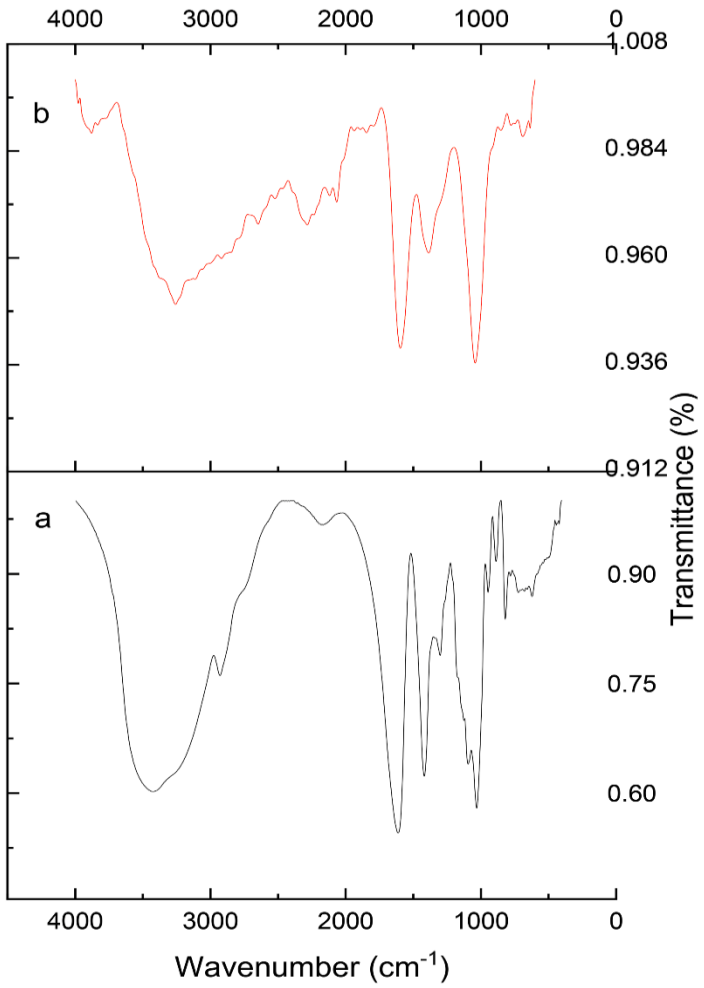


Fig. 1 FTIR spectra of (a) NaA and (b) NaA-g-EDA

Table 1. Infrared absorption frequencies of NaA and NaA-g-EDA

Samples / wavenumber (cm^{-1}) / Functional groups	NaA	NaA-g-EDA
H_2O, OH M, G, N-H	3419.63	3249.46
–COOH	1620.13	-
CH Bending	1411.82	1401.03
CH Vibration	2330.58	2277.51
C=O, N-H bending (Amide)	-	1605.44, 1401.04
C-N Stretching	-	1046.19

3.3. ^{1}HNMR spectral analysis

^{1}HNMR spectrum of NaA (Fig. 2) displayed typical signals at 3–4 ppm that belong to the protons on the carbon of NaA while the ^{1}HNMR spectrum of NaA-g-EDA displayed new signals in Fig. 3. In the ^{1}HNMR spectrum of NaA-g-EDA, two signals at 1.85-2.98 ppm all proved the existence of EDA. In addition, some of the characteristic signals of EDA are overlapped with signals of NaA. As seen in ^{1}HNMR spectra the strong signal at around 4.7 ppm, is attributed to D_2O [15].

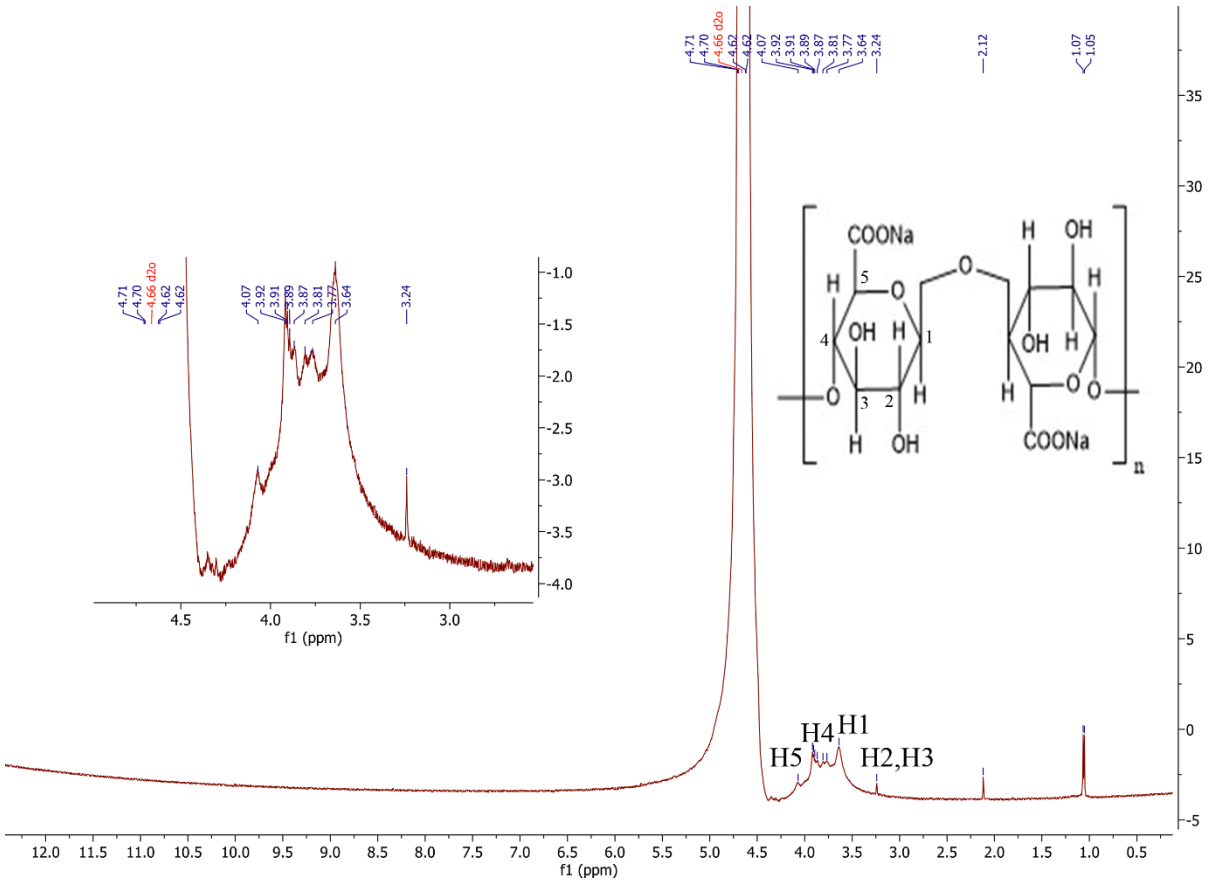


Fig. **2** ^{1}HNMR spectra of NaA in D_2O

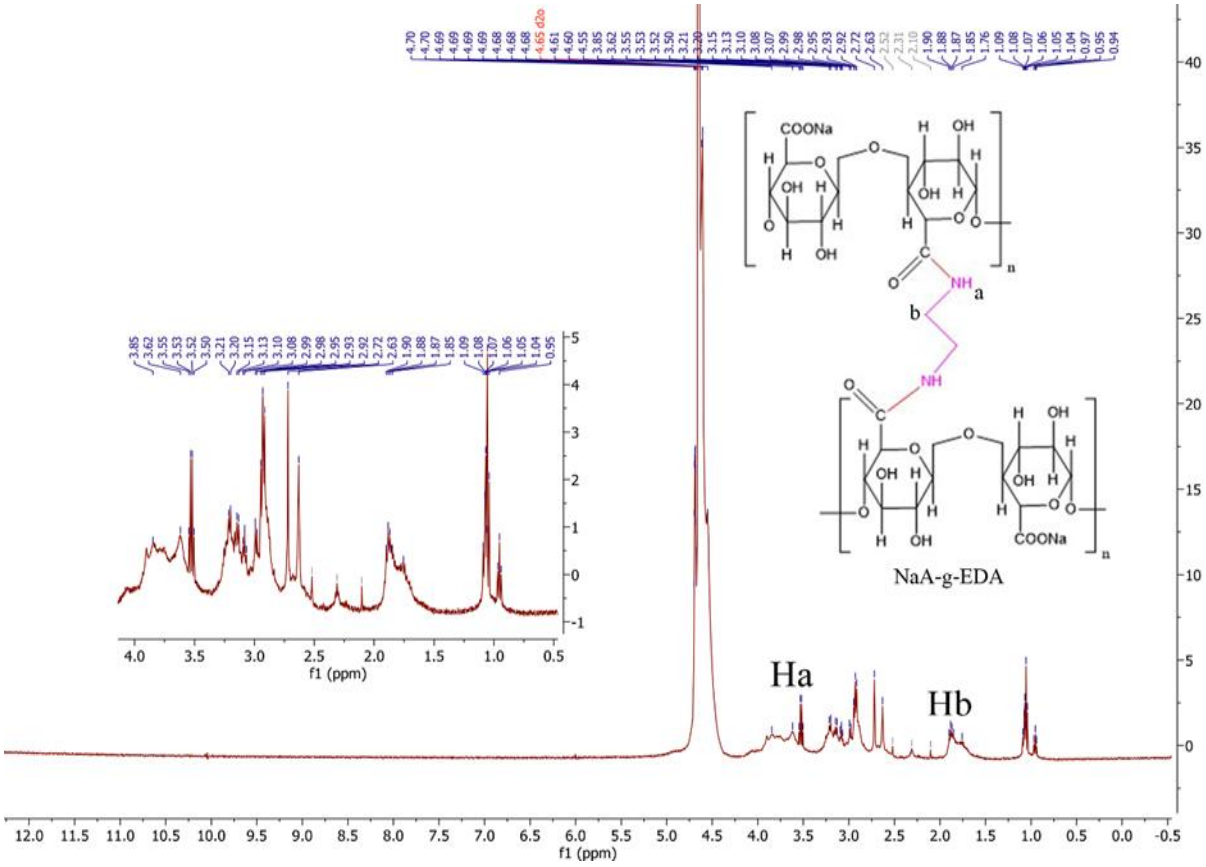


Fig. **3** [1]HNMR spectra of NaA-g-EDA in D_2O

3.4. Elemental analysis (CHNX)

An elemental analyzer was used to determine the amount of nitrogen, carbon, hydrogen, and heteroatoms (C, H, N, X) in the samples before and after amination and the results are displayed in Table 2. The nitrogen content in graft copolymers is significantly increased compared to pure sodium alginate.

Table 2. Elemental analysis of NaA and NaA-g-EDA

Sample	%C	%H	%N	%S
NaA	29.76	4.46	0.70	0.00
NaA-g-EDA	37.60	6.37	5.80	0.00

3.5. X-ray diffraction (XRD)

The crystalline structures of NaA and NaA-g-EDA were studied and shown in Fig. 4. Spectrums displayed diagnostic crystalline peaks at nearly 2θ at 9.6 °, 22.6 °,36.2,43 and 45 ° for NaA, 23 °, 29.6 ° and 45.2 ° for NaA-g-EDA. This indicates that the sigma alginate has semi-crystalline structures. Results are also in accordance with previous reports. After modification with EDA, the spectrums of a sample displayed a little fadeaway of some crystalline signals indicating the reaction performance in these parts [15].

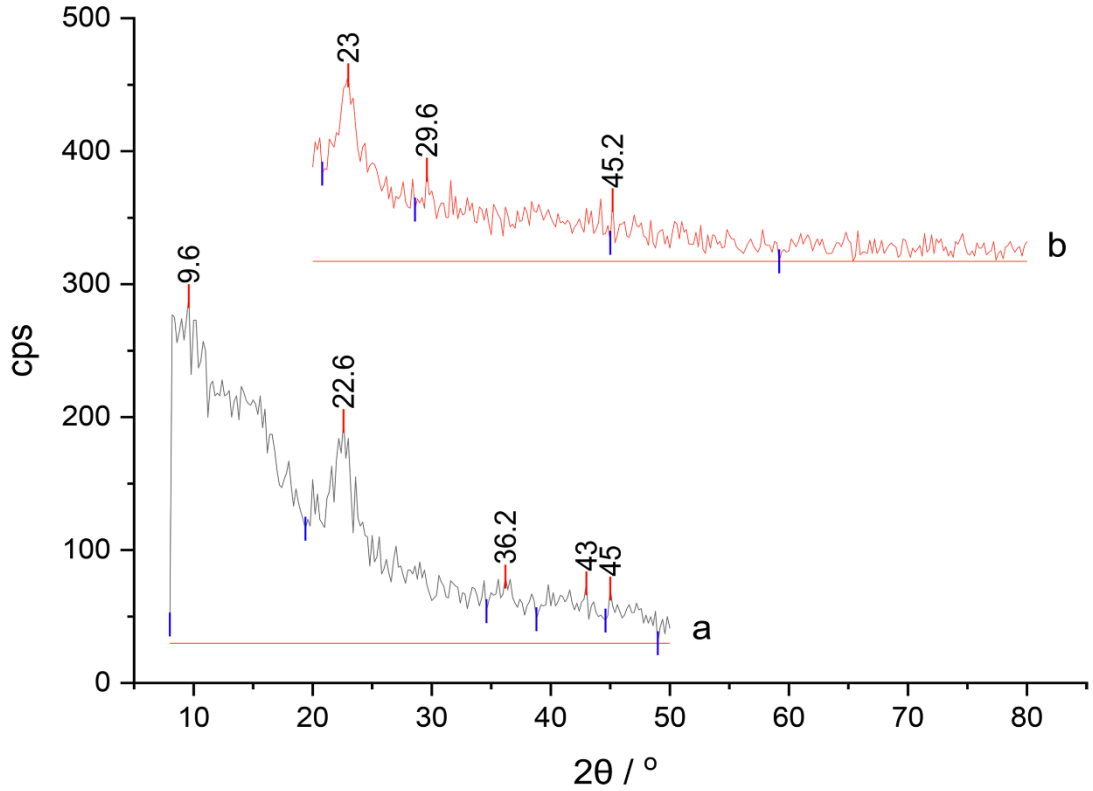


Fig 4. XRD curves of (a) NaA, (b) NaA-g-EDA.

3.6. Swelling properties of NaA and NaA-g-EDA hydrogels

NaA-g-EDA can produce robust hydrogels. The swelling ratio is a sign of hydrogel formation and capacity for water sorption that is a good character for wound exudate absorption in wound dressing. The highest percentage of swelling was related to NaA-g-EDA. Results are presented in Fig. 5. The swelling percentage of different hydrogels shows that hydrogels obtained from NaA-g-EDA had higher water absorption compared to non-

functionalized alginate after 180 minutes. It seems that hydrogels can uptake water more than 100% of their weight but in a slow manner. This character is a perfect property for wound dressing that reduces the need to exchange wound dressing every day [17,18].

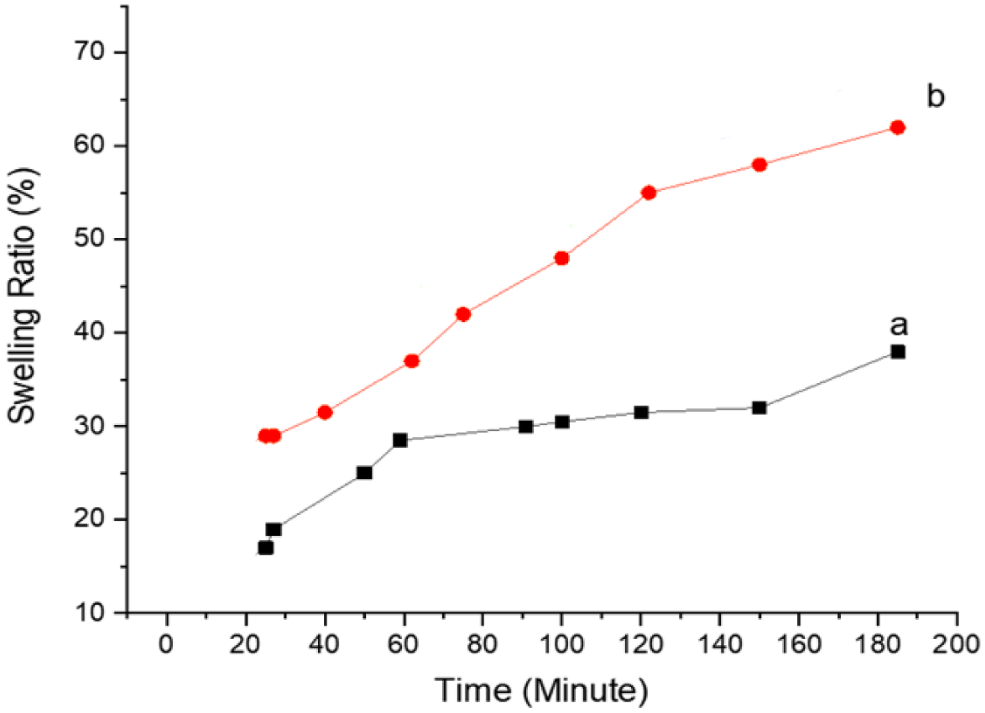


Fig 5. Water absorption profile of (a) NaA, (b) NaA-g- EDA

3.6.1. pH and temperature-responsive behavior of NaA and NaA-g-EDA

The effect of temperature and pH on the swelling ratios for all samples was investigated at a temperature range of 5 to 65 ˚C and two pH (4.5, 6.8) solutions. In this temperature and pH range, no phase transition was observed for both samples.

4. Conclusion

NaA-g-EDA hydrogels were successfully synthesized through amide bond linkages in the presence of EDC-HCl/NHS as coupling agents. Synthesized derivative showed no environmental sensitivity but the swelling percentage of hydrogels shows that hydrogels obtained from NaA-g-EDA had higher water absorption compared to non-functionalized

sodium alginate. It seems that hydrogels can uptake water more than 100% of their weight but in a slow manner. This character is a perfect property for wound dressing. Biodegradable hydrogels can self-degrade and become non-toxic gradually, so the synthesized hydrogel can be decomposed into non-toxic by-products. The obtained modified alginate was investigated using FTIR, elemental analysis, and ^{1}HNMR spectra. Crystalline structures of the hydrogels were examined utilizing X-ray diffraction. The elemental analysis studies showed that the percentage of nitrogen in the NaA-g-EDA has increased significantly compared to NaA, which indicates the amination reaction on the sodium alginate structure. Other spectral studies confirm these findings.

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