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# **ORIGINAL ARTICLE**

# Fabrication of sustainable hydrogels-based chitosan Schiff base and their potential applications



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# **KEYWORDS**

Chitosan; Isonicotinic aldehyde; Epichlorohydrin; Nanohydrogel; Biological activity; Metal removal; Ionotropic gelation **Abstract** Chemical modifications of chitosan were of interest to scientific researchers for its wide applications. Chitosan has been widely used for synthesis of unique compounds with potential biological activity and also effective for wastewater treatment. In the current study fabrication of new chitosan-based Schiff base hydrogels were fabricated through modification of chitosan with isonicotinic aldehyde to give hydrogel I or with epichlorohydrin or sodium tripolyphosphate via ionotropic gelation processes under the same reaction conditions to give hydrogels Ie, and nanohydrogels II, IIe respectively. FTIR, XRD, TGA, DSC, SEM, and TEM tools were used for characterization of the fabricated hydrogels I, Ie, II, and IIe hydrogels. The swelling behavior of the fabricated hydrogels in different solvents were determined. Evaluation of the hydrogels for leaching metal ions and biological activity towards different Gram-positive and Gram-negative of microorganisms were studied. The results showed that the highest efficiency for adsorption of cobalt and mercuric ions was revealed for hydrogels Ie, IIe (91.3%, 95.9%) and (92.5%, 95.9%) respectively. However, hydrogel IIe showed remarkable MIC and MBC towards Gram-positive (*B. subtilis*) (19.5, 38)  $\mu$ g/ml compared to the standard antibiotic Ciprofloxacin (19, 38)  $\mu$ g/ml respectively.

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# 1. Introduction

Water is the most important substance to humans and all living organisms. However, some harmful chemical compounds cause water pollution that gives negative effects on species living in water and also on the widely biological community (Guo et al., 2015). There are many industries released metal ions in water (Chen et al., 2010).

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Non-essential heavy metal ions such as [Mercury, Arsenic, and Lead] are highly toxic, non-degradable and have a tendency to bioaccumulate and constitute a serious health threat for humans and other species when existing in water, even at very low concentrations (Liu, D., et al., 2019; Kim et al., 2019; Qu and Luo, 2020; Sharma and Kandasubramanian, 2020). Essential heavy metal ions such as [Copper, Cobalt, and Zinc] are act as coenzyme in biological process at low concentrations but are toxic at higher concentrations (Kim et al., 2019).

Adsorption procedures are the most widely techniques used for removing metals and organic compounds from industrial effluents because of its convenience of operation, low cost, and availability of a diversity of adsorbent materials (Crini et al., 2019; Liu, L., et al., 2019; Yao et al., 2019; Shayegan et al., 2020). The hydrogels commonly applied in water/ wastewater treatment for the removal of heavy-metal ions (Jing et al., 2013).

Chitosan is one of the most naturally abundant and renewable polymers, it is a linear cationic polysaccharide composed of glucosamine and N-acetyl glucosamine units linked by  $\beta$  (1– 4) glycosidic bonds, is prepared by alkaline deacetylation of the N-acetyl groups from second most abundant biopolymer in nature chitin (Kandile et al., 2018; Elsoud and El Kady, 2019; Moreno-Sader et al., 2020). Chitosan is useful in wastewater treatment because of its functional groups and natural chelating properties by allowing for the binding and removal of metal ions from wastewater (Sarode et al., 2019; Kumar et al., 2020; Vera et al., 2020).

Antibacterial and antimicrobial agents are very important in reducing infectious diseases (Bhatia and Narain, 2010). They have been studied for possible use in healthcare environments, industries, and laboratories (Ali et al., 2015; Hosseinnejad and Jafari, 2016). Antimicrobial activity that can be effectively inhibit the growth of bacteria, and may destroy microorganisms (Pham et al., 2019).

Chitosan and its derivatives are widely studied in medical and pharmaceutical fields due to their biological properties like biocompatibility, biodegradability, nontoxicity, antitumor, antimicrobial, and antioxidant properties (Ways et al., 2018). Modification of chitosan has been gathered much attention for expanding its potential applications due to the presence of amine and hydroxyl groups in its structure. Amine groups of chitosan for example could react with carbonyl groups such as aldehydes and ketones groups to form Schiff bases (Kenawy et al., 2019; Antony et al., 2019). Schiff bases linkage including imine group have good thermal stability (Zhang et al., 2018) and have excellent prospects in the biomedical field due to its simplicity, reversibility, pH-sensitivity, and biocompatibility (Huang et al., 2018). Chitosan can cross-link with crosslinking reagents such as formaldehyde, glyoxal, epichlorohydrin, and glutaraldehyde to give the corresponding hydrogel (Gutha et al., 2017).

Epichlorohydrin is a very versatile chemical compound that is found to be used in a wide range of applications. Epichlorohydrin is used as cross-linking agent for synthesis of crosslinked polymeric hydrogels; it is also used to synthesis of cross-linked chitosan possessing two novel polymeric ligands (Morin-Crini and Crini, 2013; Gao et al., 2014; Hasanah et al., 2015). Thus, by modification, chitosan can be applied for different biomedical applications (Ferreira et al., 2016; Wardani and Sudjarwo, 2018; Ahmadi et al., 2015). Polymer's nanoparticles contain distinctive properties such as hydrophilicity, elasticity, and high-water absorption. Modified chitosan nanoparticles have unique properties such as biocompatibility, high permeability, biodegradability, excellent film-forming ability, cost-effectiveness, and emulsion-fixing properties (Koosha et al., 2015; Matshe et al., 2018; Abdeltwab et al, 2019; Montaser et al., 2019; Anush and Vishalakshi, 2019; Xiang et al., 2019). Chitosan nanogels have been prepared by several approaches, including crosslinking by the ionotropic gelation technique between chitosan and sodium tripolyphosphate (Ashrafi and Azadi, 2016; Vijayalakshmi et al., 2016; Yu et al., 2019; Pacheco et al., 2020).

A pyridine nucleus is an important class of heterocyclic compounds. It occurs naturally in many important compounds like vitamins niacin, pyridoxine and several alkaloids including nicotine, which is important for a wide range of biological activities and is found in many products such as medicines, food, flavors, dyes, adhesives, pesticides, and herbicides (Habibi et al., 2017; Triana-Guzmán et al., 2018; Wei et al., 2018). Thus, heterocyclic aldehydes including 3-pyridine carboxyl aldehyde are reacted with chitosan to form a Schiff base by intermediate reaction (EI Badawy, 2008).

In continuation to our previous work for the preparation of unique modified chitosan for different applications (Kandile et al., 2009; Kandile and Nasr, 2009; Abou-Zeid et al., 2011; Kandile and Nasr, 2011; Kandile and Nasr, 2014; Kavianinia et al., 2014; Kandile and Mohamed, 2019; Kandile et al., 2020; Ahmed et al., 2020; Kandile et al., 2021; Kandile and Mohamed, 2021; Elzamly et al., 2021), in the current study, new Schiff base hydrogels I, Ie were synthesized from the reaction of chitosan with isonicotinic aldehyde in absence and/or presence of epichlorohydrin, however nano hydrogels II, IIe were prepared via ionotropic gelation technique using sodium tripolyphosphate under the same reaction conditions to improve the physicochemical properties of chitosan and increase its potential applications. The new hydrogels were characterized. Evaluation of the efficiency of the prepared hydrogels for their leaching of heavy-metal ions  $(Co^{+2} \text{ and } Hg^{+2})$  from the aqueous media under different parameters was investigated. Also, their biological activities (Bacterial inhibition zones, MIC and MBC) toward some bacteria were studied.

#### 2. Materials and methods

#### 2.1. Materials

Low molecular weight chitosan (Cs) (M.W. = 60 KDa, DD 91%) (Acros Organics), isonicotinic aldehyde (107.112 g/mol) (Alpha), epichlorohydrin (92.52 g/mol) (Fluka), sodium tripolyphosphate (367.864 g/mol) (TPP) (Aldrich), glacial acetic acid (60.052 g/mol) (Alpha), ethyl alcohol (46.07 g/mol) (Aldrich), dimethylsulphoxide (78.13 g/mol) (DMS) (Aldrich), and dimethylformamide (73.09 g/mol) (DMF) (Edwic).

#### 2.2. Methods

#### 2.2.1. Fabrication of hydrogels I and Ie

Chitosan (0.25 g) was dissolved in 1% acetic acid solution (50 ml), stirring at room temperature for 30 min until the solu-

tion became clear. Isonicotinic aldehyde (0.57 g, 0.5 ml) (0.1 mol) was added to the solution in the absence and presence of epichlorohydrin (0.1 ml), and the mixture was refluxed for 12 h, where the hydrogels were formed after evaporating the solvent in air at room temperature. The prepared hydrogels were washed with1% acetic acid, distilled water and left to dry at room temperature to give the hydrogels **I**, and **Ie** respectively.

#### 2.2.2. Fabrication of hydrogels II and IIe

Nanogels were prepared via the ionotropic gelation procedure by dissolving chitosan (0.25 g) in 1% acetic acid solution (50 ml), stirring at room temperature for 30 min until the solution became clear. Sodium tripolyphosphate (0.2 g) was dissolved in distilled water (27 ml) and was added drop wise with stirring well for about 30 min. A milky colored emulsion was formed. Isonicotinic aldehyde (0.57 g, 0.5 ml) (0.1 mol) was added during the stirring in the absence and presence of epichlorohydrin (0.1 ml) and the mixture was refluxed for 12 h, where the hydrogels were formed after evaporating the solvent in air at room temperature. The hydrogels were washed with 1% acetic acid and distilled water then left to dry at room temperature to give the new hydrogels II and IIe respectively.

# 2.3. Characterizations of hydrogels

#### 2.3.1. Determination of the average molecular weight of chitosan

Using viscometry measurement (Brookfield viscometer type at 25 °C). The viscosity of chitosan solutions was measured after dissolving different concentrations of chitosan in mixture of 0.1 M acetic acid and 0.2 M sodium chloride. Molecular weight of chitosan was calculated based on the intrinsic viscosity  $[\eta]$  by Mark–Houwink–Sakurada's empirical equation (Kavianinia et al., 2014)

$$[\eta] = \mathbf{k}\mathbf{M}^{\alpha} \tag{1}$$

where k =  $1.81 \times 10^{-3}$  and  $\alpha$  = 50.93 for the buffer solution at 25 °C.

#### 2.3.2. Elemental analysis

Elemental analysis (C, H, N) was performed using FLASH 2000 CHNS/O analyzer (Thermo Scientific). The degree of deacetylation (DDA %) for (CS) and degree of substitution (DS) for (CS) derivatives were calculated using the following (Eq. (2)) (Ahmed et al., 2020) and (Eq. (3)) ((de Araújo Braz et al., 2018) respectively.

$$DD = \left[1 - \frac{C/N - 5.145}{6.861 - 5.145}\right] \times 100 \tag{2}$$

$$DS = \left[\frac{(C/N)f - (C/N)i}{n}\right]$$
(3)

where the value of 5.145 is related to the completely N-deacetylated (CS) ( $C_6H_{11}O_4N$ ) repeat unit and the value of 6.861 is related to the fully N-acetylated chitin ( $C_8H_{13}O_5N$ ) repeat unit. (C/N)<sub>f</sub> and (C/N)<sub>i</sub> are the ratio of the synthesized polymers and (CS) respectively, also n was the number of carbons introduced into the synthesized polymers.

# 2.3.3. FTIR spectra

The absorption bands of functional groups of samples were determined by FTIR. A Perkin Elmer 200 FTIR spectrophotometer instrument was utilized in recording the FT-IR spectra of the samples in the wave number range from 4000 cm<sup>-1</sup> to  $450 \text{ cm}^{-1}$  during 64 scans, with 2 cm<sup>-1</sup> resolution.

# 2.3.4. X-Ray diffraction

X-ray diffraction analysis indicated the physical properties of samples. X-ray powder diffract meter with Ni filter Cu K $\alpha$  radiation source ( $\lambda = 0.154$  nm), set at scan rate =  $10^{\circ}$ /min, using a voltage of 40 kv and a current of 30 mA was for measuring the diffraction patterns of hydrogels.

## 2.3.5. Thermal stability

2.3.5.1. Thermogravimetric analysis. Thermogravimetric analysis (TGA) indicated the thermal stability of the samples which performed at a temperature starting from 25 °C to 1000 °C under inert nitrogen atmosphere with heating rate of 10 °C min<sup>-1</sup> using the instrument: SDT Q600 V20.9 Build 20, USA.

2.3.5.2. Differential Scanning Calorimetric analysis. Differential Scanning Calorimetric DSC131 Evo (SETARAM Inc., France), the instrument was calibrated using the standards (Mercury, Indium, Tin, Lead, Zinc and Aluminum). Nitrogen and helium were used as the purging gases. The test was programed including the heating zone from 25 °C to 500 °C with a heating rate 10 °C/min. The samples were weighted in an aluminum crucible 0.12 ml and entered into the DSC. The thermo gram results were processed using (CALISTO Data processing software v.149).

# 2.3.6. Surface morphology studies

2.3.6.1. Scanning Electron Microscope (SEM). The surface morphology of chitosan and the new hydrogels was obtained by using Scanning Electron Microscope (SEM) images. The samples were mounted on a metal stub with double stick adhesive tape and coated under vacuum with gold. The gold film thickness was 150 Å. The samples were then viewed in a Quanta 250 FEG (Field Emission Gun) attached with EDX Unit (Energy Dispersive X-ray Analyses) with accelerating voltage 30 kV, magnification at 10, 20 and 40 µm and resolution from the Gun, FEI Company, Netherlands.

2.3.6.2. Transmission Electron Microscope (TEM). Shape and size of the nanoparticles were practically obtained using (TEM); JEOL- JEM-1200. The preparation of specimens for (TEM) measurements occurred by Sonication for 15 min. then placed onto a carbon-coated copper grid. The coated grids were viewed under a JEM – 1200 EX 2, Electron Microscope Japan Specimens for (TEM) measurements operating at 120 kV and 100 nm.

# 2.3.7. Solubility

Fabricated hydrogels (0.1 g) were immersed in different solvents (water, acetic acid (99.85%), dimethyl sulphoxide, and dimethyl formamide) and continuously shaken by using a shaker at 25 °C and at 100 °C for 1 h.

## 2.3.8. Swelling behaviors

2.3.8.1. Determination of the soluble and gel fraction for fabricated hydrogels in different organic solvents. A weighed

sample of hydrogels was boiled for 1 h in 20 ml of the following solvents: acetic acid, DMF, DMSO, and water. The swollen sample was dried in oven at 70 °C to remove the solvent (Alla et al., 2007). The drying process was continued to constant weight ( $W_1$ ). The soluble and gel fraction was calculated according to the following Eq. (4), (5)

Soluble fraction (SF%) = 
$$\frac{(W0 - W1)}{W0} \times 100$$
 (4)

where  $W_0$  is the initial weight of hydrogel and  $W_1$  is the weight of dried hydrogel.

2.3.8.2. Determination of the solvent uptake in different organic solvents. The swelling behavior was carried out by taking a known weight of hydrogels at 25 °C and immersed in different solvents using a cyclic swelling procedure. The swollen hydrogels were weighed at intervals after removal of liquid until equilibrium swelling was attained. The swollen hydrogels were then completely dried at 25 °C and reweighed (Mohy Eldin et al., 2015; Marjub et al., 2019). The ratio of swelling was calculated by the following Eq. (6)

Solvent uptake(%) = 
$$\frac{(W2 - W1)}{W1} \times 100$$
 (6)

where  $W_1$  is the weight of dry hydrogel and  $W_2$  is the weight of swollen hydrogel.

The swelling ratio experiments were repeated in triplicate and the results are presented as the mean  $\pm$  standard deviation (SD).

# 2.3.9. Biodegradability

The biodegradability of the fabricated hydrogels was studied through a soil burial method for several weeks. Soil for the purpose of the soil burial method was collected from the garden. 1 g of synthesized hydrogels was buried equidistant (3 cm apart) in the pot containing soil. The weight of each sample was taken by removing samples at an interval of 7 days, followed by rinsing with water and drying at 70 °C. Percentage degradation (%D) in the form of %Wt. loss as a function of the number of days was determined (La Mantia et al., 2020).

$$(\%\mathbf{D}) = \frac{(\mathbf{W}_{i} - \mathbf{W}_{f})}{\mathbf{W}_{i}} \times 100 \tag{7}$$

where  $W_i$  = initial weight of sample;  $W_f$  = final weight of sample.

# 2.4. Evaluation of fabricated hydrogels for different applications

#### 2.4.1. Evaluation of metal ions uptake for fabricated hydrogels

Absorption studies were proceeding by a concentration of 100 mg/l of hydrogel in water with stirring. The equilibrated hydrogels were withdrawn and immersed in aqueous solution [ cobalt acetate, and mercuric acetate] (0.025 g in 25 ml water). The absorbance of heavy metal ions ( $Co^{+2}$  and  $Hg^{+2}$ ) in solution was measured using UV–visible apparatus. The adsorbed metal ions were calculated from the difference between the initial metal concentration and that measured at each interval (Kandile and Nasr, 2014). The efficiency of metal ions uptake of hydrogels was calculated using the following Eq. (8)

$$\mathbf{F} = \left[1 - \frac{\mathbf{C}}{\mathbf{Co}}\right] \times 100\tag{8}$$

where F is the efficiency (%), C is the concentration of  $M^{+2}$  in the solution after a certain time period and  $C_o$  is the initial concentration of  $M^{+2}$  in solution.

2.4.1.1. Effect of contact time. The adsorption capacity of chitosan and hydrogels **I**, **Ie**, **II**, and **IIe** was studied for 10 h by using 100 mg swollen sorbent at room temperature and was measured by taking samples from the solution after 5 and 10 h for analysis of residual metal ions  $Hg^{+2}$  and  $Co^{+2}$ concentration in solution.

2.4.1.2. Effect of initial metal ions concentration. The effect of initial metal ions  $Hg^{+2}$  and  $Co^{+2}$  concentration on the uptake by hydrogel **He** was processed at definite concentrations 0.4, 0.6, 0.8, 1 g/l, using 0.05 g of swollen hydrogel **He** at room temperature and pH 7 for 24 h. After adsorption, the residual concentration of the metal ions  $Hg^{+2}$  and  $Co^{+2}$  in solution was detected.

2.4.1.3. Desorption of metal ions and reusability of the adsorbent. The hydrogels adsorbed  $\text{Co}^{+2}$  and  $\text{Hg}^{+2}$  ions were immersed in 20 ml of 2.0 mol  $1^{-1}$  hydrochloric acid solution for 8 h at room temperature, filtered and the desorption mount of metal ions was determined. Hydrogels were reused in adsorption experiment, and the process was repeated for six cycles.

The amount of adsorbed ion (A), desorption efficiency and retention rate of the investigated hydrogels were calculated using the following Eqs. (9)–(11) (Ge et al., 2012; El Halah et al., 2018)

$$\mathbf{A} = \frac{[V(C_1 - C_2)]}{W} \tag{9}$$

where V was the volume of the solution in (l), W was the weight of the hydrogel in (g),  $C_1$  and  $C_2$  were the concentrations of the metal ion before and after adsorption in (mg  $l^{-1}$ ) respectively.

Desorption efficiency = 
$$\frac{\text{Desorption mount of metal ion}}{\text{adsorbed mount of metal ion}} \times 100$$

Retention rate = 
$$\frac{\text{Adesorption mount in the sixth cycle}}{\text{adesorption mount in the first cycle}} \times 100$$

(10)

2.4.2. Evaluation of biological activities for fabricated hydrogels 2.4.2.1. Bacterial inhibition zone activity. The disc diffusion procedure (Abou-Zeid et al., 2011) was used for assessing the antibacterial activity of hydrogels. Briefly, discs of 10 mm diameter were cut from the hydrogels. Nutrient agar plates were inoculated with microbial culture. The 10 mm discs of hydrogels were placed onto the surface of inoculated plates. The plates were incubated at 37 °C for 48 h. The inhibition zone (distance from disc circumference in mm) was determined for each disc.

2.4.2.2. Minimum inhibition concentration and minimum bactericidal concentration. Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of hydrogels were evaluated according to broth tube dilution method with slide (Kasaai et al., 2000).

# 3. Results and discussion

Fabrication of the new hydrogels was carried out from reaction of chitosan with isonicotinic aldehyde via Schiff base formation under different reaction conditions. Therefore, chitosan was modified with isonicotinic aldehyde to give fabricated hydrogel I. Also, it was reported that the crosslinking reaction was one of the successful alternatives for modifying chitosan to enhance its physiochemical properties (Abd Malek et al., 2020) and nanoparticles, in particular were more efficient, easy to process and have gained growing interest for nanomedicine fields (Hipalaswins et al., 2016). Thus, chitosan was modified with epichlorohydrin as crosslinker to give hydrogel Ie, and nanohydrogels II and IIe were prepared by in-situ via ionotropic gelation processes by reaction of chitosan with isonicotinic aldehyde and sodium tripolyphosphate in absence and/or presence of epichlorohydrin respectively (Schemes 1,2).

# 3.1. Characterization of fabricated hydrogels

The prepared hydrogels were characterized by elemental analysis, IR, X-Ray, DSC, TGA, SEM, TEM, and swelling behavior.

# 3.1.1. Viscometry and elemental analysis

The average molecular weight (MW) of chitosan was determined by Mark–Houwink–Sakurada's viscometry method. It was approximately 60 KDa. Elemental analysis data was utilized to determine the degree of deacetylation (DD) of chitosan and the degree of substitution (DS) of hydrogels **I**, **Ie**, **II**, and **IIe**. The degree of substitution (DS) of fabricated hydrogels couldn't be determined using potentiometric titration due to their insolubility in 0.1 N HCl but calculated by (C/N) ratios. The results were calculated and reported in Table 1.

The degree of deacetylation for (CS) used was 91%, and the (C/N) ratios of the prepared hydrogels were higher than (C/N) ratio for chitosan (Ahmed et al., 2020) due to formation of Schiff base between aldehyde and amine groups in chitosan which showed increasing amount of carbon in the hydrogels relative to nitrogen.

# 3.1.2. FTIR spectra

FTIR spectra were used to confirm the structures of chitosan (Cs) and fabricated hydrogels I, Ie, II and IIe Fig. 1. The spectrum of chitosan showed a strong broad absorption band at 3441 cm<sup>-1</sup> could be assigned to the stretching vibration of O-H and N-H, absorption peak at 2869 cm<sup>-1</sup> related to (C-H stretch), 1590 cm<sup>-1</sup> (N-H bend), and 1149 cm<sup>-1</sup> (bridge-O stretch) (Haj et al., 2020) In addition, some shifts were showed in the spectra of the corresponding hydrogels I, Ie, II and IIe. The hydrogels I, Ie, II and IIe showed shifted absorption bands of O-H and N-H groups corresponding to the following wavenumbers  $3427 \text{ cm}^{-1}$ ,  $3423 \text{ cm}^{-1}$ ,  $3424 \text{ cm}^{-1}$ , and  $3433 \text{ cm}^{-1}$ , and C-H stretch band at 2920 cm<sup>-1</sup>, 2917 cm<sup>-1</sup>, 2923, and 2924 cm<sup>-1</sup> respectively. The appearance of a characteristic bands at 1602  $\text{cm}^{-1}$ . 1610 cm<sup>-1</sup>, 1608 cm<sup>-1</sup>, and 1632 cm<sup>-1</sup> indicate strong stretching absorption band of imine (C=N) for hydrogels I, Ie, II, IIe respectively confirming the formation of Schiff base due to the reaction of amine groups in chitosan with isonicotinic aldehyde. The appearance of absorption bands at 1250 cm<sup>-1</sup> and 1252 cm<sup>-1</sup> corresponding to the vibrations of C-O-C for the hydrogels Ie and IIe respectively.



Scheme 1 Fabrication of hydrogels I, and Ie.



Scheme 2 Fabrication of hydrogels II, and IIe.

#### 3.1.3. X-ray diffraction

X-ray diffraction analysis helped in describing the physical properties of samples in terms of crystalline structure. X-ray diffraction pattern of chitosan represented a broad peak of chitosan at  $2\theta$  at  $20^{\circ}$  (Xu et al., 2021), indicated that chitosan was semi crystalline polysaccharide. Fabricated hydrogels Ie and IIe were revealed higher crystallinity than hydrogel I and II due to modification of chitosan with isonicotinic aldehyde in presence of epichlorohydrin for hydrogels Ie and IIe. Sharp peaks of hydrogels I, II, Ie and IIe showed sharp reflections at different angles in their diffractograms, which meant that the crystalline structure of chitosan was changed after modification Fig. 2. Increasing the degree of crystallinity increases hardness and density (Sashikala and Syed Shafi, 2014; Olaru et al., 2018).

# 3.1.4. Thermal stability

3.1.4.1. Thermogravimetric analysis. The thermograph of chitosan and hydrogels I, Ie, II, and IIe were obtained under air atmosphere with heating ratio 10 °C/min to indicate the thermal stability and to determinate the temperatures of degradation of the fabricated hydrogels, Fig. 3.

The thermograph of chitosan displayed three steps of thermal degradation (Vlasceanu et al., 2020). The first step of thermal decomposition was started from 49.5 °C to 200 °C accompanied by the weight loss of 12.2%, this may be due

| Table 1   | The degree of deacetylation of | chitosan and the d | egree of substitution | of fabricated hydr | ogels I, Ie, II, and I | le.  |
|-----------|--------------------------------|--------------------|-----------------------|--------------------|------------------------|------|
| Sample co | ode (C)%                       | (H)%               | (N)%                  | C/N                | DD%                    | DS%  |
| Cs        | 40.72                          | 7.47               | 7.68                  | 5.30               | 91                     | _    |
| Ι         | 53.59                          | 6.37               | 8.13                  | 6.59               | -                      | 20.8 |
| Ie        | 52.21                          | 5.78               | 8.16                  | 6.39               | -                      | 11.7 |
| II        | 35.08                          | 6.02               | 6                     | 5.85               | -                      | 8.5  |
| IIe       | 24.89                          | 5.99               | 3.65                  | 6.82               | -                      | 16.4 |

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**Fig. 1** FTIR spectra of fabricated hydrogels (a) Cs, (b) I, (c) Ie, (d) II and (e) IIe.

to the moisture. Step two of weight loss of 40.7% for chitosan was observed between 200 and 400 °C referring to degradation of acetylated groups and the end loss of 12.7% at 400-900 °C due to degradation of backbone. The thermograph curves of hydrogels I and Ie also undergoes three stages of weight loss, the first step of loss of 10.4% starting from 60 °C to 223 °C and weight loss of 8.7% from 75 °C to 300 °C respectively. The second step of weight loss of 37.6% between 223 and 380 °C for hydrogel I and 52% between 300 and 675 °C for hydrogel Ie with high weight loss of hydrogels may be attributed to decomposition of imine groups. The third step of decomposition loss of both hydrogels were 21.4% from 380 °C to 998 °C and 12.4% from 675 °C to 1000 °C respectively. Both hydrogels II, IIe were revealed first step of weight loss 12% from 50 °C to 190 °C and 13% from 79 °C to 200 °C respectively. The second step of weight loss was 37.2% from 190 °C to 380 °C and 52% from 200 °C to 750 °C for the



**Fig. 2** X-ray diffraction of fabricated hydrogels (a) Cs, (b) I, (c) Ie, (d) II and (e) IIe.



Fig. 3 TGA of fabricated hydrogels (a) Cs, (b) I, (c) Ie, (d) II and (e) IIe.

hydrogels **II**, **IIe** respectively, this high loss of weight at this temperature of hydrogels due to decomposition of imine groups. The third stage of weight loss was 23.7% and 13.8% from 380 °C to 1000 °C and from 750 °C to 1000 °C respectively, the third step of weight loss of hydrogels **II**, **IIe** representing high temperature due to the degradation of backbone. The data showed that hydrogels **Ie** and **IIe** were more stable than hydrogels **I**, **II** due to the formation of cross-linked structure (Sashikala, and Syed Shafi, 2014; Vlasceanu et al., 2020).

3.1.4.2. Differential Scanning Calorimetric analysis. Differential Scanning Calorimetry (DSC) of chitosan, and hydrogels I, Ie, II and IIe indicated that: the glass transition temperature ( $T_g$ ) of chitosan 89 °C, and the decomposition temperature ( $T_d$ ) 247.9 °C due to the degradation of backbone (Omer et al., 2016; Liang et al., 2019). The glass transition temperature ( $T_g$ ) of the hydrogels I, II, and IIe was recorded at 109.6 °C, 117 °C, and 158 °C respectively, which indicated that the modification of chitosan with isonicotinic aldehyde effectively increase the glass transitional temperature of chitosan. These results showed that Schiff base formation could improve the thermal stability of fabricated hydrogels due to the reaction between amino group of chitosan with aldehydic group of isonicotinic aldehyde to form new functional group (imine) and increase of degree of substitution.

Hydrogels I, Ie, II and IIe were showed ( $T_d$ ) 280.8 °C, 231.3 °C, 233.5 °C and 254 °C respectively Fig. 4.

# 3.1.5. Surface morphology studies

3.1.5.1. Scanning Electron Microscope (SEM). SEM was one of the most analysis to study the surface morphology of polymers. The surface morphology of chitosan showed some clefts in the surface. The images showed masses surface of needles particles of hydrogel I due to the interaction between chitosan and isonicotinic aldehyde, whereas hydrogel Ie showed rocks shape in surface area attributed to the presence of epichlorohydrin and formation of crosslinked structure (Drzymalska et al.,



**Fig. 4** DSC of fabricated hydrogels (a) Cs, (b) I, (c) Ie, (d) II and (e) IIe.

2020). The homogeneous structure of various hydrogels demonstrated the formation of chitosan Schiff bases Fig. 5.

*3.1.5.2. Transmission Electron Microscope (TEM).* To investigate the nanostructure of fabricated hydrogels **II** and **IIe**, transmission electron microscope measurement was carried out at 120 kV and 100 nm.

The TEM image of hydrogel II was given in Fig. 6 using Magnification at  $40000\times$ ,  $50000\times$  which showed spherical shape with nanoparticles size from 21 nm to 43 nm, confirming formation of nano structure between chitosan with isonicotinic aldehyde using sodium tripolyphosphate (Alshehri et al., 2020). While hydrogel IIe using Magnification at  $25000\times$ ,  $60000\times$  was spherical in shape and nanoparticles size from 25 nm to 64 nm which confirm the success of modification of

chitosan with isonicotinic aldehyde in presence of epichlorohydrin as crosslinker Fig. 6.

# 3.1.6. Solubility

Chitosan was soluble in 1% acetic acid solution, however hydrogels **I**, **Ie**, **II** and **IIe** were insoluble in different solvents (water, acetic acid (99.85%), dimethyl sulphoxide, and dimethyl formamide) at 25 °C and by heating at 100 °C for 1 h, confirmed modification of chitosan and formation of the new hydrogels (Timur and Paşa, 2018).

# 3.1.7. Swelling behaviors

3.1.7.1. Determination of the soluble-gel fraction and solvent uptake for hydrogels in different organic solvents. At room temperature the soluble fraction of the fabricated hydrogels showed low values in weight loss whereas gel fraction increased by decreasing soluble fraction indicated increase of the degree of crosslinking Fig. 7a, b. Fabricated hydrogels showed high values of swelling capacity, the highest degree of swelling was observed with water for chitosan and hydrogel I rather than the hydrogels Ie, II and IIe due to intermolecular hydrogen bonding and the presence of high porosity in their structures (Olivera et al., 2020), Fig. 7c.

# 3.1.8. Biodegradability

The biodegradability was carried out on fabricated hydrogels through a soil burial method for several weeks. The weight loss of the hydrogels increases with burial degradation time, and the functional groups and chain ends of the polymers are easily removed from the hydrogels surface which susceptible to the attacks of microorganisms in the soil (La Mantia et al., 2020).

The hydrogels **I**, **Ie**, **II**, and **IIe** lost (15%, 10%, 13.5% and 8.5%) respectively of their weights at the end of the first week. The hydrogels **I**, **Ie**, **II**, and **IIe** lost (30%, 17.5%, 21.3% and 15.3%) respectively of their weights at the end of the second week. At the end of third week, the hydrogels **I**, **Ie**, **II**, and



Fig. 5 SEM at 20 µm of hydrogels (a) Cs, (b) I, (c) Ie, and at 40 µm of hydrogels (d) Cs, (e) I, (f) Ie.

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Fig. 6 TEM images of nanohydrogels a) II at Magnification:  $40000\times$ ,  $50000\times$ , and b) IIe at Magnification:  $25000\times$ ,  $60000\times$ .

**He** the lost (55%, 40.2%, 51% and 38.3%) respectively. However, at the end of fourth week, the hydrogels **I**, **Ie**, **II**, and **IIe** lost (85.5%, 66.5%, 79.7% and 59.7%) respectively Fig. 8. These data showed that hydrogel **I** had the highest value of biodegradability due to Schiff base formation in absence of crosslinkers (epichlorohydrin and sodium tripolyphosphate) which increase its biodegradability using soil burial method.

# 3.2. Evaluation of fabricated hydrogels for different applications

3.2.1. Evaluation of metal ions uptake for fabricated hydrogels 3.2.1.1. Effect of contact time. Chitosan and its fabricated hydrogels **I**, **Ie**, **II**, and **IIe** were evaluated for their efficiency to leaching of  $\text{Co}^{+2}$  and  $\text{Hg}^{+2}$  ions from aqueous solutions.

The results showed that the concentration of  $\text{Co}^{+2}$  and  $\text{Hg}^{+2}$  ions from its solution decreased with increasing the time of immersion of Cs and hydrogels **I**, **Ie**, **II**, and **IIe** (Kandile and Mohamed, 2019) Fig. 9a. The data indicated that the highest efficiency for adsorption of cobalt and mercuric ions was observed for hydrogels **Ie** and **Ie** at 10 h reached to (91.3%, 95.5%) and (92.5%, 95.9%) respectively, these results showed high values relative to other modification of chitosan (Rahmani et al., 2017). This may be attributed to the formation of complexes by chelation of metal ions with heteroatoms as (O, N) in the structures of the hydrogels.

3.2.1.2. Effect of initial metal ions concentration. The effect of initial metal ions  $Hg^{+2}$  and  $Co^{+2}$  concentrations on the adsorption capacity of the hydrogel **IIe** was studied. According to Fig. 9b, the adsorption percentage increased with increasing the metal ions concentration. The results suggest that using 1 g/l of metal ions could give the highest adsorption capacity of hydrogel **IIe** (Kandile and Mohamed, 2019; Shahraki et al., 2020).

3.2.1.3. Adsorption isotherms of metal ions. The equilibrium adsorption isotherm was the interactive behavior between solute and adsorbent, and it was used for clarified an adsorption system. Langmuir and Freundlich isotherm models were used to determinate the quantity of the sorption capacity of  $Co^{+2}$  and  $Hg^{+2}$  ions onto hydrogel **IIe** (Shahraki et al., 2020).

Langmuir and Freundlich models were defined in the following linear forms as Eq. (12, 13) respectively.

$$C_e/q_e = 1/K_L Q_m + C_e/Q_m \tag{12}$$

where  $C_e$  was the equilibrium concentration of  $M^{+2}$  ions in the solution in (mg  $l^{-1}$ ),  $q_e$  was adsorbent in (mg  $g^{-1}$ ),  $Q_m$  represented the maximum adsorption amount in (mg  $g^{-1}$ ), and  $k_L$ 



Fig. 7 (a) Soluble fraction (b) Gel fraction (c) Solvent uptake of fabricated hydrogels Cs, I, Ie, II and IIe.



**Fig. 8** The biodegradability of fabricated hydrogels I, Ie, II, and IIe.

was the Langmuir constant. The values of  $Q_m$  and  $k_L$  were determined from the slope and intercept of the plots of  $C_e/q_e$  versus  $C_e$  and represented in Table 2.

$$\log q_e = \log K_F + 1/\mathrm{nlog}C_e \tag{13}$$

Equation (13) explained the linearized Freundlich isotherm, where  $q_e$  and  $C_e$  were the equilibrium concentrations of  $M^{+2}$ ions in the adsorbent in (mg/g) and solution in (mg/l) respectively,  $K_F$  was Freundlich constant, and n was intensity factors. The values of n and  $K_F$  were calculated from the slope and intercept of the plots of log  $q_e$  versus log  $C_e$  and represented in Table 2. It was clear that the Langmuir adsorption isotherm was more suitable to describe the adsorption equilibrium ( $R^2 > 0.86$ ). The essential characteristics of Langmuir isotherm could be interpreter in terms of dimensionless constant separation factor ( $R_L$ ). This was defined by Eq. (14)

$$R_L = 1/(1 + K_L C_o) \tag{14}$$

where,  $K_L$  (l mg<sup>-1</sup>) was the Langmuir constant related to the energy of adsorption and  $C_o$  (mg l<sup>-1</sup>) was the initial concentration of both Co<sup>+2</sup> and Hg<sup>+2</sup> ions. The  $R_L$  value indicated the type of the isotherm, and values of  $R_L$  set between 0.0917 and 0.3357 for Co<sup>+2</sup> and between 0.0915 and 0.3508 for Hg<sup>+2</sup> indicating the favorable sorption of Co<sup>+2</sup> and Hg<sup>+2</sup> ions by hydrogel **IIe**.

3.2.1.4. Desorption of metal ions and reusability of the adsorbent. Desorption efficiency and retention rate of chitosan and hydrogels I, Ie, II, and IIe were studied to illustrate the times for reused the hydrogels for removal of heavy metal ions, Table 3. Hydrogels I, Ie, II, and IIe were showed high desorption efficiency and retention rate than chitosan which remain above 75% and 80% respectively by the sixth cyclic (Shahraki et al., 2020; Upadhyay et al., 2021). This indicated that the hydrogels could be used for removal of heavy metal ions for several times. The adsorption amount of Co<sup>+2</sup> and Hg<sup>+2</sup>ion for chitosan and hydrogels I, Ie, II, IIe were shown in Fig. 10. The results showed that the fabricated hydrogel He had the highest value of desorption efficiency and retention rate due to the high crosslinking structure formation of Schiff base in presence of crosslinkers (epichlorohydrin and sodium tripolyphosphate). The reusability of prepared hydrogels for metal ions uptake showed high adsorption capacity after six cycles. From these promising results, the prepared hydrogels can be potential to apply as membranes for treatment of contaminated water with cobalt and mercuric ions.

# 3.2.2. Evaluation of biological activities for fabricated hydrogels

The results of inhibition zone, MIC and MBC of chitosan, fabricated hydrogels I, Ie, II and IIe against the growth of (Gram-positive) B. subtilis (ATCC-6633) & S. aureus (ATCC-6538) and (Gram-negative) E. coli (ATCC-11229) & proteus (ATCC-33420), were investigated to study the effect of the hydrogels on the bacterial strains (de Araújo Braz et al., 2018). Ciprofloxacin was used as standard drug, as shown in Table 4 and Fig. 11. Bacterial inhibition zone for the tested bacteria were more sensitive to hydrogel IIe than hydrogels I, Ie, and II, reached (24 mm, 22 mm) and (19.5 mm, 18 mm) respectively. This indicated the inhibition growth of bacteria zone of hydrogel IIe more similarity to the value of ciprofloxacin antibiotic as a remarkable result. The highest value of MIC and MBC for Gram- positive (S. aureus & B. subtilis) and Gram-negative (E. coli & proteus) was showed also in hydrogel IIe. MIC and MBC for hydrogel IIe revealed the same value in Gram-positive equal to19.5 ppm, 38 ppm and Gram-negative equal to 125 ppm, 351 ppm respectively. This indicated that hydrogel IIe represented the highest inhibitory effect and the ability to prevent the growth of organisms.



Fig. 9 (a) Efficiency of fabricated hydrogels Cs, I, Ie, II, and IIe for metal ions uptake at 10 h, (b) Efficiency of hydrogel IIe on initial metal ions concentration.

**Table 2** Adsorption isotherm model parameters for the adsorption of  $Co^{+2}$  and  $Hg^{+2}$  on hydrogel IIe.

| Metal ion        | Langmuir isotherm                         |                |                |        | Freundlich isotherm             |                |        |                |  |
|------------------|---|----------------|----------------|--------|---------------------------------|----------------|--------|----------------|--|
|                  | Equation                                  | $\mathbb{R}^2$ | K <sub>L</sub> | Qm     | Equation                        | $\mathbb{R}^2$ | Ν      | K <sub>F</sub> |  |
| Co <sup>+2</sup> | $C_e/q_e = 7.2388 C_e + 0.7315$           | 0.8693         | 9.896          | 0.1381 | $Log q_e = 1.3115 C_e + 0.9663$ | 0.7797         | 0.7625 | 9.253          |  |
| Hg <sup>+2</sup> | $C_e/q_e \ = \ 39.592 \ C_e \ + \ 10.543$ | 0.9393         | 3.755          | 0.0253 | $Log q_e = 0.3866 C_e + 1.5783$ | 0.7085         | 2.587  | 37.87          |  |

Table 3 Desorption efficiency and retention rate of fabricated hydrogels Cs and (I), (Ie), (II) and (IIe) in Co<sup>+2</sup> and (Hg<sup>+2</sup>) ions.

| Code | Mount (1)             |              | Mount (2)             |                | Mount (3)             |                | Mount (4)             |                | Mount (5)             |                | Mount (6)             |                |
|------|-----------------------|--------------|-----------------------|----------------|-----------------------|----------------|-----------------------|----------------|-----------------------|----------------|-----------------------|----------------|
|      | Desorp.<br>efficiency | Ret.<br>rate | Desorp.<br>Efficiency | Ret.<br>rate   | Desorp.<br>Efficiency | Ret.<br>rate   | Desorp.<br>Efficiency | Ret.<br>rate   | Desorp.<br>Efficiency | Ret.<br>rate   | Desorp.<br>Efficiency | Ret.<br>rate   |
| Cs   | 76.7 (90.6)           | 100<br>(100) | 73.9 (90.6)           | 92<br>(98.5)   | 68.8 (87.8)           | 92<br>(95.7)   | 64 (86.6)             | 84<br>(94.3)   | 50 (85.7)             | 80<br>(88.8)   | 50 (83.3)             | 71<br>(83.3)   |
| Ι    | 80.7 (93.4)           | 100 (100)    | 76.9 (93)             | 92.8<br>(97)   | 76 (92)               | 92.7<br>(97)   | 76 (91)               | 89<br>(91.6)   | 75.6 (90)             | 84<br>(88.8)   | 75 (82.6)             | 80<br>(86.5)   |
| Ie   | 95 (95.7)             | 100<br>(100) | 91 (95.6)             | 97.9<br>(98.9) | 90.6 (94.7)           | 93.7<br>(98.9) | 86 (94.7)             | 93.7<br>(97.9) | 83 (92.7)             | 89.5<br>(95.8) | 76.5 (92.5)           | 89.5<br>(90)   |
| Π    | 86.6 (94.7)           | 100<br>(100) | 85.7 (94.3)           | 97.8<br>(98)   | 82.6 (93.7)           | 93.4<br>(96)   | 80.9 (92.4)           | 91.3<br>(89.2) | 78 (90.9)             | 89<br>(88.4)   | 76 (88.7)             | 84.7<br>(82.6) |
| IIe  | 98.6 (97)             | 100<br>(100) | 98.5 (94.6)           | 96<br>(98.2)   | 97.3 (94.3)           | 93.5<br>(96.9) | 94.5 (94.2)           | 92.3<br>(94.6) | 92.9 (94)             | 91<br>(94.6)   | 89.7 (91.4)           | 89.7<br>(94.6) |

Data in Table 4 showed that biological activities of modified hydrogels were higher than chitosan hydrogel.

# 4. Conclusions

In summary the goal of the present work is fabrication of **I**, **Ie**, **II**, **IIe** hydrogles via modification of chitosan with isonicotinic aldehyde under different reaction conditions to improve its physiochemical properties to be suitable for wide range of applications. The fabricated hydrogels **I**, **Ie**, **II**, and **IIe** were characterized by various tools. X-ray diffraction results indicated that modified hydrogels more crystalline than chitosan, whereas hydrogel **He** showed the highest thermal stability. The swelling behavior data indicated that the fabricated hydrogels revealed high values in solvent uptake for different solvents. The surface morphology of the hydrogels showed morphological changes, which confirm the modification of chitosan. Transmission electron microscope confirmed that hydrogels **H**, **He** represented nano particles size in their structures. Efficiency of fabricated hydrogels for removal of cobalt and mercuric ions showed that the adsorption capacity increased with increasing the time of immersion of the hydrogels, fabricated hydrogel **He** exhibited the highest efficiency for removal of cobalt and mercuric ions at 10 h reached (92.5,



Fig. 10 The adsorption amount of (a) Co<sup>+2</sup> ion, (b) Hg<sup>+2</sup> ion for fabricated hydrogels Cs, I, Ie, II, and IIe.

| Gram-negative |             | Gram-positive |            | Sample        |
|---------------|-------------|---------------|------------|---------------|
| Proteus       | E. coli     | B. subtilis   | S. aureus  |               |
| 468.5 [1875]  | 625 [2500]  | 117 [234]     | 78 [156]   | Cs            |
| 195 [1250]    | 195 [1250]  | 58.5 [117]    | 58.5 [114] | Ι             |
| 175.5 [750]   | 125 [750]   | 29.25 [114]   | 19.5 [78]  | Ie            |
| 195 [750]     | 175.5 [950] | 58.5 [117]    | 19.5 [78]  | II            |
| 125 [351]     | 125 [351]   | 19.5 [38]     | 19.5 [38]  | IIe           |
| 38 [72]       | 31 [50]     | 19 [38]       | 12.5 [25]  | Ciprofloxacin |

Table 4 MIC and [MBC] of fabricated hydrogels Cs and I, Ie, II and IIe.



**Fig. 11** Bacterial Inhibition Zone of fabricated hydrogels Cs, I, Ie, II, IIe and Ciprofloxacin.

95.9%) respectively. The adsorption capacity increased with increasing the metal ions concentration, and hydrogel **IIe** showed the highest adsorption capacity using 1 g/l of metal ions concentration. The hydrogels reusability showed high adsorption capacity after six cycles which is considered promising results to be potential membrane for treatment of contaminated water with cobalt and mercuric ions. The results of evaluation of biological activity of fabricated hydrogels indicated that hydrogel **IIe** revealed the highest antibacterial activities toward (Bacterial Inhibition Zones, MIC and MBC) against Gram-positive (*S. aureus & B. subtilis*) and Gram-negative (*E. coli & proteus*) compared to standard antibiotic Ciprofloxacin. Thus, hydrogel **IIe** is effective and promising, and it can be potential as antibacterial drug.

#### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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