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Green Synthesis of Smart Hydrogels via Radiation Crosslinking of Sodium Alginate and Citric Acid for pH-Sensitive Doxycycline Hyclate Release

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Highlights:

- Green synthesis methodology
- pH-responsive behavior
- Extended in vitro drug release at chronic wound pH

Keywords:

- Hydrogel
- Sodium alginate/citric acid
- pH sensitive drug release
- polysaccharide
- gamma radiation

ABSTRACT:

Doxycycline hyclate (DH) is a second-generation tetracycline antibiotic with lower toxicity than its predecessors, used for bacterial infections and topically for mucosal and diabetic ulcers. Healthy skin's pH is mildly acidic (4.0-6.0), regulating bacterial flora and preventing infections. Wounds disrupt this pH, revealing the tissue's neutral pH of 7.4, necessitating pH-sensitive controlled drug release for effective chronic wound treatment. This study explores polysaccharide-based hydrogels synthesized by crosslinking sodium alginate/citric acid (NaAlg/CA) solutions using gamma radiation with varying citric acid concentrations for pH-sensitive DH release. The citric acid-modified polysaccharide hydrogels were created using a green method, free of additional chemicals. Citric acid significantly influenced swelling, critical for drug loading and release, with the highest swelling capacity (3500% mass) observed at a 5:1 NaAlg/CA ratio. Hydrogels were tested for pH-dependent swelling and DH drug release profiles at pH 5.5, 7.4, and 9.0. The results indicate that at pH 7.4, which replicates the pH of chronic wounds, the release of DH showed a prolonged profile up to 40 hours, distinct from the results at pH 5.5 and 9.0. These results highlight the capabilities of NaAlg/CA hydrogels created through gamma radiation, combining the biocompatibility and low toxicity of sodium alginate/citric acid, for efficient and sustainable drug delivery, especially valuable in acute wound care where pH-specific therapeutic effectiveness is essential.

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INTRODUCTION

Hydrogels are a unique class of materials characterized by their ability to retain a significant amount of water within their polymeric networks. These three-dimensional structures are formed through the physical or chemical crosslinking of hydrophilic polymers, which allows them to swell in water while maintaining their distinct form (Hoffman, 2002). Hydrogels are highly versatile and can mimic the natural extracellular matrix, making them extremely useful in a range of biomedical applications, from drug delivery systems and wound healing dressings to tissue engineering and regenerative medicine (Drury & Mooney, 2003; Klouda & Mikos, 2008). The remarkable properties of hydrogels stem from their hydrophilic nature and the porosity of their structure. This allows for the controlled release of small molecules and drugs, responsiveness to environmental stimuli (such as pH, temperature, and ionic strength), and the ability to promote cell adhesion and proliferation (Koetting et al., 2015; Sood et al., 2014). Moreover, their soft tissue-like consistency and low interfacial tension with biological fluids minimize irritation when in contact with tissue, which is critical for medical implants and injectable therapies (Möller et al., 2007; Tan et al., 2009). Scientific interest in hydrogels has grown due to their potential to be tailored for specific applications by manipulating their chemical composition and physical structure. Hydrogels are commonly synthesized using chemical crosslinking agents, which create a network of polymer chains through covalent bonds. These agents can include molecules such as formaldehyde, glutaraldehyde (GA), genipin, diglycidyl ether, and N,N′-methylenebisacrylamide, which facilitate the formation of stable, three-dimensional structures (Ali et al., 2022; Bashir et al., 2020). While effective, these chemical crosslinking methods often involve toxic reagents and by-products that may pose biocompatibility issues, particularly for medical and pharmaceutical applications. Additionally, the residual crosslinking agents might remain in the hydrogel, potentially leading to adverse reactions when applied to sensitive tissues or open wounds (Hennink & Van Nostrum, 2002; Maiti et al., 2024; Mashabela et al., 2022). Therefore, developing alternative crosslinking methods that avoid these drawbacks is crucial for advancing hydrogel technology.

In this context, radiation-induced crosslinking emerges as a promising green alternative. Gamma radiation, in particular, offers a clean and efficient method to induce crosslinking without the need for additional chemical agents. This technique relies on high-energy photons to create free radicals within the polymer matrix, leading to the formation of covalent bonds between polymer chains (Bray & Merrill, 1973; Rosiak & Ulański, 1999). The absence of toxic crosslinkers enhances the biocompatibility and safety profile of the resulting hydrogels, making them highly suitable for biomedical applications. Furthermore, radiation-induced crosslinking can be precisely controlled by adjusting the radiation dose, enabling the customization of hydrogel properties such as swelling capacity and mechanical strength (Lugao & Malmonge, 2001; Rosiak et al., 1995; Yang et al., 2022). This environmentally friendly approach not only simplifies the hydrogel synthesis process but also ensures that the final product is free from harmful contaminants, aligning with the principles of green chemistry and sustainable development (Ahmed et al., 2024). In biomedical applications, radiation-synthesized hydrogels are employed in wound healing (Demeter et al., 2023), tissue engineering scaffolds (Kim et al., 2017), and controlled drug delivery systems (Ghobashy et al., 2021) due to their biocompatibility and ability to mimic biological environments. They also find use in environmental applications for water purification and pollutant remediation, showcasing their versatility and broad utility across different disciplines (Haque et al., 2024).

pH-sensitive hydrogels play a crucial role in various biomedical and pharmaceutical applications due to their unique ability to respond to changes in pH. These hydrogels are designed to swell or shrink

in response to the pH of their environment, allowing for controlled release of drugs or other therapeutic agents (Singh et al., 2020a; Sood et al., 2014). This capability is particularly beneficial for targeted drug delivery, where the hydrogel releases its payload at specific sites in the body with different pH levels, such as the neutral or slightly alkaline environment of a wound. This targeted approach not only improves the efficacy of the treatment but also minimizes side effects on healthy tissues (Li et al., 2021a, Al-Arjan et al., 2022). Healthy skin maintains a mildly acidic pH ranging from 4.0 to 6.0, which is essential for regulating bacterial flora and preventing infections. However, when a wound occurs, this acidic environment is disrupted, revealing the underlying tissue's neutral pH. Particularly, for acute wounds, there is a fluctuation in pH ranging from 7.20 to 8.35 (Kruse et al., 2017; Schreml et al., 2010). This alkaline environment in chronic wounds can foster bacterial proliferation and trigger a cycle of inflammation that exacerbates the wound's severity, potentially resulting in tissue death or even amputation (Kuo et al., 2020). Therefore, it is crucial to control the drug delivery mechanisms to actively modulate the pH of the wound. In wound care, pH-sensitive hydrogels can respond to the altered pH of the wound environment, releasing antimicrobial agents or other therapeutics to promote healing and prevent infection. At the neutral pH of a wound, these hydrogels can provide a sustained, controlled release of medication, which is critical for maintaining therapeutic levels over an extended period and promoting efficient healing (Zong et al., 2023). Additionally, these hydrogels enable sustained drug release over extended periods, enhancing therapeutic outcomes and reducing the need for frequent dosing, which improves patient compliance (Al-Arjan et al., 2022). The environmentally friendly synthesis of pH-sensitive hydrogels, such as through radiation-induced crosslinking, further enhances their appeal by ensuring safety and sustainability.

Hydrogels can be synthesized from a diverse array of materials, including monomers, synthetic polymers, and naturally derived polysaccharides (Maiti et al., 2024). In this study, sodium alginate was used to create pH-sensitive hydrogels. Sodium alginate is a natural polysaccharide derived from brown seaweed and is widely recognized for its safety and biocompatibility. Its approval for use in food products by the Food and Drug Administration (FDA) underscores its non-toxic nature and suitability for consumption. This inherent safety profile makes sodium alginate an excellent candidate for medical and pharmaceutical applications, particularly for creating drug delivery systems and wound dressings (Augst et al., 2006; Bidarra et al., 2014; Rowley et al., 1999). In this study, hydrogels were synthesized by crosslinking sodium alginate with citric acid using gamma radiation. The pH sensitivity of these hydrogels is primarily due to the chemical structure of sodium alginate and its interaction with citric acid. Citric acid, bearing three carboxylic and one hydroxyl group, offers an ideal platform for pH-sensitive materials (Franklin & Guhanathan, 2015) and has also been found to possess antimicrobial properties (Pooresmaeil et al., 2021). On the other hand, sodium alginate contains carboxyl groups, which can ionize and form carboxylate ions in aqueous solutions. The degree of ionization and the overall charge of the hydrogel network depend on the pH of the surrounding environment. At different pH levels, the carboxyl groups within the sodium alginate matrix undergo ionization changes. At acidic pH (low pH), fewer carboxyl groups are ionized, resulting in less swelling of the hydrogel. Conversely, at neutral or slightly basic pH, more carboxyl groups ionize, leading to increased swelling of the hydrogel (Zhang et al., 2022). This behavior also promotes controlled drug release, offering particular benefits at the natural pH typical of wound environments, thereby ensuring sustained and targeted therapeutic effects. Here, the synthesized hydrogel with optimum parameters was used for the in vitro release study of Doxycycline hyclate (DH). This model drug was employed to investigate the pH-sensitive release mechanisms of gamma-synthesized hydrogels. DH, a second-generation broad-spectrum antibiotic in

the tetracycline class, was developed to provide improved effectiveness and lower toxicity compared to earlier tetracycline antibiotics. In addition to its oral use, doxycycline hyclate can also be applied topically for treating certain skin and mucosal infections, including those caused by ulcers and diabetic wounds. Its broad-spectrum activity, combined with its lower toxicity, makes it a versatile and widely used antibiotic in both human and veterinary medicine (Gabriele et al., 2019; Saliy et al., 2024). Various strategies have been proposed for the use of DH in biomedical applications, including the employment of in situ gel systems capable of transitioning from a sol state to a gel form under physiological conditions. For example, Atridox® (Atrix Laboratories), a commercially available in situ gel formulation containing DH, has been effective in treating periodontitis and other inflammatory conditions affecting dental support tissues and muscoskeletal wounds (Javali & Vandana, 2012). Other in situ gel formulations for DH delivery have also been explored using polymers such as ethyl cellulose, eudragit RS, shellac, bleached shellac, cholesterol, benzyl benzoate, and N-methyl-2-pyrrolidone (Saliy et al., 2024). Limited hydrogel studies synthesized by conventional methods on DH delivery were based on polyethylene glycol (PEG)-based hydrogels (Anumolu et al., 2011), collagen-based hydrogels crosslinked with glutaraldehyde (Sanapalli et al., 2021) and hydrogel films composed of chitosan and polyvinyl alcohol (Hedayatyanfard et al., 2020). Other formulations include alginate and pectin core-shell aerogel beads where DH is encapsulated into the pectin core and protected by an alginate shell (De Cicco et al., 2016), alginate microgels embedded in Pluronic F127 thermogels for intradermal sustained delivery (Giovagnoli et al., 2010), and electrospun nanofibers of polylactic acid-hydroxyapatite (Farkas et al., 2022). Clearly, sodium alginate holds promise for DH as a next-generation drug delivery system, yet studies specifically focusing on sodium alginate hydrogels (NaAlg) have been notably absent. On the other hand, radiation crosslinking has been explored in only two studies for DH formulations: hydrogels of O-carboxymethyl chitosan conjugated with caffeic acid and its composite with polyacrylamide synthesized using electron beam irradiation (Moghaddam et al., 2020), and gamma-irradiated poly (vinyl alcohol)/methylcellulose blend hydrogels (El-Naggar et al., 2016). This study pioneers the synthesis of sodium alginate hydrogels using gamma irradiation, integrating citric acid to control the swelling mechanism, not yet documented in the literature for the controlled and pH specific release of DH.

MATERIALS AND METHODS

Materials

Alginic acid sodium salt from brown algae (Sodium alginate, NaAlg, low viscosity, Sigma-Aldrich), citric acid monohydrate (CA,≥99.0%, Sigma-Aldrich), doxycycline hyclate (DH, Kunshan Chemical & Pharmaceutical Co., Ltd, China) were used as received. Hydrochloric acid (HCl, 37%, Merck), sodium hydroxide (NaOH, pellets ACS/Reag. Ph. Eur., Sigma-Aldrich) and phosphate-buffered saline (PBS, Sigma-Aldrich) were used to adjust the pH of the solutions. Deionized water (conductivity 0.01 μS, filtered through 0.2 μm pore-size Durapore filters (Millipore Corp.)) was used to prepare NaAlg/CA solutions.

Synthesis of NaAlg/CA Hydrogels by Gamma İrradiation

Hydrogel synthesis was conducted by preparing 10 mL solutions of 1% sodium alginate (NaAlg) and 0.2%, 0.1%, and 0.05% citric acid (CA) in the following ratios: 5:1 NaAlg/CA (w/w), 10:1 NaAlg/CA (w/w), and 20:1 NaAlg/CA (w/w), respectively. These solutions were then transferred to 38 mm PP syringes and sealed, ensuring the removal of excess air. The samples were irradiated in air at ambient temperature using a Co-60 gamma source (Gammacell 220 Nordion, Canada) at a dose rate of

1 kGy/h for 10 h to achieve a total absorbed dose of 10 kGy.The irradiated samples conformed precisely to the shape of the mold (syringe), demonstrating complete and uniform gelation of the solutions, as illustrated in Figure 1. The samples were then sliced into hydrogel discs using a razor blade for subsequent studies. The synthesized hydrogels were purified over two days by immersing them in excess water and agitating at 150 rpm on an orbital shaker, with periodic replenishment of water. The purified hydrogels were dried overnight at 30°C in a vacuum oven for further studies.

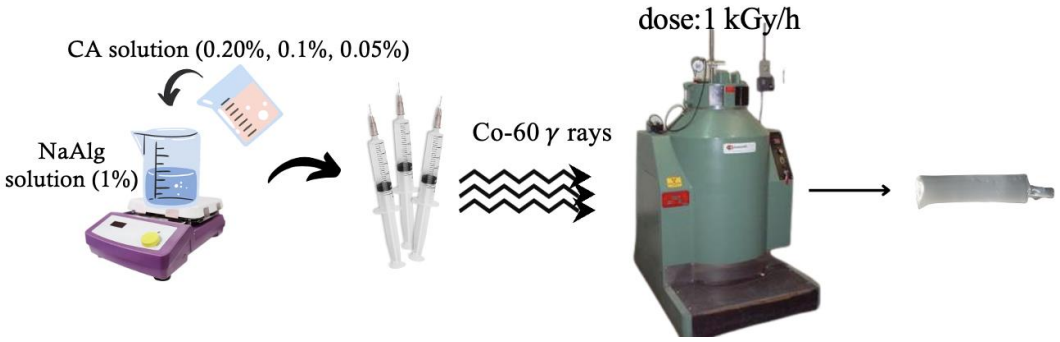


Figure 1. The synthesis of NaAlg/CA hydrogels

Characterization of NaAlg/CA Hydrogels

NaAlg, CA, and dried NaAlg/CA hydrogels were analyzed using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR). Spectra were acquired using a PerkinElmer Spectrum Two™ spectrometer in ATR mode with 4 scans and a resolution of 4 cm^{-1}, in the range of 500–4000 cm^{-1} from solid or dried hydrogel samples.

The surface morphology of the dried hydrogel was examined by environmental scanning electron microscopy (FEI Quanta 200F ESEM) operating at 10 kV. Cross sections of freeze-dried hydrogel were prepared by using a sharp razor blade and the samples were gold-sputtered (5 nm) prior to imaging.

In order to determine the gel fraction of hydrogels, samples were placed in distilled water within a shaker incubator at room temperature for 24 hours to remove the soluble fraction. Subsequently, the gel was dried under vacuum until a constant weight and the insoluble fraction was determined gravimetrically. The gel fraction was calculated using the Equation 1:

$$\text{Gel Fraction (\%)} = w_e/w_o \times 100 \quad (1)$$

where w_e is the weight of dry gel after extraction and w_o is the initial weight of the dry gel after irradiation.

For the swelling measurements, dried hydrogel samples (0.02 g) were immersed in various solutions with different pH levels. After an equilibration period of 24 hours, the swollen hydrogels were removed, gently blotted to remove any excess surface solvent, and then weighed. The percent swelling (%S) of the hydrogel was calculated using Equation 2:

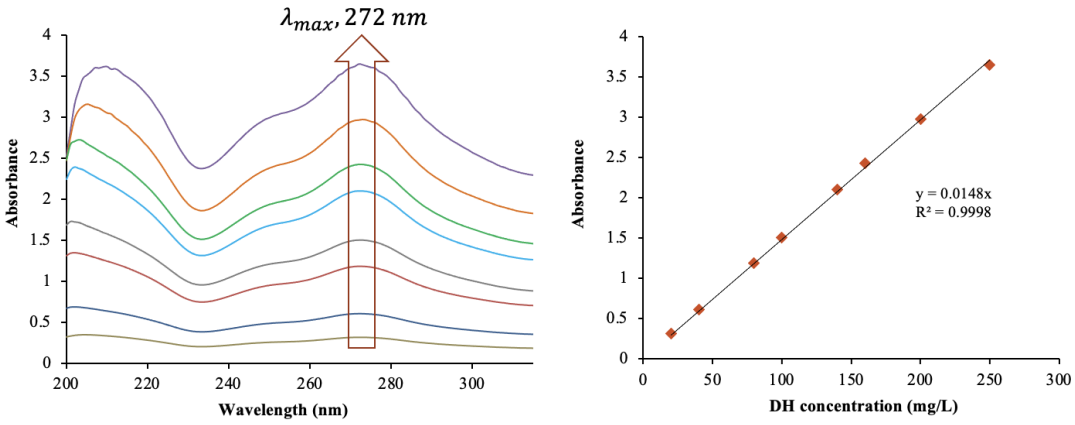
$$\%S = \frac{(w_s - w_i)}{w_i} x\ 100 \quad (2)$$

where w_i is the initial dry weight and w_s is the final weight of the hydrogel at specific time intervals.

Loading of DH to NaAlg/CA Hydrogels

To investigate the release profile of Doxycycline Hyclate (DH) from hydrogels at different pH levels, a specific experimental setup was designed. Initially, a DH solution was prepared at a

concentration of 5 mg/mL, with a total volume of 20 mL. Hydrogels with a dry mass of approximately 10 mg each were then immersed in three different buffer solutions, corresponding to pH values of 5.5, 7.4, and 9.0 and kept in a rotator for 24 hours for DH loading. The supernatant was collected to assess the amount of unloaded and hence loaded drug using a UV/vis spectrophotometer (Varian Cary100) operating at a wavelength range of 250−800 nm at room temperature. The absorbance of DH at 272 nm was measured to determine its concentration from the UV/Vis spectra of standard DH solutions given in Figure 2a. A calibration curve for calculating drug loading was created using the UV absorbance of standard DH concentrations (20–250 mg/L) as depicted in Figure 2b.



(a) (b)

Figure 2. UV/Vis spectra of standard DH solutions (20–250 mg/L) (a) and linear calibration curve of standard DH solutions, derived from the maximum absorbance of DH at 272 nm (b)

The formulas used to calculate drug loading capacity (DLC) and drug loading efficiency (DLE) were presented in Equations 3 and 4:

$$\text{Drug loading capacity (DLC)} = \frac{\text{Weight of the drug in the hydrogel}}{\text{Total weight of the hydrogel}} \text{ x } 100 \quad (3)$$

$$\text{Drug loading efficiency (DLE)} = \frac{\text{Weight of the drug in the hydrogel}}{\text{Initial weight of the drug used for loading}} \text{ x } 100 \quad (4)$$

The pH sensitive drug release of DH@NaAlg/CA Hydrogels

The DH release from hydrogels was performed in an orbital shaking water bath at 37°C at 150 rpm, lasting up to 40 hours. In detail, DH loaded NaAlg/CA hydrogels were transferred into 20 mL of release medium at pH values of 5.5, 7.4 and 9.0. Periodically, 1 mL of solution was extracted from the medium and immediately replenished with fresh pH solution to keep the volume constant. The retrieved samples were examined using UV/Vis spectroscopy, focus on DH's absorbance peak emission at 272 nm (Figure 2a) which was correlated with DH concentration using the linear calibration equation given in Figure 2b. All the release studies were studied in triplicates.

RESULTS AND DISCUSSION

The use of gamma radiation for crosslinking vinyl polymers and polysaccharides in solution has been well-documented (Ghobashy et al., 2021; Makuuchi, 2010; Tranquilan-Aranilla et al., 1999; Rosiak et al., 1995). Exposure of polymer aqueous solutions to ionizing radiation leads to the formation of reactive intermediates on the macromolecules, as described in Equation 5, arising both from direct radiation effects on polymer chains where the radiation can directly break the chemical bonds within

polymer chains resulting in the formation of radicals on the polymers themselves and from the indirect reaction of water radiolysis generating highly reactive species such as hydroxyl radicals (OH•), hydrogen atoms (H•), and hydrated electrons (e_{aq}^-) as the main products. These reactive intermediates subsequently interact with the polymer chains (Makuuchi, 2010; Rosiak et al., 1995).

$$H_2O \xrightarrow{\text{ionizing radiation}} \cdot OH, e_{aq}^-, H^{\cdot}, H^+, H_2, H_2O_2 \quad (5)$$

These radicals would then transfer to sodium alginate, increasing the number of alginate radicals, consequently increasing the rate of crosslinking and gelation. This crosslinking results in the formation of a gel, improving the material's mechanical properties and stability up to a certain dose (Chang et al., 2022; Huq et al., 2012). Several studies demonstrate the fact that higher doses such as 20-40 kGy may result in radiolytic degradation which negatively affects the physicochemical properties of sodium alginate (Chang et al., 2022; Craciun et al., 2023, Şen, 2011). Therefore, a 10 kGy dose was selected for this study for the crosslinking of NaAlg/CA solutions to minimize this degradation behavior but also promote adequate crosslinking.

Citric acid, on the other hand, was also reported to promote the crosslinking of polymers. Thermoresponsive hydrogels based on xanthan and poly(N-isopropyl acrylamide) were crosslinked using citric acid (Chen et al., 2019) and pectin and sodium alginate were crosslinked through crosslinking with citric acid (CA) and tartaric acid (TA) and proposed as alternative green packaging materials (Singh et al., 2020b). The incorporation of citric acid for crosslinking various polymers with ionizing radiation has also been extensively explored for polymers such as poly(vinyl alcohol) (Bodugöz et al., 1999), poly(N-vinyl pyrrolidone) (Caykara et al., 2000), poly(acrylamide) (Karadag et al., 2001), and poly(N-isopropyl acrylamide) (El-Arnaouty et al., 2015). However, there appears to be a lack of research exploring the radiation synthesis of hydrogels specifically using the combination of sodium alginate and citric acid.

Briefly, NaAlg-CA hydrogels were prepared by dissolving 1% sodium alginate (NaAlg) and varying concentrations (0.2%, 0.1%, and 0.05%) of citric acid (CA) in ratios of 5:1, 10:1, and 20:1 NaAlg/CA (w/w) in water subsequently irradiated under atmospheric conditions using a Co-60 gamma source at a dose rate of 1 kGy/h. The resulting hydrogels conformed to the mold shape, indicating uniform gelation (Figure 1). The samples were then cut into discs for further studies.

The gel fractions of hydrogels were calculated using Equation 1 and the results were given in Table 1. The results indicate that increasing the ratio of sodium alginate to citric acid generally leads to higher gel fractions, suggesting a higher degree of crosslinking and gel formation in the hydrogel samples. Even though the citric acid were expected to promote crosslinking, similar behavior was observed in the studies with poly(vinyl alcohol) hydrogels synthesized in the presence of citric acid where gelation was decreased with CA addition (Bodugöz et al., 1999). To better understand the structural behavior of hydrogels, it is important to examine the relationship between their crosslinking density and water absorption capabilities. Therefore swelling measurements were performed in DI water. Table 1 presents the equilibrium swelling ratios of samples determined after 24 hours, where swelling reaches a maximum and stabilizes. The results suggest an inverse relationship between gel fraction and equilibrium swelling: hydrogels with higher crosslinking densities (higher gel fraction) tend to exhibit lower equilibrium swelling capacities. This phenomenon is due to the tighter network structure formed by increased crosslinking, which restricts the hydrogel's ability to absorb water. The % swelling reached as high as 3880 % for NaAlg/CA with 5:1 NaAlg/CA ratio where it decreased to 2763 % for 20:1 NaAlg/CA ratio.

Therefore, these preliminary tests showed that ratio of 5:1 was would be optimum providing highest swelling capacity.

Table 1. The compositions, gel fraction (%) and equilibrium swelling (%) of NaAlg/CA hydrogels (Dose=10 kGy)

Entry	NaAlg (%)	CA (%)	NaAlg/CA ratio	Gel Fraction (%)	Equilibrium Swelling (%)
1	1	0.2	5:1	81.2 ± 1.6	3880 ± 33
2	1	0.1	10:1	83.4 ± 2.1	3201 ± 46
3	1	0.05	20:1	88.7 ± 1.9	2763 ± 71

The swelling kinetics of the hydrogels in water are illustrated in Figure 3. The samples exhibited a typical swelling profile over time. Initially, the hydrogels absorbed water rapidly, reaching their maximum swelling capacity within the first few hours. Following this initial phase, the swelling rate stabilized, and the hydrogels maintained nearly constant volume, indicating that equilibrium was reached within 8 hours with minimal additional water uptake observed up to 24 hours. This behavior is typical of hydrogel materials, demonstrating their ability to swell rapidly and retain a substantial amount of water relative to their dry mass. Furthermore, the influence of different concentrations of citric acid (CA) on swelling kinetics aligns with earlier discussions.

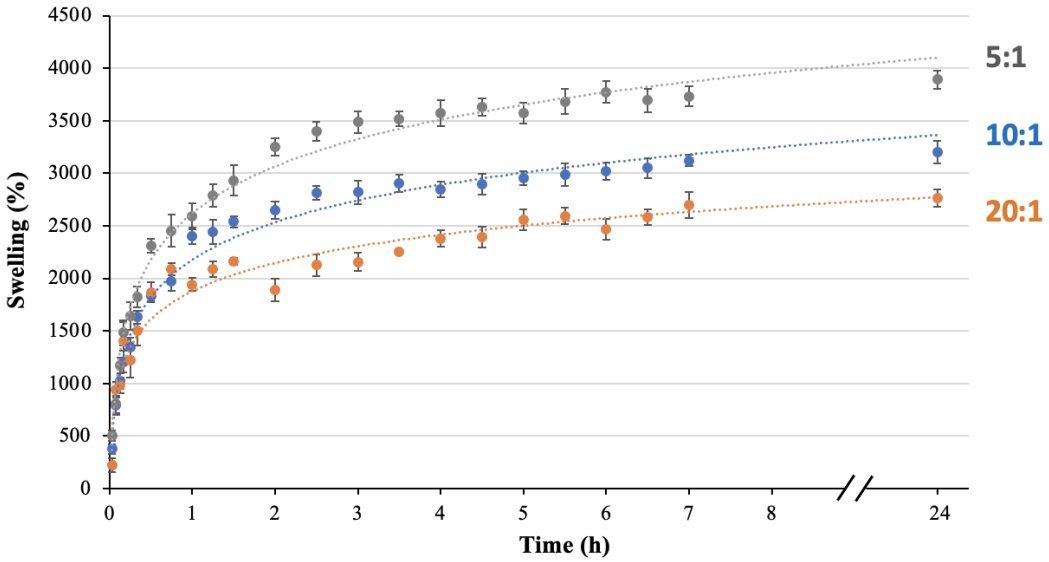


Figure 3. Swelling kinetics of NaAlg/CA hydrogels with NaAlg/CA ratios 5:1, 10:1 and 20:1 in water (dose=10 kGy)

The swelling experiments were also conducted across various pH levels, spanning from pH 1 to pH 10, as depicted in Figure 4. The swelling behavior of hydrogel based on NaAlg is intricately linked to pH-induced changes in the ionization state of its carboxyl groups along its molecular chains. In acidic conditions, carboxyl groups ($-COO^-$) protonate to form carboxylic acid groups (-COOH), leading to strengthened hydrogen bonding and reduced hydrogel swelling. As pH becomes neutral to basic, -COOH groups ionize into $-COO^-$ ions, weakening hydrogen bonds and allowing the hydrogel to expand due to increased hydrophilicity and water absorption. This pH-responsive property was also depicted in Figure 4. where all NaAlg/CA hydrogels show similar pH-responsive property. Specifically, the hydrogels with the highest citric acid ratio (5:1) displayed greater swelling across all pH levels as compared to hydrogels with lower citric acid amount except for pH 10. This could be attributed to the enhancement of the pH sensitivity of the hydrogel due to CA. At lower pH levels, CA facilitates stronger interactions between sodium alginate chains through hydrogen bonding and electrostatic attractions. As pH levels increase,

the ionization of citric acid and sodium alginate carboxyl groups decreases these interactions, allowing the hydrogel to swell further.

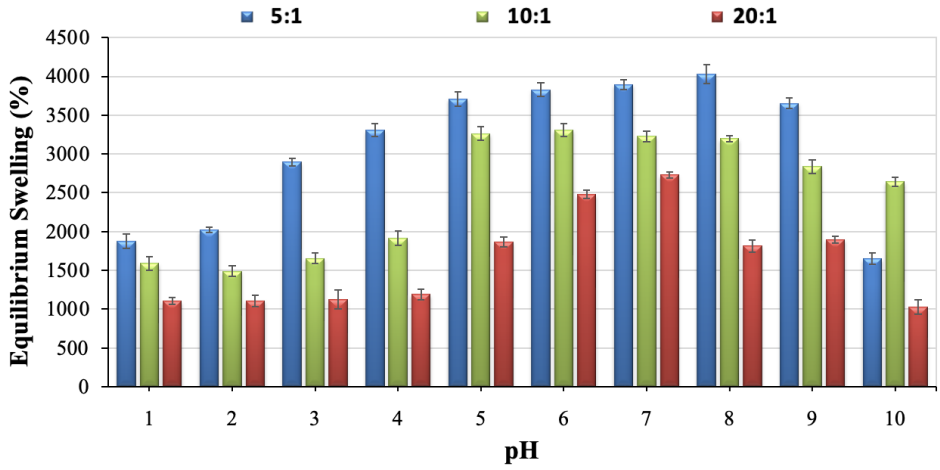


Figure 4. Equilibrium swelling (%) of NaAlg/CA hydrogels with NaAlg/CA ratios 5:1, 10:1 and 20:1 at different pHs (Dose=10 kGy)

The structural characterizations of NaAlg/CA hydrogels were performed using FTIR spectroscopy. As shown in Figure 5, the FTIR spectrum of pure SA revealed a broad absorption band at 3249 cm^{-1} attributed to –OH stretching vibrations, a peak at 2910 cm^{-1} associated with C–H stretching, and a peak at 1593 cm^{-1} assigned to asymmetric stretching vibrations of COO− groups, the peak at 1404 cm^{-1} corresponded to the C–H deformation and 1024 cm^{-1} was attributed to C–O symmetric stretching vibrations of the glycosidic bond typical for polysaccharides (Li et al., 2021b; Sezen et al., 2021). The characteristic peaks of citric acid were as follows: absorption bands in the region of 3100-3500 cm^{-1} due to O-H stretching vibration, peaks at 1662 and 1695 cm^{-1} are associated with the stretching vibration of the C=O bond of -COOH, and 779 cm^{-1} due to CH_2 rocking (Li et al., 2021b). NaAlg/CA hydrogels present all the characteristic peaks of NaAlg in addition to the specific peaks corresponding to citric acid (CA). As the concentration of CA increases, the intensity of peaks corresponding to the CA also increases clearly visible for peaks at 1662 and 1695 cm^{-1} (C=O) and 779 cm^{-1} (CH_2), indicating greater incorporation of CA into the hydrogel matrix.

As the concentration of CA increases, the intensity of peaks corresponding to CA becomes more pronounced, particularly notable at peaks around 1662 and 1695 cm^{-1} (C=O stretching) and 779 cm^{-1} (CH_2 rocking). This phenomenon reflects the progressive interaction and integration of citric acid molecules within the sodium alginate network, influencing the structural and chemical properties of the hydrogels.

The optimal conditions for NaAlg/CA hydrogels were presented and ratio of 5:1 was selected for prior in vitro studies of DH. DH is a tricarboxylic acid and present multiple pKa values as 3.02, 7.97 and 9.15 (Kogawa & Salgado, 2012) as illustrated in Figure 6a. Therefore, drug loading experiments were performed in different pHs to assess the effect of pH on DLC and DLE of DH using Equations 2 and 3. Figure 6b illustrates the effect of dissolution medium pH on these parameters. The lowest drug loading was attributed to the pH 5.5 condition, where DH tends to be more protonated due to its lower pKa values. This protonation potentially reduced its interaction with sodium alginate, resulting in lower drug loading compared to neutral pH conditions. On the other hand, at pH 7.4, DH exists in a mixture of protonated and deprotonated forms. Sodium alginate, in its deprotonated form ($-COO^-$), possibly interacted more effectively with DH due to electrostatic attractions resulting in highest drug loading. At

pH 9.0, DH becomes predominantly deprotonated, its interaction with the hydrogel matrix was expected to be enhanced. However, excessive alkalinity may have affected DH's stability or solubility, potentially influencing its loading efficiency despite increased electrostatic interactions.

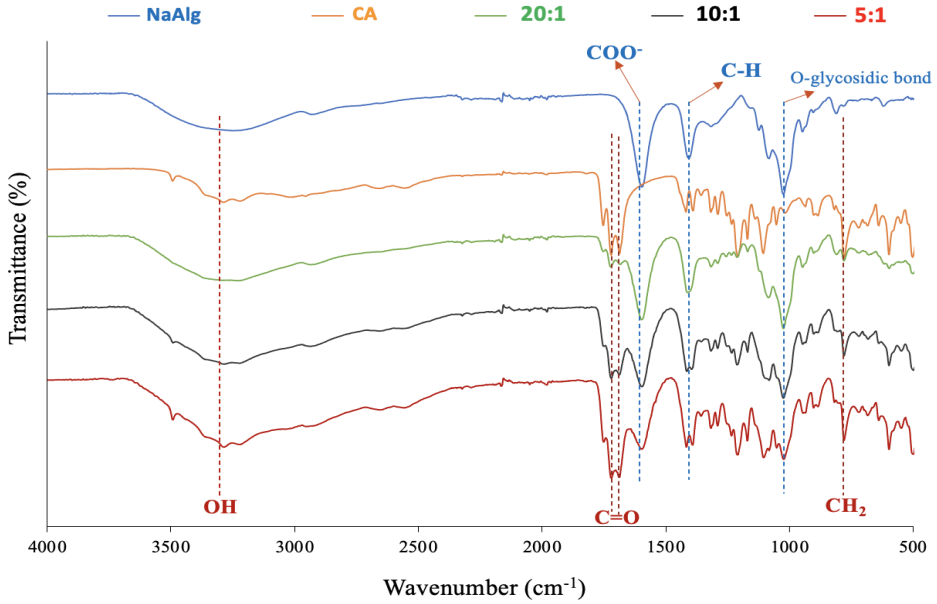


Figure 5. FTIR spectra of NaAlg, CA and NaAlg/CA hydrogels with different CA amount (NaAlg/CA=5:1, Dose=10 kGy)

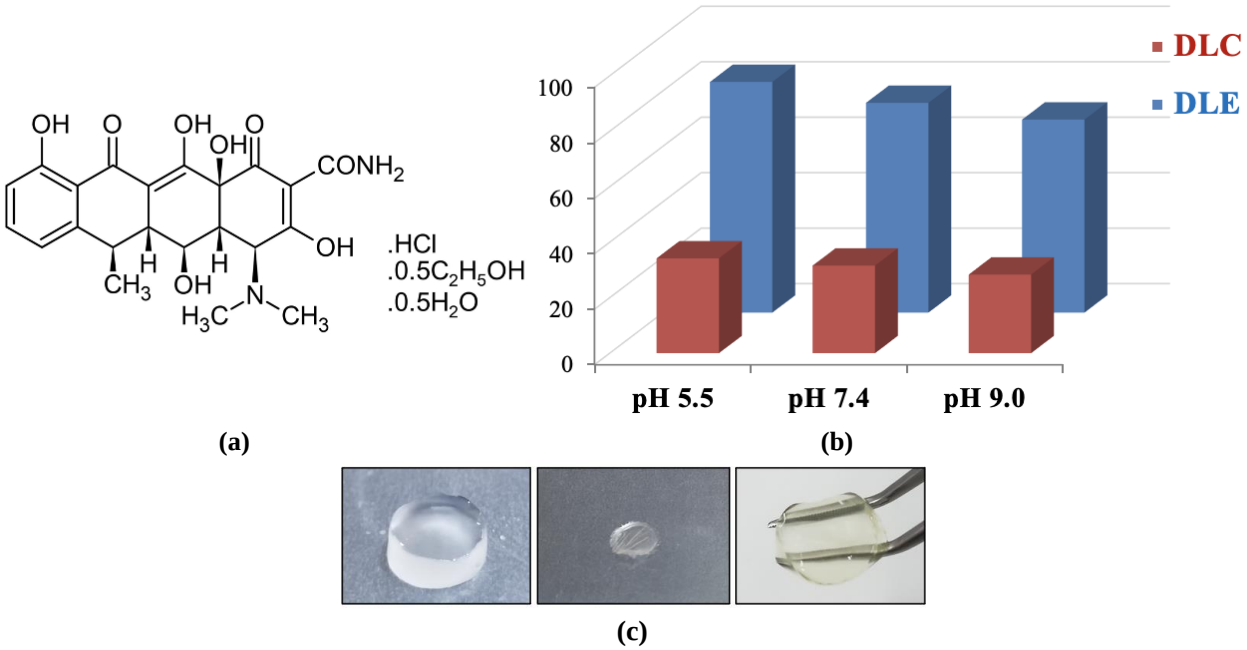


Figure 6. Chemical structure of doxycycline hyclate (DH) (a), DLC and DLE values for DH loading into NaAlg/CA hydrogels at different pHs (b), and photographs of swollen, dried and DH loaded NaAlg/CA hydrogels (from left to right) (c) (NaAlg/CA=5:1, Dose=10 kGy)

DH loading into hydrogels was characterized using FTIR spectroscopy, as illustrated in Figure 7. The results revealed the integration of DH into the hydrogel structure, evidenced by the presence of specific DH peaks as the C–N stretching band at 1039 cm^{-1}, the amide I band attributed to C=O stretching at 1610 cm^{-1}, and the amide II band due to N–H bending at 1554 cm^{-1}, all of which were distinctly observed in the DH-loaded hydrogels (Yadav et al., 2017).

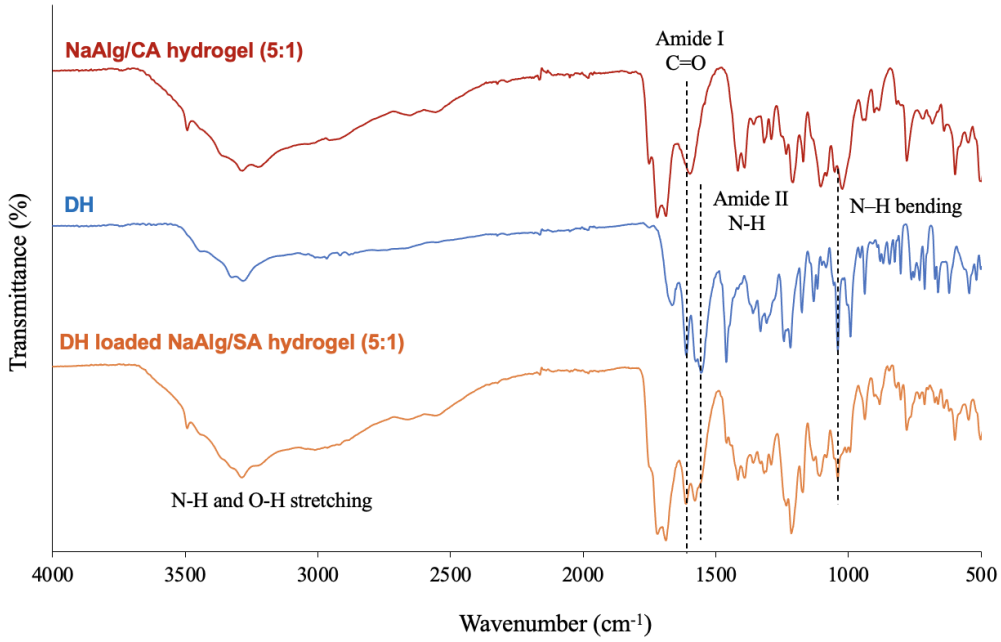
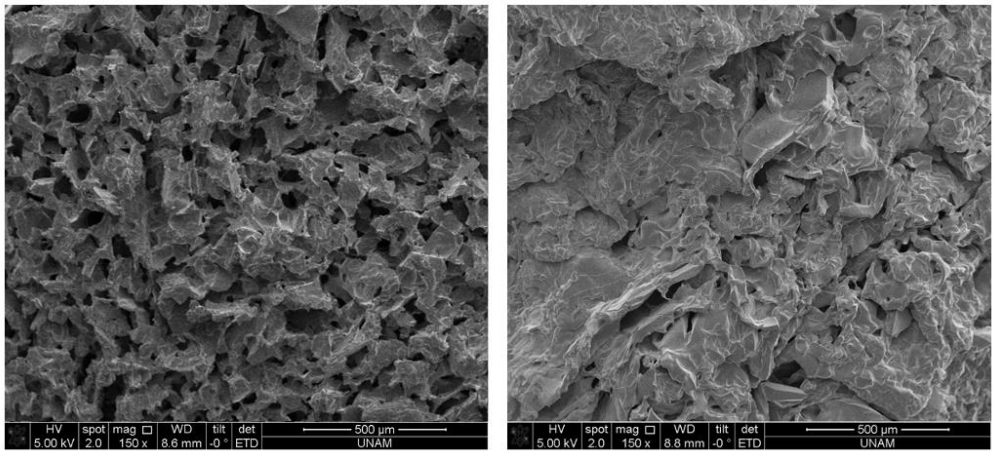


Figure 7. FTIR spectra of NaAlg/CA hydrogel, DH, and DH loaded NaAlg/CA hydrogel (NaAlg/CA=5:1, Dose=10 kGy)

Figure 8 depicts the SEM images of freeze-dried bare and DH loaded NaAlg/CA hydrogel (NaAlg/CA=5:1, Dose=10 kGy) where unloaded hydrogel possessed porous 3D structure and loading with DH revealed a significant reduction in pore size, indicating effective incorporation and closure of pores due to the drug loading process.



(a) **(b)**

Figure 8. SEM images of dried (a) and DH loaded NaAlg/CA hydrogel (b) (NaAlg/CA=5:1, Dose=10 kGy)

Acute wounds typically have a higher pH ranging from 7.20 to 8.35 compared to healthy skin, which remains mildly acidic (pH 4.0 to 6.0) (Kruse et al., 2017). Several pH sensitive materials were proposed in the literature for enhanced delivery in acidic or basic conditions but achieving sustained drug release specifically tailored to neutral to alkaline pH ranges is limited (Al-Arjan et al., 2022; Li et al., 2021a). Figure 9. illustrates the in vitro release profiles of DH from NaAlg/CA hydrogels in dissolution media at pH levels 5.5, 7.4, and 9.0. The results indicated that drug release from the hydrogels is pH-dependent. At pH 5.5 and pH 9, half of the drug was released within just 20 minutes, demonstrating a burst release, with a subsequent decrease in release rate at these pH levels as compared to pH 7.4. At acidic pH, the carboxylate group of NaAlg protonates, leading to strong hydrogen bonds with DH and a consequent

reduction in drug release. At high pH, NaAlg transforms into its sodium salt form, enhancing hydrophilicity and increasing drug release due to electrostatic repulsion. However, the most effective release occurred at pH 7.4, typical of chronic wound environments, where the highest release was observed reaching 85% at 3 hours. Unlike other conditions, at pH 7.4, a slower and biphasic release pattern was noted, lasting up to 40 hours, following a conventional profile.

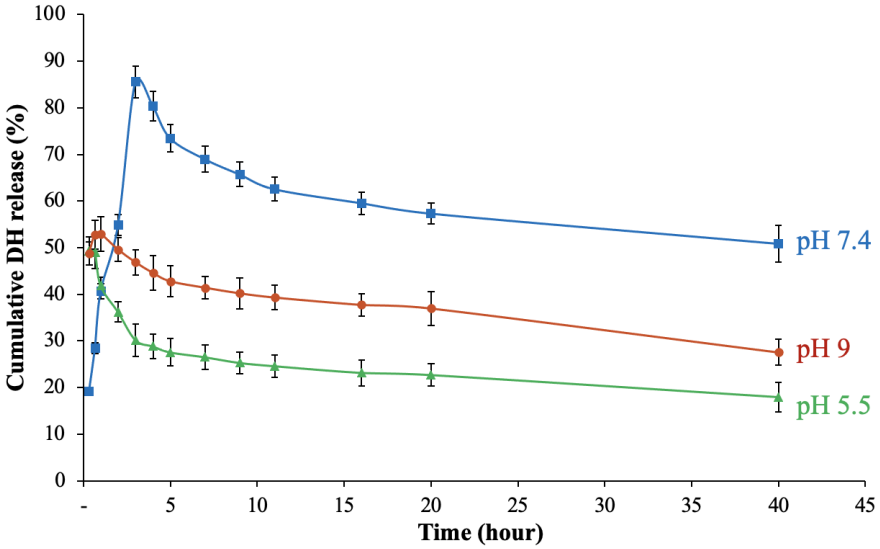


Figure 9. In vitro release profiles of DH from NaAlg/CA hydrogels in dissolution media with pH values of 5.5, 7.4 and 9.0

Various studies have examined doxycycline hyclate (DH) release, including nanoparticles, in-situ gelling systems, and hydrogels. However, most of these systems do not exhibit any pH sensitivity and/or the studies do not consider the effect of pH. Kazmi et al. studied the pH-dependent release of DH from gold nanoparticles showing 60% release at pH 4.30 and only 5% at pH 7.34 (Kazmi et al., 2019). Another study focused on DH release from crosslinked and non-crosslinked chitosan sponges, observing that the non-crosslinked sponge exhibited a slower initial release due to gel formation, while both systems could prolong drug release, but without pH sensitivity (Phaechamud, Charoenteeraboon, 2008). Guglava et al. studied niosomes with various surfactants and cholesterol for ocular delivery of doxycycline hyclate, aiming for prolonged release. While the release duration was extended, the study did not address the effect of pH (Gugleva et al., 2019). Ranch et al. designed an in situ gel-forming system using Poloxamer 407, chitosan, and polyethylene glycol 600, which achieved sustained drug release above the minimum inhibitory concentration (MIC) and high drug retention in the periodontal cavity. Although this system offered ease of administration and sustained release, pH sensitivity was not a focal point (Ranch et al., 2021). Patlolla et al. investigated hydrogels composed of Poloxamer 407 and Poloxamer 188, observing an increasing release profile up to 50 hours. However, similar to the previous studies, pH sensitivity was not considered (Patlolla et al., 2019). Phaechamud at al. explored doxycycline hyclate-loaded bleached shellac in situ forming gel achieving maximum release within 1–2 hours and rapid equilibrium, without addressing pH-dependent release (Phaechamud et al., 2019). Ardica et al. presented a hydrogel system based on crosslinks between carboxymethyl chitosan (CMC) and aldehyde hyaluronic acid (AHA). This study achieved up to 85% DH release and examined the release at pH 5.5 and 7.4, but found no significant differences in the release profiles between these pH levels (Ardica et al., 2023). Overall, pH-sensitive in-vitro drug release profiles of DH-based systems, especially at neutral pH, are uncommon. This highlights the unique advantage of the proposed NaAlg/CA hydrogels.

CONCLUSION

This study showcased the environmentally friendly synthesis of sodium alginate/citric acid (NaAlg/CA) hydrogels using gamma radiation, offering a sustainable and safe approach devoid of toxic chemicals, thus minimizing environmental impact. The inclusion of citric acid significantly influenced the cross-linking and subsequent swelling behavior of the hydrogels in aqueous environments across varying pH conditions, crucial for developing robust and pH-responsive structures. The hydrogel with an optimal NaAlg/CA ratio of 5:1 was selected for in vitro studies, revealing that the release profile of doxycycline hyclate (DH) was strongly influenced by pH conditions. At pH 7.4, simulating the pH of chronic wounds, DH release exhibited an enhanced release profile lasting up to 40 hours, in contrast to the release behavior at pH 5.5 and 9 conditions. These findings underscore the potential of NaAlg/CA hydrogels synthesized via gamma radiation for sustainable and effective drug delivery, particularly in acute wound healing applications where pH-dependent therapeutic efficacy is crucial.

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Conflict of Interest

The article author declare that there is no conflict of interest.

REFERENCES

Ahmed, M. S., Islam, M., Hasan, M. K., & Nam, K. W. (2024). A Comprehensive Review of Radiation-Induced Hydrogels: Synthesis, Properties, and Multidimensional Applications. *Gels*, *10*(6), 381.

Al-Arjan, W. S., Khan, M. U. A., Almutairi, H. H., Alharbi, S. M., & Razak, S. I. A. (2022). pH-Responsive PVA/BC-f-GO Dressing Materials for Burn and Chronic Wound Healing with Curcumin Release Kinetics. *Polymers*, *14*(10), 1949.

Ali, F., Khan, I., Chen, J., Akhtar, K., Bakhsh, E. M., & Khan, S. B. (2022). Emerging Fabrication Strategies of Hydrogels and Its Applications. *Gels*, *8*(4), 205.

Ardika, K.A.R., Marzaman, A.N.F., Kaharuddin, K.M., Parenden, M.D.K., Karimah, A., Musfirah, C.A., Pakki, E. and Permana, A.D. (2023). Development of chitosan-hyaluronic acid based hydrogel for local delivery of doxycycline hyclate in an ex vivo skin infection model. *Journal of Biomaterials Science, Polymer Edition*, *34*(16), 2274-2290.

Anumolu, S. S., Menjoge, A. R., Deshmukh, M., Gerecke, D., Stein, S., Laskin, J., & Sinko, P. J. (2011). Doxycycline hydrogels with reversible disulfide crosslinks for dermal wound healing of mustard injuries. *Biomaterials*, *32*(4), 1204–1217.

Augst, A. D., Kong, H. J., & Mooney, D. J. (2006). Alginate Hydrogels as Biomaterials. *Macromolecular Bioscience*, *6*(8), 623–633.

Bashir, S., Hina, M., Iqbal, J., Rajpar, A. H., Mujtaba, M. A., Alghamdi, N. A., Wageh, S., Ramesh, K., & Ramesh, S. (2020). Fundamental Concepts of Hydrogels: Synthesis, Properties, and Their Applications. *Polymers*, *12*(11), 2702.

Bidarra, S. J., Barrias, C. C., & Granja, P. L. (2014). Injectable alginate hydrogels for cell delivery in tissue engineering. *Acta Biomaterialia*, *10*(4), 1646–1662.

Bodugöz, H., Pekel, N., & Güven, O. (1999). Preparation of poly(vinyl alcohol) hydrogels with radiation grafted citric and succinic acid groups. *Radiation Physics and Chemistry*, *55*(5–6), 667–671.

Bray, J. C., & Merrill, E. W. (1973). Poly(vinyl alcohol) hydrogels. Formation by electron beam irradiation of aqueous solutions and subsequent crystallization. *Journal of Applied Polymer Science*, *17*(12), 3779–3794.

Caykara, T., Oren, S., Kantoglu, M., & Guven, O. (2000). The effect of gel composition on the uranyl ions adsorption capacity of poly(N-vinyl 2-pyrrolidone-g-citric acid) hydrogels prepared by gamma rays. *Journal of Applied Polymer Science*, *77*(5), 1037–1043.

Chang, K. A., Chew, L. Y., Law, K. P., Ng, J. F., Wong, C. S., Wong, C. L., & Hussein, S. (2022). Effect of gamma irradiation on the physicochemical properties of sodium alginate solution and internally crosslinked film made thereof. *Radiation Physics and Chemistry*, *193*, 109963.

Chen, X., Li, P., Kang, Y., Zeng, X., Xie, Y., Zhang, Y., Wang, Y., & Xie, T. (2019). Preparation of temperature-sensitive Xanthan/NIPA hydrogel using citric acid as crosslinking agent for bisphenol A adsorption. *Carbohydrate Polymers*, *206*, 94–101.

Craciun, G., Calina, I. C., Demeter, M., Scarisoreanu, A., Dumitru, M., & Manaila, E. (2023). Poly(Acrylic Acid)-Sodium Alginate Superabsorbent Hydrogels Synthesized by Electron Beam Irradiation Part I: Impact of Initiator Concentration and Irradiation Dose on Structure, Network Parameters and Swelling Properties. *Materials*, *16*(13), 4552.

De Cicco, F., Russo, P., Reverchon, E., García-González, C. A., Aquino, R. P., & Del Gaudio, P. (2016). Prilling and supercritical drying: A successful duo to produce core-shell polysaccharide aerogel beads for wound healing. *Carbohydrate Polymers*, *147*, 482–489.

Demeter, M., Scărișoreanu, A., & Călina, I. (2023). State of the Art of Hydrogel Wound Dressings Developed by Ionizing Radiation. *Gels*, *9*(1), 55.

Drury, J. L., & Mooney, D. J. (2003). Hydrogels for tissue engineering: scaffold design variables and applications. *Biomaterials*, *24*(24), 4337–4351.

El-Arnaouty, M. B., Eid, M., & Ghaffar, A. M. A. (2015). Radiation Synthesis of Stimuli Responsive Micro-porous Hydrogels for Controlled Drug Release of Aspirin. *Polymer-plastics Technology and Engineering*, *54*(12), 1215–1222.

El-Naggar, A. W., Senna, M., Mostafa, T., & Helal, R. (2016). Characterization and drug delivery properties of gamma irradiated poly (vinyl alcohol)/methylcellulose (PVA/MC) blends. *The International Conference on Chemical and Environmental Engineering*, *8*(13), 2–22.

Farkas, N. I., Marincaș, L., Barabás, R., Bizo, L., Ilea, A., Turdean, G. L., Toșa, M., Cadar, O., & Barbu-Tudoran, L. (2022). Preparation and Characterization of Doxycycline-Loaded Electrospun PLA/HAP Nanofibers as a Drug Delivery System. *Materials*, *15*(6), 2105.

Franklin, D., & Guhanathan, S. (2015). Investigation of citric acid–glycerol based pH-sensitive biopolymeric hydrogels for dye removal applications: A green approach. *Ecotoxicology and Environmental Safety*, *121*, 80–86.

Gabriele, S., Buchanan, B., Kundu, A., Dwyer, H. C., Gabriele, J. P., Mayer, P., & Baranowski, D. C. (2019). Stability, Activity, and Application of Topical Doxycycline Formulations in a Diabetic Wound Case Study. *PubMed*, *31*(2), 49–54.

Ghobashy, M. M., Elbarbary, A. M., & Hegazy, D. E. (2021). Gamma radiation synthesis of a novel amphiphilic terpolymer hydrogel pH-responsive based chitosan for colon cancer drug delivery. *Carbohydrate Polymers*, *263*, 117975.

Giovagnoli, S., Tsai, T., & DeLuca, P. P. (2010). Formulation and Release Behavior of Doxycycline–Alginate Hydrogel Microparticles Embedded into Pluronic F127 Thermogels as a Potential New Vehicle for Doxycycline Intradermal Sustained Delivery. *AAPS PharmSciTech*, *11*(1), 212–220.

Gugleva, V., Titeva, S., Rangelov, S., & Momekova, D. (2019). Design and in vitro evaluation of doxycycline hyclate niosomes as a potential ocular delivery system. *International journal of pharmaceutics*, *567*, 118431.

Haque, S. N., Bhuyan, M. M., & Jeong, J. H. (2024). Radiation-Induced Hydrogel for Water Treatment. *Gels*, *10*(6), 375.

Hedayatyanfard, K., Khoulenjani, S. B., Abdollahifar, M. A., Amani, D., Habibi, B., Zare, F., Asadirad, A., Pouriran, R., & Ziai, S. A. (2020). Chitosan/PVA/Doxycycline Film and Nanofiber Accelerate Diabetic Wound Healing in a Rat Model. *Iran. J. Pharmaceut. Res.*, *19*(4), 225–239.

Hennink, W., & Van Nostrum, C. (2002). Novel crosslinking methods to design hydrogels. *Advanced Drug Delivery Reviews*, *54*(1), 13–36.

Hoffman, A. S. (2002). Hydrogels for biomedical applications. *Advanced Drug Delivery Reviews*, *54*(1), 3–12.

Huq, T., Khan, A., Dussault, D., Salmieri, S., Khan, R. A., & Lacroix, M. (2012). Effect of gamma radiation on the physico-chemical properties of alginate-based films and beads. *Radiation Physics and Chemistry*, *81*(8), 945–948.

Javali, M. A., & Vandana, K. (2012). A comparative evaluation of atrigel delivery system (10% doxycycline hyclate) Atridox with scaling and root planing and combination therapy in treatment of periodontitis: A clinical study. *Journal of Indian Society of Periodontology*, *16*(1), 43.

Karadag, E., Saraydin, D., Sahiner, N., & Güven, O. (2001). Radiation induced acrylamide/citric acid hydrogels and their swelling behaviors. *Journal of Macromolecular Science. Pure and Applied Chemistry/Journal of Macromolecular Science. Part a. Pure & Applied Chemistry*, *38*(11), 1105–1121.

Kazmi, S. A. R., Qureshi, M. Z., Ali, S., & Masson, J. F. (2019). In vitro drug release and biocatalysis from pH-responsive gold nanoparticles synthesized using doxycycline. *Langmuir*, *35*(49), 16266-16274.

Kim, M. H., Kim, B. S., Lee, J., Cho, D., Kwon, O. H., & Park, W. H. (2017). Silk fibroin/hydroxyapatite composite hydrogel induced by gamma-ray irradiation for bone tissue engineering. *Biomaterials Research/Biomaterials Research*, *21*(1).

Klouda, L., & Mikos, A. G. (2008). Thermoresponsive hydrogels in biomedical applications. *European Journal of Pharmaceutics and Biopharmaceutics*, *68*(1), 34–45.

Koetting, M. C., Peters, J. T., Steichen, S. D., & Peppas, N. A. (2015). Stimulus-responsive hydrogels: Theory, modern advances, and applications. *Materials Science & Engineering. R, Reports*, *93*, 1–49.

Kogawa, A. C., & Salgado, H. R. N. (2012). Quantification of Doxycycline Hyclate in Tablets by HPLC–UV Method. *Journal of Chromatographic Science*, *51*(10), 919–925.

Kruse, C. R., Singh, M., Targosinski, S., Sinha, I., Sørensen, J. A., Eriksson, E., & Nuutila, K. (2017). The effect of pH on cell viability, cell migration, cell proliferation, wound closure, and wound reepithelialization: In vitro and in vivo study. *Wound Repair and Regeneration*, *25*(2), 260–269.

Kuo, S. H., Shen, C. J., Shen, C. F., & Cheng, C. M. (2020). Role of pH Value in Clinically Relevant Diagnosis. *Diagnostics*, *10*(2), 107.

Li, L., He, Y., Zheng, X., Yi, L., & Nian, W. (2021a). Progress on Preparation of pH/Temperature-Sensitive Intelligent Hydrogels and Applications in Target Transport and Controlled Release of Drugs. *International Journal of Polymer Science*, *2021*, 1–14.

Li, Y., Wang, Z., Wang, X., Yan, B., Peng, Y., & Ran, R. (2021b). Fe3+-citric acid/sodium alginate hydrogel: A photo-responsive platform for rapid water purification. *Carbohydrate Polymers*, *269*, 118269.

Lugao, A. B., & Malmonge, S. M. (2001). Use of radiation in the production of hydrogels. *Nuclear Instruments and Methods in Physics Research. Section B, Beam Interactions With Materials and Atoms/Nuclear Instruments & Methods in Physics Research. Section B, Beam Interactions With Materials and Atoms*, *185*(1–4), 37–42.

Maiti, S., Maji, B., & Yadav, H. (2024). Progress on green crosslinking of polysaccharide hydrogels for drug delivery and tissue engineering applications. *Carbohydrate Polymers*, *326*, 121584.

Makuuchi, K. (2010). Critical review of radiation processing of hydrogel and polysaccharide. *Radiation Physics and Chemistry*, *79*(3), 267–271.

Mashabela, L. T., Maboa, M. M., Miya, N. F., Ajayi, T. O., Chasara, R. S., Milne, M., Mokhele, S., Demana, P. H., Witika, B. A., Siwe-Noundou, X., & Poka, M. S. (2022). A Comprehensive Review of Cross-Linked Gels as Vehicles for Drug Delivery to Treat Central Nervous System Disorders. *Gels*, *8*(9), 563.

Moghaddam, R. H., Dadfarnia, S., Shabani, A. M. H., Amraei, R., & Moghaddam, Z. H. (2020). Doxycycline drug delivery using hydrogels of O-carboxymethyl chitosan conjugated with caffeic acid and its composite with polyacrylamide synthesized by electron beam irradiation. *International Journal of Biological Macromolecules*, *154*, 962–973.

Möller, S., Weisser, J., Bischoff, S., & Schnabelrauch, M. (2007). Dextran and hyaluronan methacrylate based hydrogels as matrices for soft tissue reconstruction. *Biomolecular Engineering*, *24*(5), 496–504.

Patlolla, V. G. R., Peter Holbrook, W., Gizurarson, S., & Kristmundsdottir, T. (2019). Doxycycline and monocaprin in situ hydrogel: Effect on stability, mucoadhesion and texture analysis and in vitro release. *Gels*, *5*(4), 47.

Phaechamud, T., & Charoenteeraboon, J. (2008). Antibacterial activity and drug release of chitosan sponge containing doxycycline hyclate. *Aaps PharmSciTech*, *9*, 829-835.

Phaechamud, T., Senarat, S., Puyathorn, N., & Praphanwittaya, P. (2019). Solvent exchange and drug release characteristics of doxycycline hyclate-loaded bleached shellac in situ-forming gel and-microparticle. *International journal of biological macromolecules*, *135*, 1261-1272.

Pooresmaeil, M., Javanbakht, S., Namazi, H., & Shaabani, A. (2021). Application or function of citric acid in drug delivery platforms. *Medicinal Research Reviews*, *42*(2), 800–849.

Ranch, K. M., Maulvi, F. A., Koli, A. R., Desai, D. T., Parikh, R. K., & Shah, D. O. (2021). Tailored doxycycline hyclate loaded in situ gel for the treatment of periodontitis: Optimization, in vitro characterization, and antimicrobial studies. *AAPS PharmSciTech*, *22*, 1-11.

Rosiak, J., & Ulański, P. (1999). Synthesis of hydrogels by irradiation of polymers in aqueous solution. *Radiation Physics and Chemistry*, *55*(2), 139–151.

Rosiak, J., Ulański, P., Pajewski, L., Yoshii, F., & Makuuchi, K. (1995). Radiation formation of hydrogels for biomedical purposes. Some remarks and comments. *Radiation Physics and Chemistry*, *46*(2), 161–168.

Rowley, J. A., Madlambayan, G., & Mooney, D. J. (1999). Alginate hydrogels as synthetic extracellular matrix materials. *Biomaterials*, *20*(1), 45–53.

Saliy, O., Popova, M., Tarasenko, H., & Getalo, O. (2024). Development strategy of novel drug formulations for the delivery of doxycycline in the treatment of wounds of various etiologies. *European Journal of Pharmaceutical Sciences*, *195*, 106636.

Sanapalli, B. K. R., Gounder, K. C., Ambhore, N. S., Kuppuswamy, G., Krishnamurthy, P. T., & Karri, V. V. S. R. (2021). Doxycycline Loaded Collagen-Chitosan Composite Scaffold for the Accelerated Healing of Diabetic Wounds. *Journal of Visualized Experiments*, *174*.

Schreml, S., Szeimies, R., Karrer, S., Heinlin, J., Landthaler, M., & Babilas, P. (2010). The impact of the pH value on skin integrity and cutaneous wound healing. *JEADV. Journal of the European Academy of Dermatology and Venereology/Journal of the European Academy of Dermatology and Venereology*, *24*(4), 373–378.

Şen, M. (2011). Effects of molecular weight and ratio of guluronic acid to mannuronic acid on the antioxidant properties of sodium alginate fractions prepared by radiation-induced degradation. *Applied Radiation and Isotopes*, *69*(1), 126–129.

Sezen, S., Thakur, V. K., & Ozmen, M. M. (2021). Highly Effective Covalently Crosslinked Composite Alginate Cryogels for Cationic Dye Removal. *Gels*, *7*(4), 178.

Singh, R., Pal, D., & Chattopadhyay, S. (2020a). Target-Specific Superparamagnetic Hydrogel with Excellent pH Sensitivity and Reversibility: A Promising Platform for Biomedical Applications. *ACS Omega*, *5*(34), 21768–21780.

Singh, P., Baisthakur, P., & Yemul, O. S. (2020b). Synthesis, characterization and application of crosslinked alginate as green packaging material. *Heliyon*, *6*(1), e03026.

Sood, N., Bhardwaj, A., Mehta, S., & Mehta, A. (2014). Stimuli-responsive hydrogels in drug delivery and tissue engineering. *Drug Delivery*, *23*(3), 748–770.

Tan, H., Chu, C. R., Payne, K. A., & Marra, K. G. (2009). Injectable in situ forming biodegradable chitosan–hyaluronic acid based hydrogels for cartilage tissue engineering. *Biomaterials*, *30*(13), 2499–2506.

Tranquilan-Aranilla, C., Yoshii, F., Rosa, A. D., & Makuuchi, K. (1999). Kappa-carrageenan–polyethylene oxide hydrogel blends prepared by gamma irradiation. *Radiation Physics and Chemistry*, *55*(2), 127–131.

Yang, J., Rao, L., Wang, Y., Zhao, Y., Liu, D., Wang, Z., Fu, L., Wang, Y., Yang, X., Li, Y., & Liu, Y. (2022). Recent Advances in Smart Hydrogels Prepared by Ionizing Radiation Technology for Biomedical Applications. *Polymers*, *14*(20), 4377.

Zhang, J., Hurren, C., Lu, Z., & Wang, D. (2022). pH-sensitive alginate hydrogel for synergistic anti-infection. *International Journal of Biological Macromolecules*, *222*, 1723–1733.

Zong, Y., Zong, B., Zha, R., Zhang, Y., Li, X., Wang, Y., Fang, H., Wong, W., & Li, C. (2023). An Antibacterial and Anti-Oxidative Hydrogel Dressing for Promoting Diabetic Wound Healing and Real-Time Monitoring Wound pH Conditions with a NIR Fluorescent Imaging System. *Advanced Healthcare Materials/Advanced Healthcare Materials*, *12*(24).