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ARTÍCULO DE INVESTIGACIÓN

Chitosan hydrogels crosslinked with glyoxal as controlled drug release systems

Hidrogeles de quitosano entrecruzados con glioxal como sistemas de liberación controlada de fármacos

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ABSTRACT

Chitosan hydrogels have garnered attention as transdermal drug delivery systems due to their ability to facilitate controlled release of bioactive compounds, achieving systemic therapeutic effects. In this study, we synthesized and characterized chitosan hydrogels crosslinked with glyoxal, targeting their potential as controlled drug release systems in animal production. The hydrogels were extensively characterized using Fourier Transform Infrared Spectroscopy to identify functional groups and confirm the successful incorporation of insulin. Swelling studies demonstrated a maximum swelling ratio of 4155 % at acidic pH, indicating significant water uptake and potential for drug delivery applications. Biocompatibility assessments, including hemolysis and MTT assays, revealed a hemolysis percentage below 10 % and cell viability exceeding 95 % after 24 and 48 hours, underscoring the hydrogels' safety profile. Insulin incorporation efficiency was 95.99 %, with a sustained release profile reaching a maximum of 13.19 % at 27 hours. These findings suggest that chitosan-based hydrogels crosslinked with glyoxal hold promise as effective platforms for slow or sustained transdermal drug delivery, particularly for therapeutic agents requiring prolonged release.

Keywords: (chitosan), (hydrogels), (controlled drug release), (insulin)

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RESUMEN

Los hidrogeles de quitosano han generado interés como sistemas de administración transdérmica de fármacos debido a su capacidad para la liberación controlada de compuestos bioactivos. En este estudio, se sintetizaron y caracterizaron hidrogeles de quitosano entrecruzados con glioxal, como un sistema de liberación controlada de fármacos en producción animal. Los hidrogeles fueron caracterizados mediante Espectroscopía Infrarroja por Transformada de Fourier para identificar grupos funcionales y confirmar la incorporación de insulina. Los estudios de hinchamiento demostraron una relación de hinchamiento máxima del 4155 % en pH ácido, lo que indica una significativa absorción de agua y potencial para aplicaciones en la administración de fármacos. Las evaluaciones de biocompatibilidad, que incluyeron ensayos de hemólisis y MTT, revelaron un porcentaje de hemólisis inferior al 10 % y una viabilidad celular superior al 95 % después de 24 y 48 horas, evidenciando su biocompatibilidad. Se encontró una eficiencia de incorporación de insulina del 95.99 %, con un perfil de liberación sostenida que alcanzó un máximo del 13.19 % a las 27 horas. Estos hallazgos sugieren que los hidrogeles de quitosano entrecruzados con glioxal tienen potencial como plataformas para la administración transdérmica lenta o sostenida de fármacos en la producción animal, particularmente para agentes terapéuticos que requieren una liberación prolongada.

Palabras clave: (quitosano), (hidrogeles), (liberación controlada de drogas), (insulina)

INTRODUCTION

The administration of different drugs often presents some drawbacks, such as poor control over plasma drug levels and side effects, highlighting the need for alternative approaches. Controlled drug release systems have gained significant attention in the last two decades, particularly in materials science and pharmaceuticals. The goal is to design intelligent systems loaded with bioactive molecules that respond to environmental stimuli. In recent years, polymers have garnered much attention because their properties can be tailored to the specific needs of each drug[1, 5]. Among polymers, hydrogels exhibit excellent drug-loading properties due to their three-dimensional network structure formed by flexible, crosslinked polymer chains that absorb large amounts of water. They are characterized by hydrophilicity, softness, elasticity, and the ability to swell in aqueous solution, significantly increasing their volume while maintaining shape. Natural polymers, in particular, provide advantages such as enhanced biocompatibility, biodegradability, and reduced antigenicity, facilitating their application in various biomedical contexts[23, 4]. One of the most studied natural polymers for drug administration is chitosan (Chi). It is a linear cationic polysaccharide composed of units of β-(1-4)-2-deoxy-2-amino-D-glucopyranose (D-glucosamine) and β-(1-4)-2-deoxy-2-acetamido-D-glucopyranose (N-acetyl-D-glucosamine). Chi possesses a three-dimensional helical configuration stabilized by hydrogen bonds between its constituent monomers[23, 24, 21]. Among the most commonly used crosslinking agents are dialdehydes such as glyoxal, a small molecule with high water solubility and low cytotoxicity compared to most chemical crosslinking agents. Crosslinking between chitosan and glyoxal occurs via covalent bonds between the aldehydes present in glyoxal and the amines of chitosan[7, 25].

Insulin-like growth factor I (IGF-I) is a single-chain polypeptide composed of 70 amino acids, with a molecular weight of 7.6 kDa, an isoelectric point of 8.5, and three disulfide bonds that stabilize its structural conformation, organized into four domains. Its structural homology with insulin, particularly in the A and B chains, explains its similar functional properties[19]. IGF-I plays a fundamental role in fetal growth, participating in the modulation of maternal metabolism, placental differentiation, growth stimulation, and facilitating nutrient transport across the placenta, processes essential for proper fetal development[20]. Numerous studies have demonstrated a positive correlation between fetal IGF-I concentrations and birth weight in various species, including pigs, sheep, and primates[6]. In ovine models, exogenous administration of IGF-I has shown an increase in body weight and the size of vital organs such as the heart, lungs, liver, and kidneys, even under conditions of intrauterine growth restriction[22]. Considering its biological relevance, the development of effective strategies for its administration represents a significant therapeutic challenge. In this context, hydrogel-

based transdermal delivery systems are emerging as a promising alternative, enabling sustained and controlled release of IGF-I.

Moreover, the use of drug delivery systems for encapsulating and releasing drugs via transdermal routes offers numerous advantages. The transdermal route allows for systemic effects, bypassing the gastrointestinal tract and reducing the potential hepatic metabolism of the drug. Other benefits include an extended therapeutic action time, the possibility of decreasing the dosage, and controlled release. For this route of administration, either synthetic or natural, are recommended[1, 4].

This work aimed to synthesize and characterize hydrogels based on chitosan crosslinked with glyoxal for potential use as a controlled drug release system of insulin, as a model protein of IGF-I, in animal production.

MATERIALS AND METHODS

Synthesis of chitosan hydrogels

Chitosan (Sigma-Aldrich) was dissolved in a 2 % v/v glacial acetic acid (99,5 %, Cicarelli) aqueous solution at room temperature, with continuous mechanical stirring for 12 h to obtain two solutions: 2 % w/v and 3 % w/v, both with a pH of 4. Different concentrations of glyoxal (Sigma-Aldrich) were studied: 2 and 3 % v/v (for hydrogels with 3 % chitosan) and 4 % v/v (for hydrogels with 2 % chitosan). The solution was placed in 1 mL syringes and allowed to react for 48 h. The obtained hydrogels were cut into 2 mm discs and washed in distilled water for 7 days. Finally, they were dried at 37 °C for 3 weeks until their weight remained constant.

Fourier Transform Infrared Spectroscopy (FTIR)

The chemical structure of insulin, Chi hydrogels, and those loaded with insulin were studied through FTIR. Measurements were performed using a Nicolet Impact 400 FTIR spectrometer, with a resolution of 4 cm^{-1}. Spectra were recorded between 500 and 4000 cm^{-1}.

Swelling capacity

The swelling capacity of the hydrogels was evaluated using the gravimetric method. Swelling was evaluated in triplicate in different solutions (2 % v/v acetic acid and distilled water) over time.

The swelling percentage (% Sw) was calculated as follows:

$$\%Sw = \frac{m_s - m_d}{m_d} * 100$$

Where m_s corresponds to the mass of the swollen hydrogel and m_d corresponds to the mass of the dry hydrogel.

Hemolysis test

The integrity of the plasma membrane of erythrocytes exposed to Chi hydrogels was evaluated using a hemolysis assay. Peripheral blood from 90-day-old pigs (~60 kg, n = 3) from an intensive pig farming facility was used. The assay was performed in triplicate with hydrogel concentrations of 3, 5.5, and 10.5 µg/µL. As a positive hemolysis control (100 %), 0.1 % v/v Triton X-100 was used by adding 20 µL of Triton X-100 to 180 µL of the erythrocyte working solution. As a negative control, Triton X-100 was replaced by 20 µL of PBS. For the performance of the assay, the protocol was followed according to Pawar *et al.*[13] with modifications.

MTT assay

To evaluate the effect of Chi hydrogels on cell viability, an MTT reduction assay was performed on the Caco-2 cell line (human epithelial colorectal adenocarcinoma cells) DMEM medium supplemented with 10 % FBS was used as a negative viability control (100 %). All experiments were performed in quadruplicate. The experimental procedure followed the protocol described by Pérez *et al.*[16].

Encapsulation and release kinetics of a model protein

Loading capacity. The hydrogel was introduced into a concentrated insulin solution (Humulin N) (0.33 mL insulin/mL solution) at room temperature for 48 h. At the beginning and end of each assay, the hydrogels were weighed, and the fluorescence intensity at λ_{ex}=310 nm and λ_{em}=350 nm was determined for both the insulin solution (Ins) alone and the insulin solution after 48 h with the hydrogel. The assay was performed in triplicate. The percentage of loading capacity (*%LC*) was calculated using the following formula:

$$\%LC = \frac{Ins_i - Ins_f}{Ins_i} * 100$$

Insulin Release. The kinetics of insulin release from the hydrogels via diffusion at 37 °C for 48 h was investigated. The assay was conducted in triplicate. Hydrogels containing incorporated insulin were placed in 1 mL of PBS buffer solution (pH=7), and aliquots of 0.5 mL were taken at different time intervals over 48 h to measure the solution's fluorescence. Subsequently, the medium was replenished with 0.5 mL of PBS to maintain a

constant final volume. The release percentage was calculated using the following formula:

$$\% \, Release = \frac{mg \; Ins \; released}{mg \; Ins \; encapsulated} * 100$$

Statistical analysis

Statistical analyses were performed by one-way analysis of variance (ANOVA), followed by Fisher's LSD test (GraphPad Prism), to identify statistically significant differences between experimental groups. Statistical significance was determined at a value of $P < 0.05$.

RESULTS

Properties of the synthesized hydrogels

Chi hydrogels crosslinked with glyoxal were synthesized in different proportions. The 3 % w/v Chi hydrogels crosslinked with 2 % v/v and 3 % v/v glyoxal absorbed water during washing without breaking and maintained their shape after drying. However, the 2 % w/v Chi hydrogels with 4 % v/v glyoxal did not achieve gelation, lacking consistency 48 hours after applying the crosslinker.

Based on the synthesis of different hydrogels, observation during washing processes, and swelling tests, the 3 % w/v Chi hydrogel with 3 % v/v glyoxal exhibited the best mechanical properties. This hydrogel was further characterized in terms of swelling, incorporation, and release of insulin, hemolysis tests, and MTT assay.

Chemical structure

FTIR spectroscopy was used to evaluate the characteristic functional groups of the Chi hydrogel in the presence and absence of insulin (Figure 1).

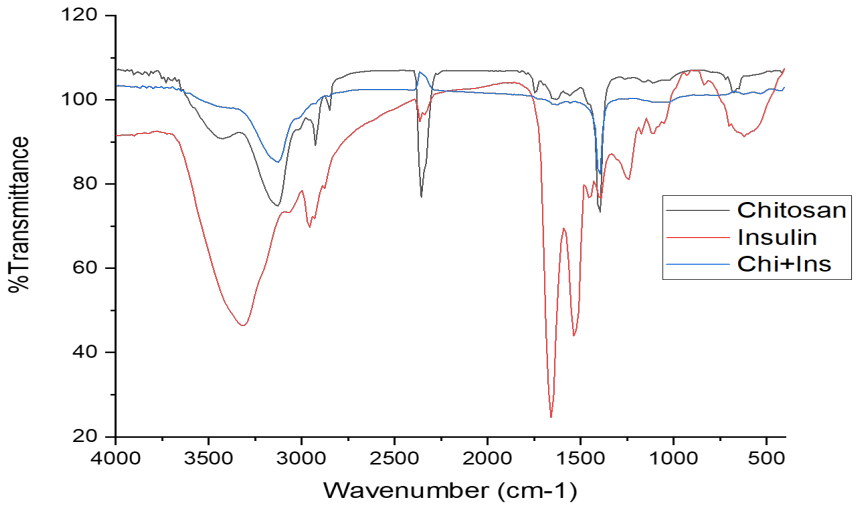

Figure 1. FTIR spectrum of chitosan hydrogels (Chi). FTIR spectrum of chitosan hydrogels in black, insulin in red, and chitosan hydrogels loaded with insulin in blue.

In the spectrum of the Chi hydrogel, the appearance of bands at 1628 and 1558 cm^{-1} corresponding to the vibration of the NH_2 group bond, and the band at 1394 cm^{-1} representing the tension of the C-C bond of the chitosan glucose molecule, can be observed. Bands of the C-H group at 2926 cm^{-1} and bands of the OH groups at 3128 cm^{-1} are also appreciated. In the spectrum of the chitosan hydrogel incorporating insulin (Chi-Ins) compared to the chitosan hydrogel without insulin (Chi), a change in the intensity of the transmittance band around 3300 cm^{-1} was observed. This event may be related to the fact that in this transmittance range, insulin contains the same functional groups, thus causing a decrease in the intensity of this band due to the increase in the concentration of OH groups.

Swelling capacity

In the Chi hydrogels placed in acetic acid (pH 5) and distilled water (pH 7), rapid initial absorption was observed at 30 min. In distilled water, equilibrium was achieved at 60 min, while in acetic acid, stabilization was achieved from 180 min onwards. The maximum value for the evaluated parameter was attained when the hydrogels were incubated in an acidic pH solution, reaching 4155 % ± 1176.2 at 18 h of incubation. In the pH 7 solution, the maximum value was 175 % ± 35.4 at the same 18-hour incubation time (Figure 2).

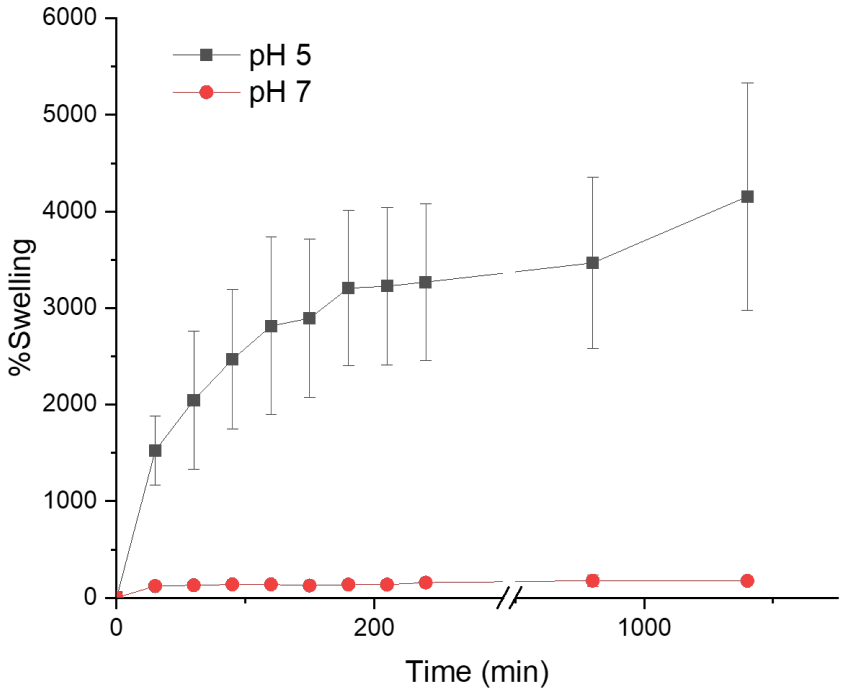

Figure 2. Percentage (%) of swelling over time, in water (pH 7) and acetic acid (pH 5)

Biocompatibility

The biocompatibility of the hydrogels was studied by hemolysis test and MTT assay. In all evaluated concentrations of hydrogels (3; 5.5; and 10.5 µg/µL), the percentage of hemolysis was less than 10 % (Figure 3a). No dose-dependent effect was observed ($p \leq 0.05$).

The evaluation of cell viability using the MTT assay showed that the exposure of cells to Chi hydrogels for 24 and 48 h did not cause significant alterations in mitochondrial functionality compared to the control group ($p > 0.05$). The cell viability percentage was 101.5 ± 2.6 % at 24 h and 97.82 ± 2.234 % at 48 h (Figure 3b). These findings suggest that Chi hydrogels exhibit high biocompatibility with the Caco-2 cell line.

Incorporation and insulin release kinetics in chitosan hydrogels

After the incubation of the chitosan hydrogels in the insulin solution, an increase in the weight of the hydrogels and a decrease in the insulin concentration (mL insulin/mL solution) in the solution were observed. The chitosan hydrogel exhibited a high degree of insulin incorporation, which was 95.99 % ± 0.11.

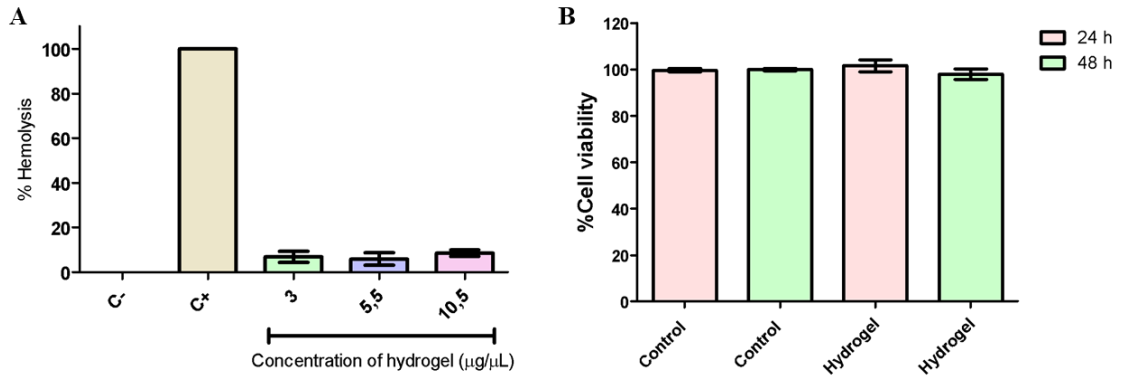

Figure 3. A) Percentage of hemolysis at three concentrations of hydrogels. C+ positive control. C- negative control. **B)** Cell viability determined by MTT assay in the Caco-2 cell line at 24 h and 48 h after incubation with the hydrogels.

Insulin release

The chitosan hydrogel exhibited a high degree of insulin retention, as the maximum percentage of release was 13.19 % ± 0.44 at 27 h of incubation. Additionally, it can be observed that insulin release reached stabilization after 60 min (Figure 4).

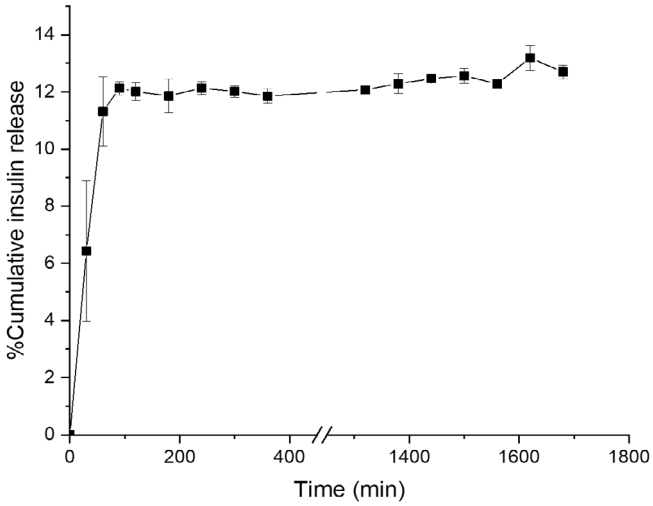

Figure 4. Cumulative insulin release over time

DISCUSSION

Chitosan hydrogels are widely used in biomedical applications due to their biocompatibility, biodegradability, and low antigenicity. Formed by covalent crosslinking, they create three-dimensional structures that swell in water or biological fluids, making them ideal for delivering bioactive molecules and releasing drugs. The degree of crosslinking significantly affects mechanical strength, swelling, diffusion, and porosity. This is influenced by factors such as crosslinker concentration, chitosan's molecular weight, degree of deacetylation, and temperature[15]. Our results revealed that chitosan hydrogels with 2 % glyoxal incubated in water broke and could not be evaluated for swelling, while those with 3 % glyoxal absorbed water rapidly within 30 minutes. Similar results were found by Sánchez *et al.*[18], who observed that lower glyoxal concentrations reduced the swelling capacity of chitosan hydrogels. This decrease in swelling capacity may be due to the hindrance of water absorption when the hydrogels are crosslinked, preventing water absorption due to the presence of an elastic force of polymer chain retraction.

In chitosan hydrogels crosslinked with 3 % glyoxal and incubated in distilled water, a maximum swelling percentage of 138 % was observed at 90 min. These results are comparable to those reported by Bahram *et al.*[2], who obtained a swelling percentage of 155 % at 60 mins with chitosan hydrogels crosslinked with glutaraldehyde. When the Chi hydrogels were incubated in acetic acid, the swelling parameter was higher than in distilled water, reaching 4155 % at 18 hours.

These findings are consistent with those of Mirzaei *et al.*[12] where chitosan hydrogels crosslinked with glutaraldehyde (chitosan: crosslinker molar ratio of 1:0.205) showed that the swelling percentage gradually decreases as the pH increases. This could be explained by the fact that at low pH, the amino groups of chitosan ($-NH_2$) are protonated ($-NH^{3+}$), leading to repulsion in the polymer chains and dissociation of secondary interactions such as intramolecular hydrogen bonds. Therefore, the network has more potential for hydrogen bonding with the surrounding water and allows more water diffusion into the hydrogel network.

In the FTIR spectrum of the hydrogels, characteristic functional group bands of the chitosan molecule can be identified. One of the characteristic bands found is the amino group and the C-C stretching bands, which coincide with the range of bands identified by Ledezma-Delgadillo *et al.*[10], being 1639 cm^{-1} and 1582 cm^{-1} for the former, corresponding to the vibration of

the NH_2 group bond, and 1374 cm^{-1} for the latter, corresponding to the C-C stretching bands of the chitosan glucose molecule. On the other hand, the observed C-H band coincides with the band range found at 2947 cm^{-1} due to typical C-H stretching vibrations. In the spectrum of chitosan hydrogels loaded with insulin, a decrease in the band at 3300 cm^{-1} attributable to hydroxyl groups was observed; similar results were reported by Lima *et al.*[11].

Chitosan hydrogels are highly suitable materials for use as drug delivery systems due to their swelling characteristics in liquid media, providing properties to absorb, retain, and release organic substances under controlled conditions, such as growth factors, drugs, and proteins, among others[14]. In this study, it was observed that the percentage of the loading capacity of insulin was 95.99 % ± 0.11. This can be explained because the choice of hydrogel material, the conformation of the covalent networks, and the insulin loading mechanism are complementary to insulin properties, such as its low molecular weight, hydrophobicity, and charge[4].

On the other hand, substance release depends simultaneously on the rate of water migration to the device, hydration, and relaxation of the polymer. In this study, the Chi hydrogel released a maximum of 13.19 % ± 0.44 of insulin in a PBS solution at 36 °C after 27 hours. These results were expected, given that the swelling percentage in distilled water was low and that the primary kinetic mechanisms regulating the controlled release rate of a substance are water diffusion into the system and polymer swelling[4]. Additionally, an increase in release is expected under relatively acidic pH conditions due to greater matrix swelling. These findings are consistent with those reported by Zhang *et al.*[27], who developed hydrogels based on chitosan modified with phenylboronic acid, formyl-terminated polyethylene glycol, and polyvinyl alcohol. In their study, they evaluated insulin release in PBS at physiological pH and acidic pH (6.5), obtaining values of 16.0 % after 36 hours in the first case and 70.2 % in the second. Since a reduction in % Swellings leads to a significant decrease in the mesh size of the polymer network, the change in the swelling rate of Chi hydrogels results in a modification of the gel's mesh size, thereby modulating the release of the substance[3].

The Hemolysis Test revealed that Chi hydrogels crosslinked with glyoxal did not affect the viability of porcine erythrocytes at the evaluated concentrations, as the hemolysis percentages did not exceed 10 %[8]. On the other hand, cell viability studies indicated that Chi hydrogels exhibit high biocompatibility with the Caco-2 cell line, with viability values exceeding 95 % at all evaluated time points[26, 9]. These results were expected, as chitosan is a naturally biocompatible polymer[23, 17].

CONCLUSION

Chitosan hydrogels present a highly promising platform for biomedical applications, particularly in drug delivery systems. Our study demonstrated that the degree of crosslinking significantly influences the swelling properties, mechanical strength, and porosity of these hydrogels. Specifically, Chi hydrogels crosslinked with 3 % glyoxal exhibited rapid initial water absorption and substantial swelling capacity, with markedly different behaviors depending on the incubation medium. The enhanced swelling in acidic environments suggests that pH plays a crucial role in modulating hydrogel properties, making them adaptable for targeted drug release in specific physiological conditions.

FTIR analysis confirmed the presence of characteristic chitosan functional groups, and the high insulin loading capacity highlights the hydrogels' potential as efficient drug carriers. The high biocompatibility observed in hemolysis and cell viability tests further supports the safety and suitability of these hydrogels for clinical applications. Moreover, the controlled release of bioactive molecules, governed by the hydrogel's swelling behavior and polymer matrix relaxation, underscores their potential for precision medicine.

Overall, Chi hydrogels crosslinked with glyoxal emerge as versatile and effective materials for biomedical use, with potential applications such as the controlled and sustained transdermal delivery of IGF-I, avoiding proteolytic degradation and improving its systemic bioavailability. Further research into optimizing their properties and understanding their interactions with biological systems will pave the way for their broader clinical adoption.

REFERENCES

1. Adepu, S.; Ramakrishna, S. Controlled Drug Delivery Systems: Current Status and Molecules. En: 2021;26:5905. https://www.mdpi.com/1420-3049/26/19/5905
2. Bahram Bahrami, S.; Kordestani, S.; Mirzadeh, H.; Mansoori, P. Poly (vinyl alcohol) - Chitosan Blends: Preparation, Mechanical and Physical Properties. *Iranian Polymer Journal*. 2016;1–23.
3. Berger, J.; Reist, M.; Mayer, JM.; Felt, O.; Peppas, NA.; Gurny, R. Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. *Eur J Pharm Biopharm*. 2004;57(1):19–34. En: https://www.sciencedirect.com/science/article/pii/S0939641103001619
4. Bhattarai, N.; Gunn, J.; Zhang, M. Chitosan-based hydrogels for controlled, localized drug delivery. *Adv Drug Deliv Rev.* 2010;62(1):83–99. En: https://www.sciencedirect.com/science/article/pii/S0169409X09002828
5. Bruneau, M.; Bennici, S.; Brendle, J.; Dutournie. P.; Limousy, L.; Pluchon, S. Systems for stimuli-controlled release: Materials and applications. *J Control Release*. 2019;294:355–71. En: https://www.sciencedirect.com/science/article/pii/S0168365918307417
6. Fowden, A. L. The insulin-like growth factors and feto-placental growth. *Placenta*. 2003;24(8-9): 803-812. En: https://www.sciencedirect.com/science/article/pii/S0143400403000808?casa_token=uk7ui4tq80AAAAAA:a7Dz3OYGM4nFHgtCep8z5tUXq9UWPkVY5UfXIplXdwyVIbcuF71fpYUDKinU5OXZrEhs7_nIAV
7. Kaczmarek-Szczepańska, B.; Pin, JM.; Zasada, L.; et al. Assessment of Melatonin-Cultured Collagen/Chitosan Scaffolds Cross-Linked by a Glyoxal Solution as Biomaterials for Wound Healing. *Antioxidants*. 2022;11(3). En: https://www.mdpi.com/2076-3921/11/3/570
8. Kaczmarek-Szczepańska, B., Mazur, O., Michalska-Sionkowska, M., Łukowicz, K., & Osyczka, A. M. The preparation and characterization of chitosan-based hydrogels cross-linked by glyoxal. *Materials*. 2021;14(9):2449. En: https://www.mdpi.com/1996-1944/14/9/2449
9. Kaufmann, G., Klein, M. P., Goettert, M. I., & Aguirre, T. A. S. Development and cytotoxicity evaluation of a cylindrical pH-responsive chitosan-genipin hydrogel for the oral delivery of diclofenac sodium. *European Polymer Journal*. 2022;181:111649. En: https://www.sciencedirect.com/science/article/pii/S001430572200653X
10. Ledezma-Delgadillo, A.; Carrillo-González, R.; Martín-Martínez, ES.; Jaime-Fonseca, MR.; Chacón-López, MA. Nanocápsulas de urea en quitosano y ácido polimetacrílico y su aplicación en cultivo hidropónico de lechuga (Lactuca sativa L). *Rev Mex Ing Química*. 2016;15(2):423–31. En: https://www.redalyc.org/pdf/620/62046829010.pdf
11. Lima, HA.; Marcus, F.; Lia, V.; Ramdayal, S. Preparation and characterization of chitosan-insulin-tripolyphosphate membrane for controlled drug release: effect of cross linking agent. *Journal of Biomaterials and Nanobiotechnology*. 2014;211–9. En: https://scirp.org/journal/PaperInformation?PaperID=50277
12. Mirzaei B, E., Ramazani SA, A., Shafiee, M., & Danaei, M. (2013). Studies on glutaraldehyde crosslinked chitosan hydrogel properties for drug delivery systems. *International Journal of Polymeric Materials and Polymeric Biomaterials*,62(11), 605-611. En: https://www.tandfonline.com/doi/abs/10.1080/00914037.2013.769165
13. Pawar, V., Dhanka, M., & Srivastava, R. (2019). Cefuroxime conjugated chitosan hydrogel for treatment of wound infections. Colloids and Surfaces B: Biointerfaces, 173, 776-787. En: https://www.sciencedirect.com/science/article/pii/S0927776518307306
14. Peers, S.; Montembault, A.; Ladavière, C. Chitosan hydrogels for sustained drug delivery. *J Control Release.* 2020;326:150–63. En: https://www.sciencedirect.com/science/article/pii/S0168365920303461
15. Pellá, MCG.; Lima-Tenório, MK.; Tenório-Neto, ET.; Guilherme, MR.; Muniz, EC.; Rubira, AF. Chitosan-based hydrogels: From preparation to biomedical applications. *Carbohydr Polym.* 2018;196:233–45. En: https://www.sciencedirect.com/science/article/pii/S0144861718305654
16. Pérez, M.L.S., Funes, J. A., Bracamonte, C. F., Ibarra, L. E., Forrellad, M. A., Taboga, O., ... & Molina, M. (2023). Development and biological evaluation of pNIPAM-based nanogels as vaccine carriers. *International Journal of Pharmaceutics*, 630, 122435. En: https://www.sciencedirect.com/science/article/pii/S0378517322009905
17. Rodríguez-Rodríguez, R.; Velasquillo-Martínez, C.; Knauth, P. Sterilized chitosan-based composite hydrogels: Physicochemical characterization and in vitro cytotoxicity. *J Biomed Mater Res* - Part A. 2020;108(1):81–93. En: https://onlinelibrary.wiley.com/doi/full/10.1002/jbm.a.36794
18. Sánchez, A.; Sibaja, M.; Vega-Baudrit, J.; Madrigal, S. Síntesis y caracterización de hidrogeles de quitosano obtenido a partir del camarón langostino (Pleuroncodes planipes) con potenciales aplicaciones biomédicas. *Rev Iberoam polímeros*. 2007;8(4):241–67. En: https://www.researchgate.net/profile/Jose-Vega-Baudrit2/publication/28181638_Sintesis_y_caracterizacion_de_hidrogeles_de_quitosano_obtenido_a_partir_del_camaron_langostino_Pleuroncodes_planipes_con_potenciales_aplicaciones_biomedicas/links/54eb3e390cf29a16cbe59c82/Sintesis-y-caracterizacion-de-hidrogeles-de-quitosano-obtenido-a-partir-del-camaron-langostinoPleuroncodes-planipes-con-potenciales-aplicaciones-biomedicas.pdf
19. Schultz, I., Wurzel, J., & Meinel, L. Drug delivery of Insulin-like growth factor I. *European Journal of Pharmaceutics and Biopharmaceutics*. 2015;97:329-337. En: https://www.sciencedirect.com/science/article/pii/S0939641115002064?casa_token=dhUa_PeCxHgAAAAA:lXcOLmST2QwaW0yuEa_UU0PVMzELrQR0uH19-ONazJ7FO7zK2DngXroCjiD2XtQ6y4FJPnqQaOwz
20. Sferruzzi-Perri, A. N., Owens, J. A., Pringle, K. G., & Roberts, C. T. The neglected role of insulin-like growth factors in the maternal circulation regulating fetal growth. *The Journal of physiology*. 2011, 589(1), 7-20. En: https://physoc.onlinelibrary.wiley.com/doi/full/10.1113/jphysiol.2010.198622

21. Shariatinia, Z. Pharmaceutical applications of chitosan. *Adv Colloid Interface Sci*. 2019;263:131–94. En: https://www.sciencedirect.com/science/article/pii/S000186861830277X

22. Stremming, J., Heard, S., White, A., Chang, E. I., Shaw, S. C., Wesolowski, S. R., Jonker, S. S., Rozance, P.J. & Brown, L. D. IGF-1 infusion to fetal sheep increases organ growth but not by stimulating nutrient transfer to the fetus. *American Journal of Physiology-Endocrinology and Metabolism*. 2021, 320(3): 527-538. En: https://journals.physiology.org/doi/full/10.1152/ajpendo.00453.2020

23. Tian, B.; Hua, S.; Tian, Y.; Liu, J. Chemical and physical chitosan hydrogels as prospective carriers for drug delivery: A review. *J Mater Chem B.* 2020;8(44):10050–64. En: https://pubs.rsc.org/en/content/articlelanding/2020/tb/d0tb01869d/unauth

24. Tian, B.; Liu, J. Smart stimuli-responsive chitosan hydrogel for drug delivery: A review. *Int J Biol Macromol.* 2023;235:123902. En: https://www.sciencedirect.com/science/article/pii/S0141813023007961

25. Wang, L.; Stegemann, JP. Glyoxal crosslinking of cell-seeded chitosan/collagen hydrogels for bone regeneration. *Acta Biomater.* 2011;7(6):2410–7. En: https://www.sciencedirect.com/science/article/pii/S1742706111000882

26. Yang, J., Liang, G., Xiang, T., & Situ, W. Effect of crosslinking processing on the chemical structure and biocompatibility of a chitosan-based hydrogel. *Food Chemistry.* 2021, 354: 129476. En: https://www.sciencedirect.com/science/article/pii/S0308814621004829

27. Zhang, J.; Chen, F.; Yu, D.; Liang, Z.; Dai, F.; Liang, H.; Dai, F.; Liang, H.; Li, H.; Tan, H.; Zhao, L. Chitosan-based injectable hydrogels with dual glucose sensors for precise control of insulin release and diabetes mellitus therapy. *International Journal of Pharmaceutics.* 2023;643:123246. En: https://www.sciencedirect.com/science/article/pii/S037851732300666X#s0080