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O-Carboxymethylchitosan Hydrogel Loaded with Dexamethasone for Intraocular Use

Hidrogel de O-Carboximetilquitosana carreado com dexametasona para uso intraocular

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ABSTRACT

This study aimed to develop hydrogels from carboxymethyl chitosan (CMC), and carboxymethyl chitosan (CMC) with dexamethasone (DEX) for intraocular application, aiming to provide structural stability to the eye and attenuation of inflammatory processes. CMC hydrogels were prepared at concentrations of 0.5%, 1% and 2% (w/v) and incorporated with 10% (v/v) DEX. The samples were characterized by FT-IR spectroscopic, thermogravimetric analysis, injectability, rheology, and cytotoxicity. The results showed that the incorporation of DEX did not significantly alter the absorption bands and thermal behavior of the CMQ hydrogels. Injectability tests revealed that hydrogels with DEX require injection force similar to the commercial material. In rheological tests, pseudoplastic and linear viscoelastic behavior was identified. Cytotoxicity tests confirmed the biocompatibility of the hydrogels. These results demonstrate that the developed hydrogels have properties suitable for biomedical applications, especially aimed at the ophthalmological context.

Keywords: Biomaterials; Ophthalmology; Anti-inflammatory; Cataract.

RESUMO

Este estudo teve como objetivo desenvolver hidrogéis de carboximetilquitosana (CMC) e carboximetilquitosana (CMC) com dexametasona (DEX) para aplicação intraocular, visando proporcionar estabilidade estrutural ao olho e atenuação de processos inflamatórios. Os hidrogéis de CMC foram preparados nas concentrações de 0,5%, 1% e 2% (p/v) e incorporados com 10% (v/v) de DEX. As amostras foram caracterizadas por espectrometria de FT-IR, análise termogravimétrica, injetabilidade, reologia e citotoxicidade. Os resultados mostraram que a incorporação de DEX não alterou significativamente as bandas de absorção e o comportamento térmico dos hidrogéis de CMQ. Os testes de injetabilidade revelaram que os hidrogéis com DEX requerem força de injeção semelhante ao material comercial. Nos testes reológicos, foi identificado comportamento viscoelástico pseudoplástico e linear. Os testes de citotoxicidade confirmaram a biocompatibilidade dos hidrogéis. Esses resultados demonstram que os hidrogéis desenvolvidos apresentam propriedades adequadas para aplicações biomédicas, especialmente voltadas ao contexto oftalmológico.

Palavras-chave: Biomateriais; Oftalmologia; Anti-inflamatório; Catarata.

INTRODUCTION

Cataracts and macular degeneration emerge as leading global causes of vision loss (Datta *et al.*, 2017). These pathologies impose a considerable burden on the ocular health of the elderly population, often requiring complex surgical interventions to preserve and restore vision. In this scenario, the use of hydrogels plays a fundamental role in improving ocular surgery, allowing successful results, promoting the protection of the corneal endothelial layer, maintaining the depth of the anterior chamber and improving the visibility of intraocular structures under the microscope (Shinde *et al.*, 2023).

Hydrogels are materials composed of interconnected three-dimensional networks of cross-linked polymer chains. They have high hydrophilicity, allowing them to absorb and retain large quantities of water and other fluids without undergoing disintegration. This swelling capacity makes hydrogels particularly useful in diverse applications, from biomaterials for controlled drug release to support systems for cell growth in tissue engineering (Hunt *et al.*, 2014). Its rheological properties play a fundamental role in the success of ophthalmic surgeries. However, the use of hydrogels has sometimes been associated with some side effects, including increased intraocular pressure (Safa *et al.*, 2021) and post-surgical inflammation due to residual amounts of viscoelastic inside the eye (Kwitko, 2000; Figueirêdo *et al.*, 2010; Safa *et al.*, 2021).

The choice of polymeric material for the hydrogel matrix is extremely important, as it directly influences its properties and, consequently, its applications. The matrix must provide a three-dimensional environment with high hydration capacity, mimicking native soft tissues (Spicer, 2020). In this context, carboxymethyl chitosan (CMC), a biopolymer derived from chitosan (CS), stands out for its solubility in physiological media and its ability to form hydrogels. Furthermore, CMC presents biocompatibility, biodegradability, drug encapsulation and release capacity, as well as antioxidant and antibacterial activities, making it a promising material for various applications (Lucio *et al.*, 2014; De Lacerda Bukzem *et al.*, 2021; Lucio *et al.*, 2022).

Dexamethasone (DEX) is a corticosteroid drug widely used in the ophthalmological field, with several applications, including the treatment of postoperative intraocular inflammation in cataract surgeries and corneal transplants. Conventional topical forms of dexamethasone administration have several disadvantages, such as inadequate routes for transporting drugs to the posterior portion of the ocular tissue and low bioavailability (Ahirrao *et al.*, 2023). Given this context, the main

objective of this study was to develop hydrogels combining carboxymethyl chitosan with dexamethasone for intraocular application. This combination sought to provide a synergistic approach to ophthalmological treatment, aiming not only at the structural stability of the eye, but also at attenuating inflammatory processes, minimizing the potential adverse effects associated with the use of conventional viscoelastic substances.

MATERIALS E METHODS

Carboxymethyl chitosan (CMC) was synthesized from medical grade Chitosan (CS) (260 KDa; degree of deacetylation > 90%), manufactured and supplied by the Biomaterials Evaluation and Development Laboratory of the Northeast – CERTBIO (Paraíba, Brazil). Isopropanol (Dinâmica), sodium hydroxide (VETEC), absolute ethanol (Neon), monochloroacetic acid and Dexamethasone were purchased from Sigma Aldrich, and a commercial viscoelastic hydrogel, Oft Visc® 2% -1.5 ml purchased from Oft vision, as reference material. All other agents used were of analytical grade.

The synthesis of carboxymethyl chitosan (CMC) was conducted using a methodology adapted from Chen e Park (2003). Initially, a solution of chitosan (CS) in isopropanol, with a concentration of 42.37 g/L, was prepared and subjected to magnetic stirring for 30 minutes. Next, 10 mL of a sodium hydroxide solution (10.2 mol/L) was added. After obtaining the initial solution, it was solubilized in a mixture of isopropanol and water in a 1:1 (v/v) ratio, maintaining stirring for 24 hours. In the second stage of the process, a solution of monochloroacetic acid in isopropanol (8.28 mol/L) was slowly added, with continuous stirring over 24 hours. Subsequently, 100 mL of absolute ethanol (Neon) was introduced, maintaining magnetic stirring. The resulting solution was then subjected to ten filtrations with ethanol, ensuring the complete removal of the CMC salt. The CMC was then washed with ethanol to eliminate any residues until a final pH close to 7 was achieved. After the filtration and washing process, the CMC was kept at room temperature for 12 hours to dry, thus completing the production of the final product.

The CMC hydrogel was prepared at concentrations of 0.5%, 1%, and 2% (w/v) with and without 10% v/v of DEX in 10 mL of ultrapure water, under magnetic stirring at 100 rpm and room temperature (~24°C) for 30 minutes. The resulting samples were then stored in a desiccator at room temperature (~24°C).

The samples were coded according to Table 1. Then, the hydrogels were characterized and compared with a commercial viscoelastic hydrogel (OFT Visc 1.5 ml / 2%.) reference.

Table 1- Sample coding.

Code	Sample
CV	Commercial Viscoelastic
CV/DEX	Commercial Viscoelastic + Dexamethasone
CMC/0.5%	Carboxymethylchitosan 0.5 %
CMC/0.5%/DEX	Carboxymethylchitosan 0.5 % + Dexamethasone
CMC/1%	Carboxymethylchitosan 1 %
CMC/1%/DEX	Carboxymethylchitosan 1 % + Dexamethasone
CMC/2%	Carboxymethylchitosan 2 %
CMC/2%/DEX	Carboxymethylchitosan 2 % + Dexamethasone

Source: Research data (2024).

CHARACTERIZATIONS

The hydrogels in different concentrations, with and without the addition of the drug, were subjected to different characterization techniques. The hydrogen potential (pH) was evaluated using a Micronal pH meter model B474, without sample dilution, with three measurements for each sample. Fourier transform infrared spectroscopy (FTIR) was conducted using a Perkin Elmer Spectrum 400 equipment, at room temperature, with a scanning range of 4000 to 400 cm^{-1}, resolution of 4 cm^{-1}, and 16 scans.

Thermogravimetric analysis (TGA) was performed using Pyris 1 TGA model equipment (Perkin Elmer), where approximately 5 mg of each sample was heated from 20 to 700 °C, at a rate of 10 °C/min in a nitrogen atmosphere, with a flow of 50 mL/min. Injectability was performed to guarantee the practical applicability of hydrogels in injectable administration systems (Halimi, Célia *et al.*, 2015). For this, support for a 5 mL syringe (Bectin Dickinson Ind, Cirúrgica Ltda) connected to a 27 Gauge needle was integrated into an Instron 3366 universal mechanical testing machine, adjusted in

compression mode, using a 500N load cell and a speed of 1 mm/min at room temperature (25ºC).

The viscosity and loss and storage moduli of carboxymethylchitosan hydrogels, with and without drug, were evaluated using a HAAKE MARS III flat plate rheometer, equipped with a modular PP 35 Ti rotor system, manufactured by Thermo Scientific. Measurements were carried out at an oscillatory frequency of 0.1 to 10 Hz, with a voltage of 0.1%, using a gap of 0.5 mm and maintaining the temperature at 25°C.

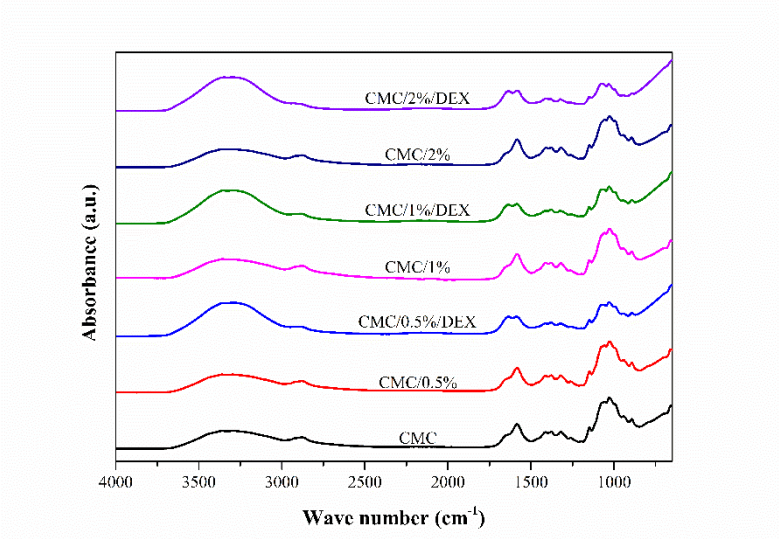
The evaluation of the in vitro cytotoxicity of the hydrogels was carried out by the fibroblast cell viability test using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide), as described in ISO 10993-5:2009 standard. The cell line used was L929, obtained from the Rio de Janeiro Cell Bank. The evaluation parameters included the percentage of cell death and the determination of IC50, which represents the concentration of the product capable of inhibiting 50% of cell growth.

RESULTS AND DISCUSSION

FT-IR Spectroscopy

Figure 1 shows the FTIR spectra of the CMC and the samples obtained from the hydrogels with and without drug. In the CMC spectrum, the absorptions observed at 1640 cm-1 and 1395 cm-1 are characteristic of the carboxymethylation reaction, which can be attributed to the symmetric and asymmetric deformations of the COO- group. It is also inferred that the chemical substitution occurred predominantly in the hydroxyl group, that is, obtaining O-carboxymethyl chitosan, as described by (Mohseni *et al.*, 2024). The band observed around 3300 cm^{-1} refers to the axial stretching of the OH bond superimposed on the N-H stretching band. The bands at 2883 and 2895 cm^{-1} are attributed to the asymmetric stretching of the C-H group (Galdino *et al.*, 2022; Hadi *et al.*, 2023; Mohseni *et al.*, 2024).

Figure 1 – FTIR spectra of carboxymethyl chitosan and the obtained hydrogels.



Source: Research data (2024).

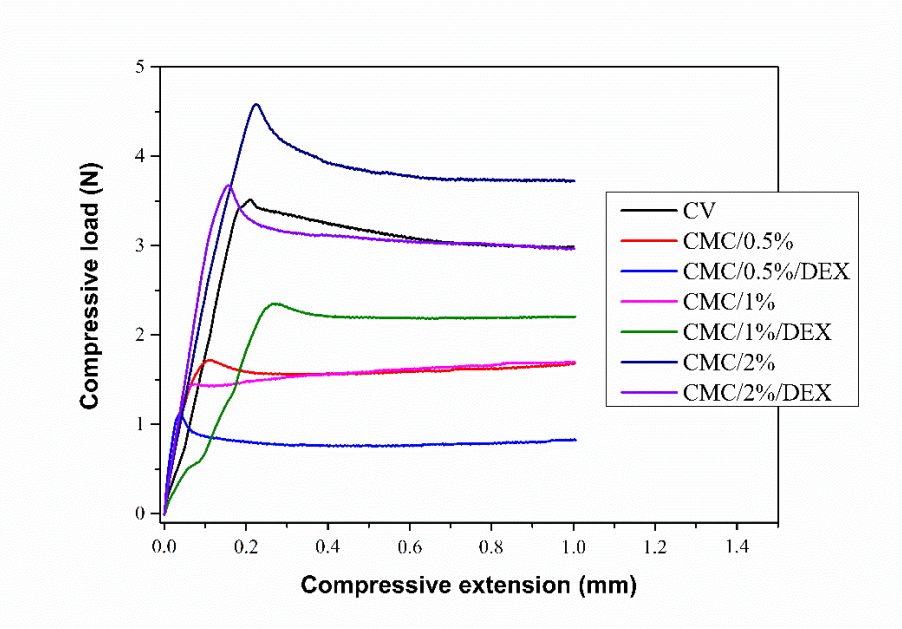
The same bands were observed in the spectra of the hydrogels, with the intensification of the bands at 2883 and 2895 cm^{-1} attributed to the asymmetric stretching of the C-H group for the CMC/2% sample, possibly due to its higher concentration. In the spectra of samples with the drug, it is observed that the introduction of the drug into the matrix caused the intensification of the vibration band around 3300 cm^{-1} and the reduction of the bands around 2883, 2895, 1640, and 1395 cm^{-1} (Hadi *et al.*, 2023; Matadh *et al.*, 2023).

The reductions in vibrations observed in FTIR suggest a possible secondary bond of the drug with the matrix, suggests that it was incorporated into the CMC matrix at the molecular level (Allen e Ansel, 2013).

Injectability

Injection force refers to the pressure or energy required to inject hydrogels into a specific system, such as biological tissues or devices. High injection force can cause damage to surrounding tissues or compromise the integrity of the hydrogel itself (Han *et al.*, 2024). The results of the injectability tests are presented in Figure 2.

Figure 2 – Injectability curves of the developed hydrogel samples.



Source: Research data (2024).

The results of measuring the injection force as a function of the displacement of the syringe plunger (constant speed of 1 mm/min) of samples with and without drug, in addition to the commercial material. Based on the results obtained, it was possible to observe that the hydrogel compositions without dexamethasone demonstrated a superior injection force. Specifically, the CMC/2% composition required a higher injection force, which was expected due to its higher CMC concentration. When comparing the values obtained for the CMC/2%/DEX composition and the commercial viscoelastic (CV), a significant similarity was observed between them.

Segundo Halimi, C. *et al.* (2015), an injection force value below 15 N is usually necessary for an uneventful injection procedure. Thus, the results demonstrate that the hydrogels obtained present injection force values suitable for intraocular application, as proposed in this work (Mohseni *et al.*, 2024).

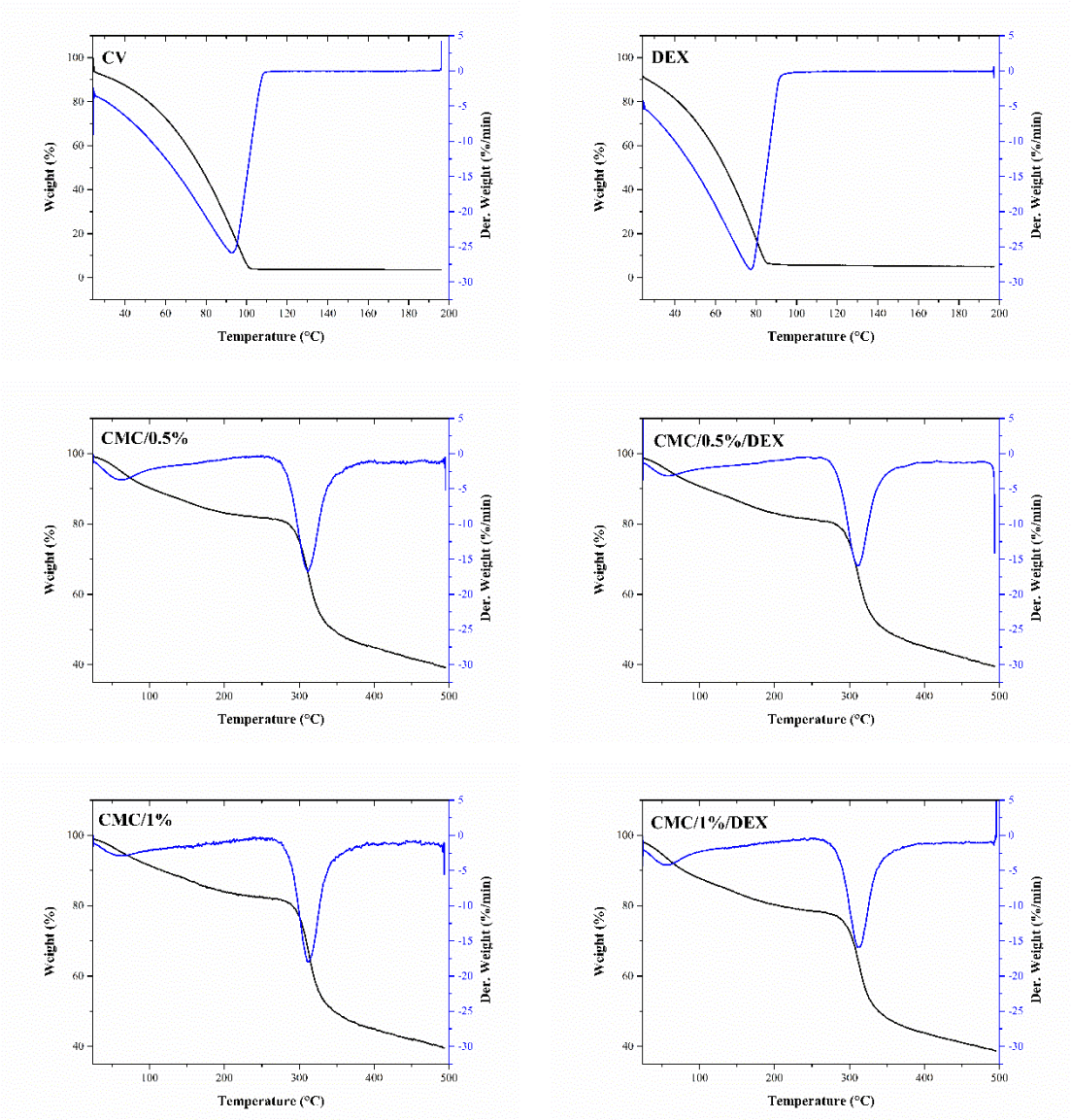
Thermogravimetric Analysis (TGA)

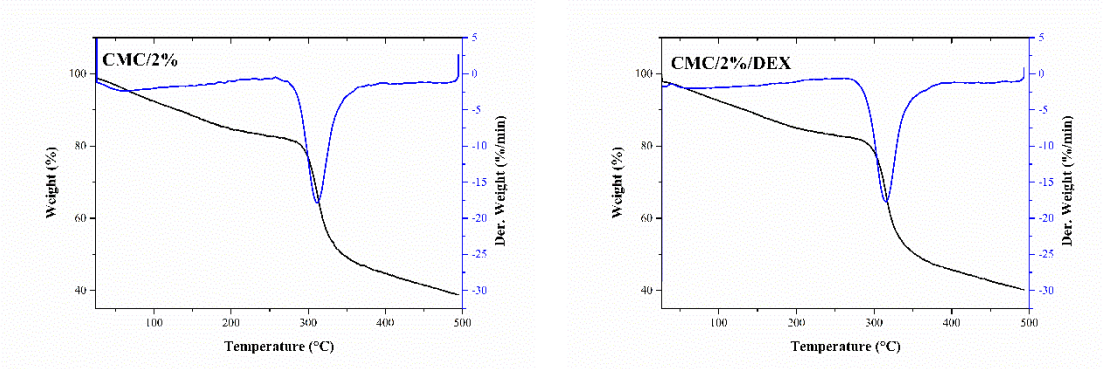
In Figure 3, the results of thermogravimetric analyses of samples with and without drug are presented, where mass losses were observed as a function of temperature. A total mass loss of approximately 95% was observed for the commercial hydrogel and drug samples, while for the hydrogel samples, the loss was around 60%.

The incorporation of dexamethasone (DEX) did not significantly alter the thermal behavior of the hydrogels (Santos *et al.*, 2021). Furthermore, increasing the concentration of CMC in the hydrogels had little influence on the decomposition of the samples.

Only two stages of decomposition were observed; the first between 30 and 260°C, and the second stage between 270 and 700°C. Degradation up to approximately 258 °C can be attributed to water loss, while above 258 °C, decomposition of the material is observed (Ladeira *et al.*, 2021). Systematically, there was a reduction in the total mass loss of hydrogels with DEX when compared to samples without drug addiction.

Figure 3 - Thermogravimetric analysis curves of the developed hydrogel samples.





Source: Research data (2024).

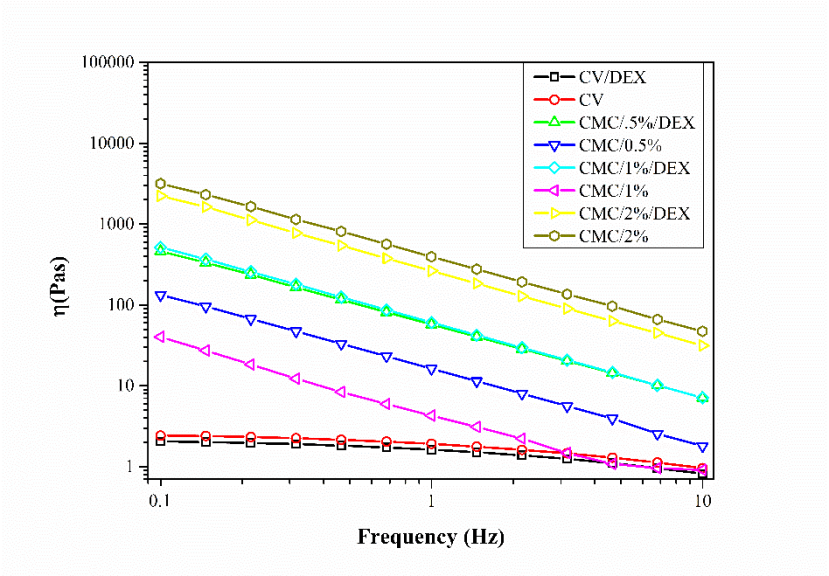
Rheological Analysis (η, G' and G")

The results of the rheological analyses of the hydrogels are presented in Figure 4, where it is possible to observe that the viscosities of the hydrogels are relatively low. These samples exhibit non-Newtonian fluid behavior, with a reduction in viscosity with increasing shear rate, regardless of CMC concentration, due to the orientation of the long-chain molecules along the flow direction (Enoch e Somasundaram, 2023). Materials that exhibit this behavior of decreasing viscosity with increasing strain rate are known as pseudoplastics.

The pseudoplastic effect is more evident for the CMC hydrogel samples, while the CV and CV/DEX samples showed less influence of frequency on their viscosity. This effect arises from interactions between CMC molecules and their high molecular mass (Nair e Roy Choudhury, 2020; Enoch e Somasundaram, 2023).

It can be seen that with increasing CMC concentration, viscosity also increases. This is because, as the CMC concentration increases, the entanglement of the polymer network containing carboxymethyl groups, held by hydrogen bonds, becomes denser, resulting in an increase in viscosity (Enoch e Somasundaram, 2023).

Figure 4 - Viscosity curves of the developed hydrogel samples.



Source: Research data (2024).

The viscoelastic behavior of hydrogels is characterized by the storage (G') and loss (G") moduli. The storage modulus (G') represents the elastic response of the material, while the loss modulus (G") reflects its viscous response to the applied deformation (Enoch e Somasundaram, 2023). Figure 5 presents the results of the storage (G') and loss (G") moduli as a function of frequency for hydrogels with and without DEX.

The results of commercial samples with and without DEX (CV and CV/DEX) indicated an increase in G' and G" with increasing frequency, demonstrating viscoelastic behavior.

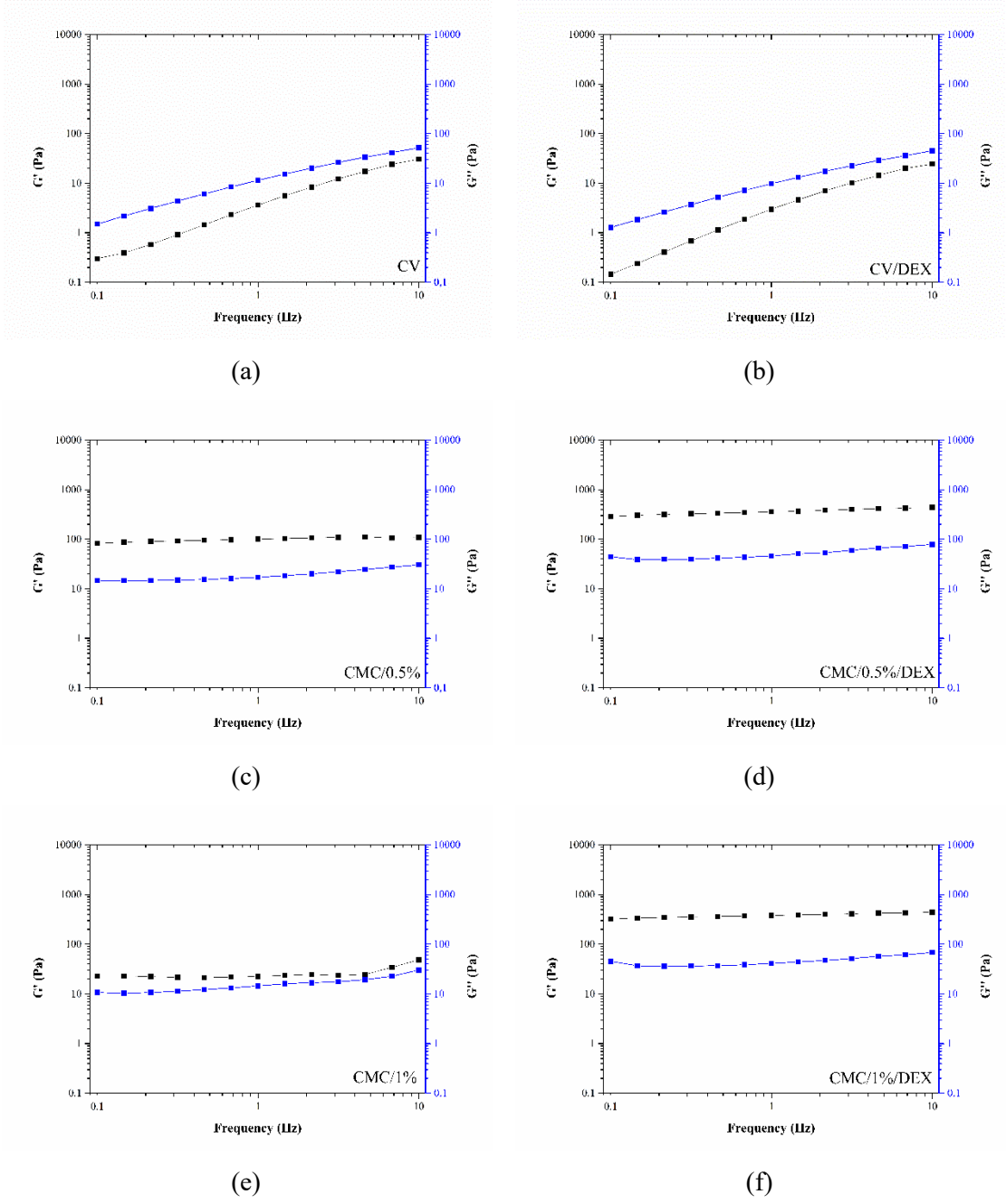
For samples with CMC, both G' and G"' are practically constant with frequency, suggesting a linear viscoelastic behavior. This indicates that the material maintains its ability to store and dissipate energy consistently regardless of frequency (Ma *et al.*, 2020). Furthermore, for these samples, the values of G' were higher than those of G", which indicates that the material has a predominantly elastic response in relation to deformation. This suggests that the material's structure is more rigid and resistant to deformation, compared to its ability to dissipate energy in the form of heat (Kong *et al.*, 2003).

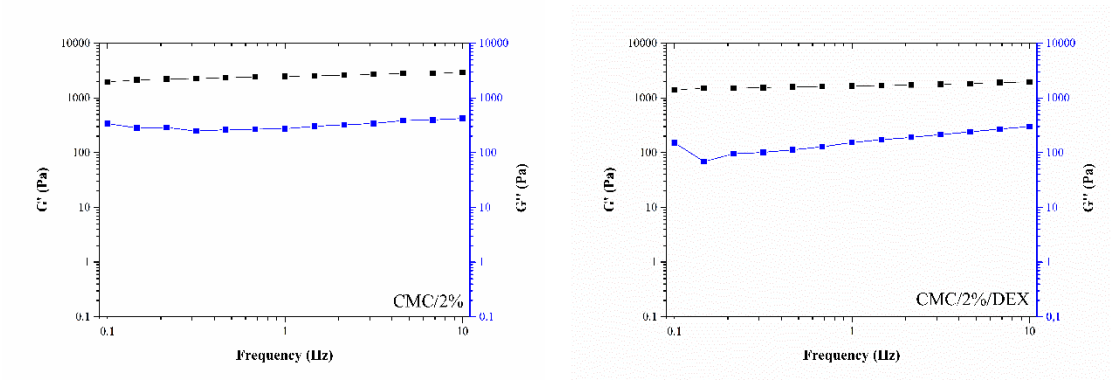
The CMC/2% hydrogel presented the highest G' value, behaving as the stiffest material among the samples, as illustrated in Figure 5g. Corroborating the values obtained for viscosity (indicate the topics) and injectability (indicate the topics).

Hydrogels with linear viscoelastic behavior can offer effective cushioning capacity against impacts and movements in the intraocular environment. This is

particularly important in ophthalmic surgery and conditions such as glaucoma, where protection against mechanical damage is essential for eye health (Shinde *et al.*, 2023).

Figure 5 - Storage modulus (G') and loss (G") curves of the developed hydrogel samples.





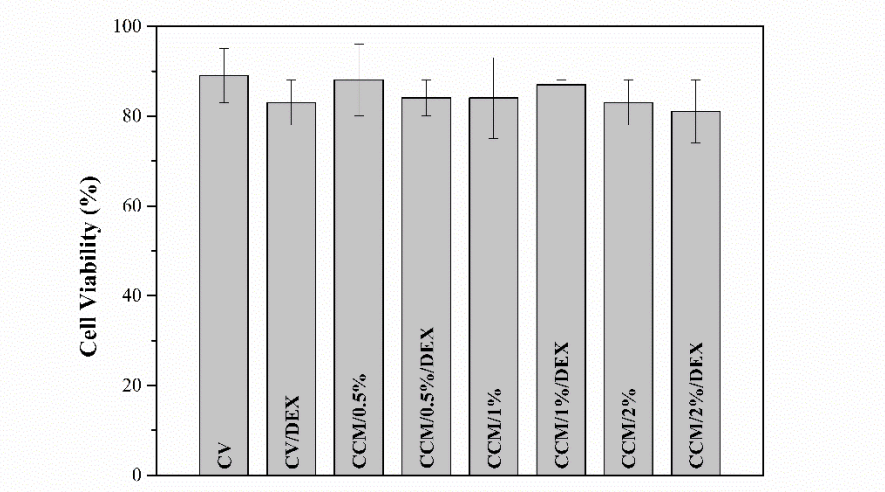
(g) (h)

Source: Research data (2024).

In Vitro Cytotoxicity Test

For the in vitro cytotoxicity assay against L929 cells, extracts from CMC and CMC/DEX hydrogels were used (Figure 6). According to ISO 10993-5:2009, samples with cell viability over 75% are generally considered noncytotoxic. The hydrogels obtained showed cell viability values above 80%, which indicates that the hydrogels developed are not cytotoxic and are suitable for ophthalmic applications. It is also noteworthy that the incorporation of the drug (DEX) into the hydrogel did not significantly alter cell viability.

Figure 6 – Cell viability of hydrogels with and without dexamethasone.



Source: Research data (2024).

These results are in line with what has been reported in the literature for CMC hydrogels and demonstrate their applicability as a biomaterial (Hao *et al.*, 2022; Kłosiński *et al.*, 2023).

CONCLUSION

The proposed methodology was effective in obtaining carboxymethylchitosan (CMC) and carboxymethylchitosan/dexamethasone (CMC/DEX) hydrogels for intraocular use. The results obtained confirm the feasibility and effectiveness of CMC hydrogels for biomedical applications. Carboxymethylation and incorporation of DEX were confirmed by FT-IR spectroscopy. Injectability tests showed that hydrogels without the drug and with a higher concentration of CMC required a higher injection force. However, the samples with DEX presented injection force similar to the commercial material, indicating greater practical applicability. The incorporation of DEX did not significantly alter the thermal behavior of the hydrogels, as demonstrated by thermal analyses. The rheological results showed that the hydrogels exhibit pseudoplastic behavior, with viscosity decreasing as the shear rate increases. Furthermore, the CMC hydrogels showed a linear viscoelastic behavior, indicating a predominantly elastic response. Cytotoxicity tests confirmed the biocompatibility of the hydrogels, with cell viability meeting the criteria of ISO 10993-5:2009. Together, these results demonstrate that the developed hydrogels have physicochemical, rheological, and biological properties promising for biomedical applications, especially in ophthalmological context.

REFERENCES

AHIRRAO, S. P.; BHAMBERE, D. S.; AGIWALE, B.; ALGAT, S.; ZOMAN, D.; CHAUDHARI, V. pH sensitive in-situ gel for ophthalmic delivery of ofloxacin and dexamethasone sodium phosphate: Formulation, development, and evaluation. **J Materials Today: Proceedings,** 2023.

ALLEN, L.; ANSEL, H. C. **Ansel's pharmaceutical dosage forms and drug delivery systems**. Lippincott Williams & Wilkins, 2013. ISBN 1451188765.

CHEN, X.-G.; PARK, H.-J. Chemical characteristics of O-carboxymethyl chitosans related to the preparation conditions. **Carbohydrate Polymers,** v. 53, n. 4, p. 355-359, 2003.

DATTA, S.; CANO, M.; EBRAHIMI, K.; WANG, L.; HANDA, J. T. J. P. I. R.; RESEARCH, E. The impact of oxidative stress and inflammation on RPE degeneration in non-neovascular AMD. **Progress in retinal eye research,** v. 60, p. 201-218, 2017.

DE LACERDA BUKZEM, A.; DOS SANTOS, D. M.; LEITE, I. S.; INADA, N. M.; CAMPANA-FILHO, S. P. Tuning the properties of carboxymethylchitosan-based porous membranes for potential application as wound dressing. **J International Journal of Biological Macromolecules,** v. 166, p. 459-470, 2021.

ENOCH, K.; SOMASUNDARAM, A. A. Rheological insights on Carboxymethyl cellulose hydrogels. **International Journal of Biological Macromolecules,** v. 253, p. 127481, 2023.

FIGUEIRÊDO, E. S. D.; MACEDO, A. C. D.; FIGUEIRÊDO, P. F. R. D.; FIGUEIRÊDO, R. S. D. J. A. B. D. O. Aplicações oftalmológicas do ácido hialurônico. **Arquivos Brasileiros de Oftalmologia,** v. 73, p. 92-95, 2010.

GALDINO, T. P.; OLIVEIRA, L. C. D.; LIMA, E. P. N.; FARIAS, R. Í. R.; JESUS, R. A. D.; QUEIROZ, S. F. A. M. D.; SANTOS, A. C. D. Q.; FOOK, M. V. L. Synthesis and characterization of O-Carboxymethylchitosan as an alternative to the use of Hyaluronic Acid. **Research, Society and Development,** v. 11, n. 5, p. e5011527634, 2022.

HADI, Z.; NAVARCHIAN, A. H.; RAFIENIA, M. Synthesis of pH-responsive carboxymethyl chitosan for encapsulating tetracycline-HCl: Morphology, drug release behavior and antibacterial activity of microcapsules. **Journal of Drug Delivery Science and Technology,** v. 84, p. 104462, 2023.

HALIMI, C.; MONTEMBAULT, A.; GUERRY, A.; DELAIR, T.; VIGUIER, E.; FULCHIRON, R.; DAVID, L. Chitosan solutions as injectable systems for dermal filler

applications: Rheological characterization and biological evidence. In: 2015 37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), IEEE, p.2596-2599, 2015.

HALIMI, C.; MONTEMBAULT, A.; GUERRY, A.; DELAIR, T.; VIGUIER, E.; FULCHIRON, R.; DAVID, L. Chitosan solutions as injectable systems for dermal filler applications: Rheological characterization and biological evidence. **Annu Int Conf IEEE Eng Med Biol Soc,** v. 2015, p. 2596-9, 2015.

HAN, X.; LI, G.; YOU, S.; SHEN, M.; XU, Y.; YANG, H.; LU, C.; ZHANG, M.; FANG, J.; ZHOU, Q.; YAO, Q. Injectable bio-multifunctional hyaluronic acid-based hydrogels loaded with poly ADP-ribose polymerase inhibitors for ovarian cancer therapy. **International Journal of Biological Macromolecules**, p. 132275, 2024.

HAO, Y.; ZHAO, W.; ZHANG, H.; ZHENG, W.; ZHOU, Q. Carboxymethyl chitosan-based hydrogels containing fibroblast growth factors for triggering diabetic wound healing. **Carbohydrate Polymers,** v. 287, p. 119336, 2022.

HUNT, J. A.; CHEN, R.; VAN VEEN, T.; BRYAN, N. Hydrogels for tissue engineering and regenerative medicine. **Journal of Materials Chemistry B,** v. 2, n. 33, p. 5319-5338, 2014.

KŁOSIŃSKI, K. K.; WACH, R. A.; GIREK-BĄK, M. K.; ROKITA, B.; KOŁAT, D.; KAŁUZIŃSKA-KOŁAT, Ż.; KŁOSIŃSKA, B.; DUDA, Ł.; PASIEKA, Z. W. **Biocompatibility and Mechanical Properties of Carboxymethyl Chitosan Hydrogels**. Polymers (Basel). 15 2023.

KONG, H. J.; WONG, E.; MOONEY, D. J. Independent control of rigidity and toughness of polymeric hydrogels. **Macromolecules,** v. 36, n. 12, p. 4582-4588, 2003.

KWITKO, S. Endotélio e cirurgia da catarata: grandes desafios. **J Arquivos Brasileiros de Oftalmologia** v. 63, p. 235-239, 2000.

LADEIRA, N. M. B.; DONNICI, C. L.; DE MESQUITA, J. P.; PEREIRA, F. V. Preparation and characterization of hydrogels obtained from chitosan and carboxymethyl chitosan. **Journal of Polymer Research,** v. 28, n. 9, p. 335, 2021.

LUCIO, D.; ZORNOZA, A.; MARTÍNEZ-OHÁRRIZ, M. C. Role of Microstructure in Drug Release from Chitosan Amorphous Solid Dispersions. **International Journal of Molecular Sciences,** v. 23, n. 23, p. 15367, 2022.

LUCIO, D.; ZORNOZA, A.; MARTÍNEZ-OHÁRRIZ, M. C. J. I. J. O. P. Influence of chitosan and carboxymethylchitosan on the polymorphism and solubilisation of diflunisal. **International Journal of Pharmaceutics,** v. 467, n. 1-2, p. 19-26, 2014.

MA, C.; WANG, Y.; JIANG, Z.; CAO, Z.; YU, H.; HUANG, G.; WU, Q.; LING, F.; ZHUANG, Z.; WANG, H.; ZHENG, J.; WU, J. Wide-range linear viscoelastic hydrogels with high mechanical properties and their applications in quantifiable stress-strain sensors. **Chemical Engineering Journal,** v. 399, p. 125697, 2020.

MATADH, A. V.; JAKKA, D.; PRAGATHI, S. G.; POORNIMA, K.; SHIVAKUMAR, H. N.; MURTHY, R. N.; RANGAPPA, S.; SHIVANNA, M.; MURTHY, S. N. Polymer coated polymeric microneedles for intravitreal delivery of dexamethasone. **Experimental Eye Research,** v. 231, p. 109467, 2023.

MOHSENI, M.; SHOKROLLAHI, P.; BARZIN, J. Gelatin/O-carboxymethyl chitosan injectable self-healing hydrogels for ibuprofen and naproxen dual release. **International Journal of Biological Macromolecules,** v. 263, p. 130266, 2024.

NAIR, R.; ROY CHOUDHURY, A. Synthesis and rheological characterization of a novel shear thinning levan gellan hydrogel. **International Journal of Biological Macromolecules,** v. 159, p. 922-930, 2020.

SAFA, B. N.; READ, A. T.; ETHIER, C. R. Assessment of the viscoelastic mechanical properties of the porcine optic nerve head using micromechanical testing and finite element modeling. **Acta Biomaterialia,** v. 134, p. 379-387, 2021.

SANTOS, W. M.; NÓBREGA, F. P.; ANDRADE, J. C.; ALMEIDA, L. F.; CONCEIÇÃO, M. M.; MEDEIROS, A. C. D.; MEDEIROS, F. D. Pharmaceutical compatibility of dexamethasone with excipients commonly used in solid oral dosage forms. **Journal of Thermal Analysis and Calorimetry,** v. 145, n. 2, p. 361-378, 2021.

SHINDE, S.; CHOUDHARY, K.; RANGU, V. Ophtalmic viscosurgical devices. **Int. J. Adv. Res.,** v. 11, n. 02, p. 328-332, 2023.