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PREPARATION AND PHYSICO-CHEMICAL CHARACTERIZATION OF A HYBRID HYDROGEL BASED ON HYALURONIC ACID AND CARBOPOL

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Abstract. Combining natural and synthetic polymers represents an effective strategy to obtain hybrid hydrogels with improved properties for biomedical applications. In this context, the purpose of this study is the preparation and characterization of a hybrid hydrogel based on hyaluronic acid and Carbopol. Although hyaluronic acid offers superior biological and regenerative properties, its utilization is limited by poor mechanical and rheological properties. To overcome these limitations, hyaluronic acid was combined with Carbopol, a synthetic polymer renowned for its gelling properties and its capacity to form stable hydrogels. The network structure and morphology were investigated using optical microscopy, SEM; EDX and FTIR. FTIR analysis demonstrated the formation of a stable network stabilized through hydrogen bonds. Optical microscopy indicated a homogeneous blending in the wet state, while SEM analysis revealed an interconnected three-dimensional porosity featuring fine fibrillar structures. The results confirm the purity and stability of the obtained biomaterial, demonstrating a promising potential for controlled drug delivery and tissue regeneration systems.

Keywords: hyaluronic acid, Carbopol, hydrogel, spectroscopy, biomedical applications.

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1. Introduction

In recent decades, hydrogels have attracted increasing interest in the biomedical field due to their capacity to retain large quantities of water or biological fluids while maintaining a stable structure. These materials have evolved into complex systems capable of responding to external stimuli and performing specific functions when interacting with biological environments (Dhara (Ganguly), 2024; Ho *et al.*, 2022; Kumar *et al.*, 2025; Vasile *et al.*, 2020). By combining natural and synthetic polymers, hybrid hydrogels integrate the biocompatibility and bioactivity of biopolymers with the predictable mechanical strength and structural stability of synthetic matrices. These systems are specifically developed to address the shortcomings of single-component hydrogels, which often fail to simultaneously meet the complex mechanical, chemical and biological requirements of biomedical applications (Choi *et al.*, 2024).

Natural polysaccharides, including cellulose, chitosan, sodium alginate, hyaluronic acid and starch, are widely utilized in hydrogel formulations. However, specific intrinsic limitations- such as water insolubility of cellulose, the restriction of chitosan solubility to acidic media or the slow and poorly predictable degradation profile of alginate- restrict their direct applicability (Yang *et al.*, 2022). Within this landscape, hyaluronic acid stands out due to its exceptional biocompatibility, biodegradability and non-immunogenicity. Coupled with its unique water-retention capacity, it has emerged as an intensively investigated biomaterial (Segneanu *et al.*, 2025; Trombino *et al.*, 2019).

Hyaluronic acid (HA) is a non-sulfated glycosaminoglycan composed of repeating disaccharide units of N-acetyl-D-glucosamine and D-glucuronic acid, linked alternately by β -1,4 and β -1,3 glycosidic bonds. This configuration enables the formation of highly flexible macromolecular chains that can reach high molecular weights, ranging from several hundred thousand to several million Daltons (Grabowski *et al.*, 2025). Low-molecular-weight (LMW) hyaluronic acid is of particular biomedical interest due to its superior tissue penetration, potent antioxidant activity and enhanced capacity to stimulate cellular regeneration compared to its high-molecular-weight counterparts (Yasin *et al.*, 2022). Hyaluronic acid is widely distributed throughout the epithelial and connective tissues of vertebrates, representing the major component of the extracellular matrix. Furthermore, it modulates complex physiological processes such as inflammation, wound healing, tissue development and morphogenesis (Trombino *et al.*, 2019).

Nevertheless, hydrogels prepared solely from native hyaluronic acid often suffer from poor mechanical strength, low structural stability over time and weak rheological properties, which inherently limit their performance in applications subjected to physical stress (Zhang *et al.*, 2025). In this context, Carbopol (cross-linked polyacrylic acid) represents an ideal candidate for

integration into a hybrid system alongside hyaluronic acid. Renowned for its remarkable gelling efficiency, bioadhesion and thermal stability, Carbopol is capable of forming hydrophilic networks with pronounced viscosity and a pseudoplastic behavior ideal for pharmaceutical and topical applications (Piau, 2007; Pourmadadi *et al.*, 2023; Shafiei *et al.*, 2018).

Therefore, the objective of this study is the fabrication and physico-chemical characterization of a hybrid system based on hyaluronic acid and Carbopol. This approach aims to combine the superior biocompatibility of hyaluronic acid with the mechanical stability, viscosity and rheological control provided by the Carbopol matrix, thereby developing an optimized biomaterial for biomedical applications.

2. Materials and methods

2.1. Materials

In this study, the materials listed in Table 1 were used to prepare the hydrogel.

Table 1
Materials used in hydrogel formulation

Compound	Chemical formula	Molecular weight	Purity	Supplier
Sodium hyaluronate (LMW)	$(C_{14}H_{21}NO_{11})_n$	10-200 kDa	$\geq 95\%$	Sigma-Aldrich
Carbopol 940	$(C_3H_4O_2)_n$	72.06 g/mol (monomer unit)	99,3%	Chimreactiv SRL
Sodium hydroxide 1N	NaOH	39.997 g/mol	100%	Chimreactiv SRL
Distilled water	H ₂ O	18.01 g/mol	100%	-

2.1. Experimental preparation protocol

The preparation of the hyaluronic acid and Carbopol 940 hybrid hydrogel involved several sequential steps to ensure homogeneity, physico-chemical stability and optimal product efficacy.

Initially, the gel components were weighed, while the required volume of distilled water was measured. Carbopol 940 (3.5 g) was weighed and 90.5 mL of distilled water was gradually added under continuous stirring to prevent the formation of aggregates. In the subsequent step, a 1 N sodium hydroxide solution was added as a neutralizing agent to adjust the pH within the optimal range for the formation of a stable and viscous polymeric network. Upon reaching the target pH, hyaluronic acid (1 g), previously dissolved in distilled water to ensure

efficient integration into the hydrogel matrix, was incorporated. The resulting mixture was homogenized to achieve a uniform distribution throughout the entire volume of the hydrogel. Subsequently, a portion of the obtained hydrogel was transferred into Petri dishes and subjected to oven-drying at 45°C to investigate the changes in the hydrogel structure induced by water elimination.

The dried hydrogel sample was then analyzed and characterized using the following methods:

- Optical microscopy;
- Scanning electron microscopy (SEM);
- Fourier-transform infrared spectroscopy (FTIR).

3. Results and discussion

Several characterization methods were employed to highlight the structural and compositional properties of the prepared hydrogel.

For the morphological characterization of the hydrogel based on 1% hyaluronic acid and Carbopol, an optical microscope with magnifications of 10x, 60x and 200x was used, both in the wet state and after complete drying. The resulting images are shown in Fig. 1. The purpose of this analysis was to highlight the changes in the hydrogel's structure resulting from water loss, given the importance of this aspect for its functional properties.

The images obtained in the wet state revealed a homogeneous distribution of voids within the hydrogel matrix. At 10x magnification, a dense, uniform network with a homogeneous dispersion of voids is observed, indicating good compatibility between Carbopol and hyaluronic acid, both of which contribute to the stabilization of the aqueous phase. At 60x magnification, the surface details of the voids become evident, highlighting the absence of major imperfections. At 200x, the analysis reveals thin and smooth void walls, confirming efficient interactions between the polymers and water.

After drying the sample, the microscopic images exhibited significant morphological changes. At 10x magnification, the overall structure appeared collapsed, with the complete disappearance of the microbubbles and reduction in the matrix volume. At 60x and 200x magnification, circular contours resulting from water evaporation and the retraction of the polymeric walls were observed, sometimes accompanied by microcracks. These changes suggest a substantial loss of mechanical stability upon dehydration, a characteristic of Carbopol - based hydrogels.

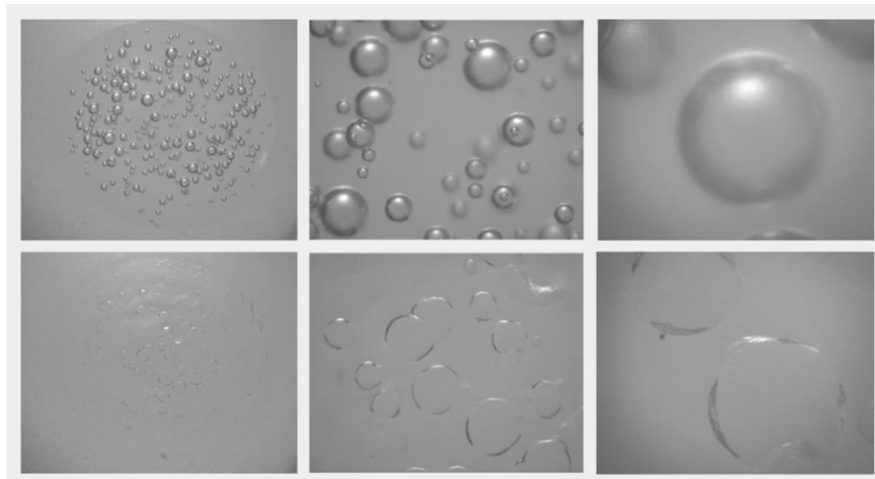


Fig. 1 – Microscopic analysis of the hyaluronic acid - Carbopol hydrogel in wet state (top) and dry state (bottom).

The obtained results confirm that the three-dimensional network of the hydrogel is directly dependent on hydration, drying leading to structural collapse and the loss of initial physico-mechanical properties. These observations are relevant for optimizing formulations intended for pharmaceutical, dermatological or cosmetic applications, where water retention and the maintenance of hydrogel stability over time are critical factors.

For a detailed structural analysis, scanning electron microscopy (SEM) was utilized, providing high-resolution images of the hydrogel surface, as presented in Fig. 2.

SEM detection performed via an Everhart-Thornley detector (ETD) enabled the acquisition of detailed surface micrographs. The obtained images highlight a complex morphology characteristic of cross-linked polymeric networks. A non-uniform structure is observable, with the clear presence of interconnected porous zones varying in size, which may indicate a good absorption capacity. In certain regions, fibrillation zones (thin, filament-like networks) are distinguishable, suggesting stable interactions and associations between the polymeric chains of the two components. These fibrillar structures contribute to the formation of a dense, three-dimensional matrix, which confers mechanical stability and the ability to sustain the controlled release of active substances. In some images, compact aggregates are noticeable, likely resulting from the drying process, which may reflect a heterogeneous distribution of the network density. No large crystals or phase-separated domains are observed, indicating that the hyaluronic acid and Carbopol are well homogenized. The surface exhibits a rough topography.

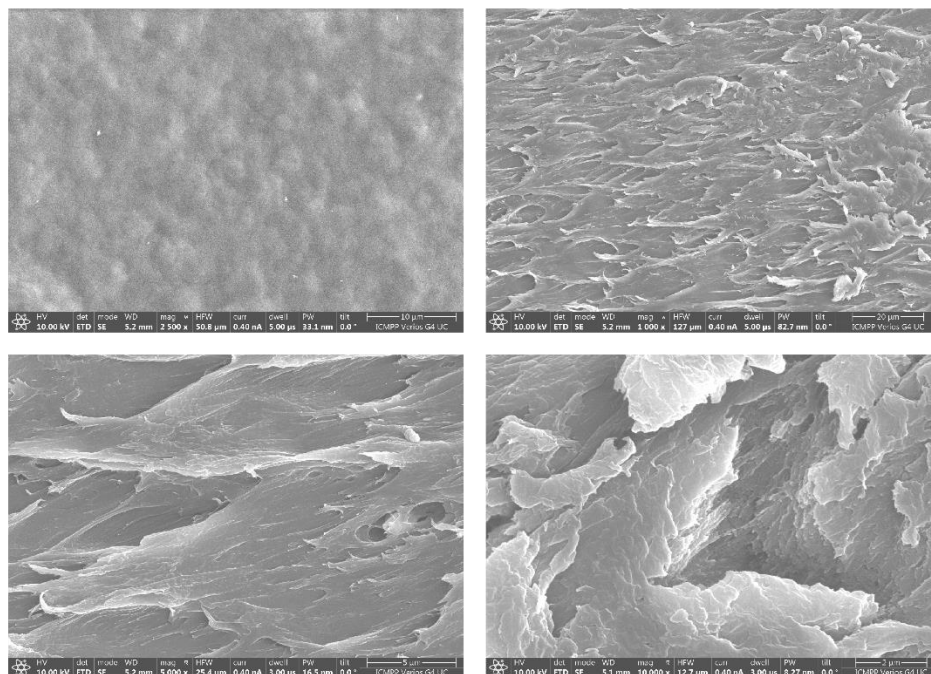


Fig. 2 – SEM analysis of the hyaluronic acid - Carbopol hydrogel in dried state.

The elemental composition was determined via scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDX) across five distinct regions of the analyzed surface, as shown in Fig. 3.

The results indicate a predominantly organic composition, characterized by a high content of carbon (ranging from 55.28% to 56.80%) and oxygen (with values between 30.63% and 33.04%). This composition reflects the chemical structure of the two components, both being polymers rich in carboxylic, hydroxyl and amide functional groups. The presence of nitrogen in proportions of 2.61 - 3.35% confirms the inclusion of amide groups, which are specific to the N-acetyl-glucosamine units within the hyaluronic acid structure. The detection of sodium (2.40 - 2.66%) verifies the use of the sodium salt form of hyaluronic acid in the preparation of the hydrogel. The presence of gold and palladium in the analyzed zones is attributed to the conductive metal coating applied to enhance surface conductivity for the SEM-EDX analysis. Lastly, the trace amounts of silicon detected can be attributed to residual contamination or interaction with the sample substrate.

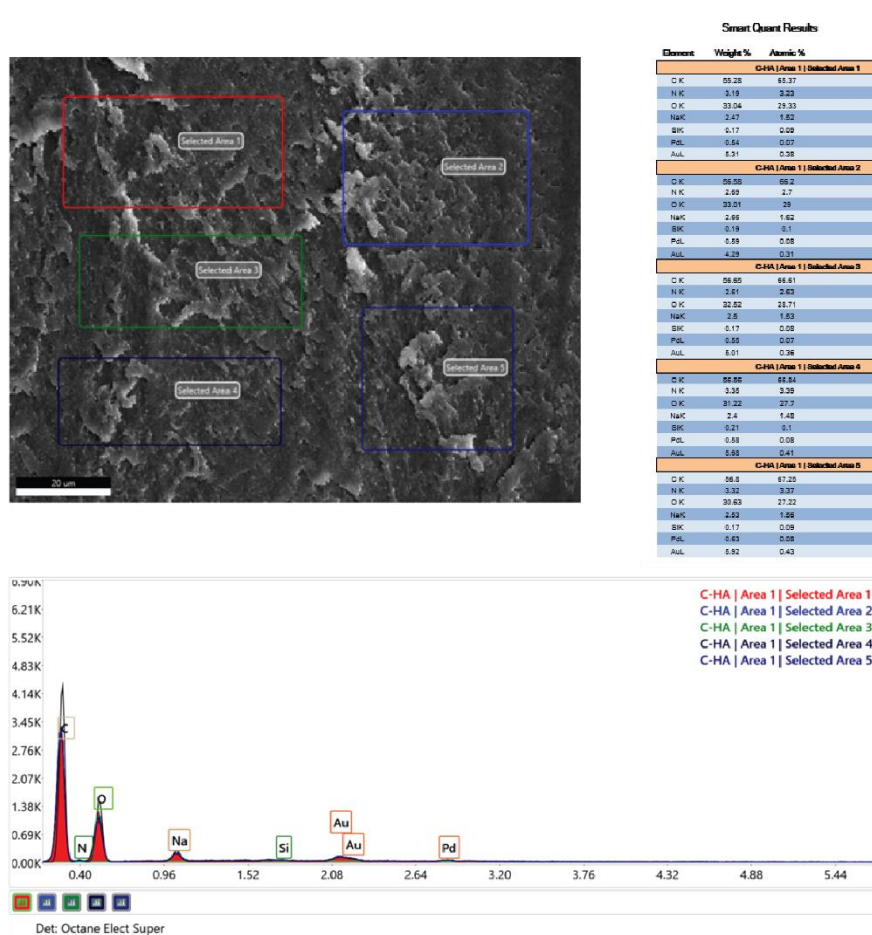


Fig. 3 – SEM-EDX analysis.

In terms of spatial distribution, the elemental composition is consistent across all examined regions, indicating a high homogeneity of the synthesized hydrogel.

To evaluate potential interactions between hyaluronic acid and Carbopol within the hydrogel matrix, the FTIR spectrum of the hyaluronic acid-Carbopol blend was recorded. Comparative analysis with the spectrum of the pure Carbopol hydrogel revealed significant modifications, highlighting the presence of characteristic functional groups and the formation of new molecular-level interactions.

The standard FTIR spectrum of hyaluronic acid (Fig. 4) exhibits several sharp peaks and characteristic bands. Among these, peak 1, located at 3436 cm^{-1} , is significantly broader than the others, spanning a convoluted frequency range between 3200 cm^{-1} and 3600 cm^{-1} , which can be attributed to the overlapping

stretching vibrations of the O–H and N–H groups. Peak 2, centered at 1628 cm^{-1} , is assigned to the carbonyl (C=O) stretching vibration, indicating the presence of the Amide I band. For most secondary amides, the weaker N–H bending absorption typically emerges within the frequency range of 1500 cm^{-1} to 1560 cm^{-1} (Amide II). Peak 3, located at 1410 cm^{-1} , corresponds to the symmetric stretching vibration of the O–C=O carboxylate group. Peaks 4 and 5, situated at 1153.41 cm^{-1} and 1046.36 cm^{-1} , are linked to the stretching vibrations of C–O and C–O–C linkages, respectively. Finally, peak 6, located at 650.68 cm^{-1} , represents a characteristic fingerprint peak of hyaluronic acid, which can be attributed to a combination of out-of-plane bending vibrations.

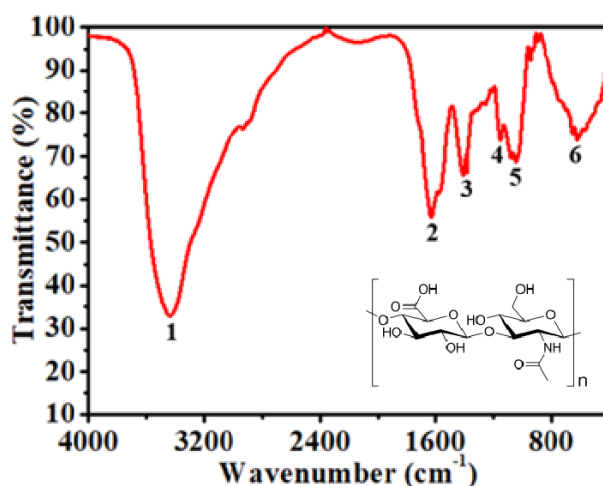


Fig. 4 – Standard FTIR spectrum of hyaluronic acid.

The FTIR spectrum of the pure Carbopol hydrogel (Fig. 5) displays a broad band at 3312 cm^{-1} , which is characteristic of the stretching vibrations of hydroxyl groups from both the carboxylic functionalities and adsorbed water molecules. The presence of an intense and well-defined band at 1708 cm^{-1} indicates the carbonyl stretching vibration of undissociated carboxylic acids, suggesting an acidic environment specific to Carbopol in its native state. Additionally, supplementary bands are observed at 2924 cm^{-1} and 2882 cm^{-1} , as well as in the $1450 - 1276\text{ cm}^{-1}$ regions, which are attributed to C–H and C–O vibrations.

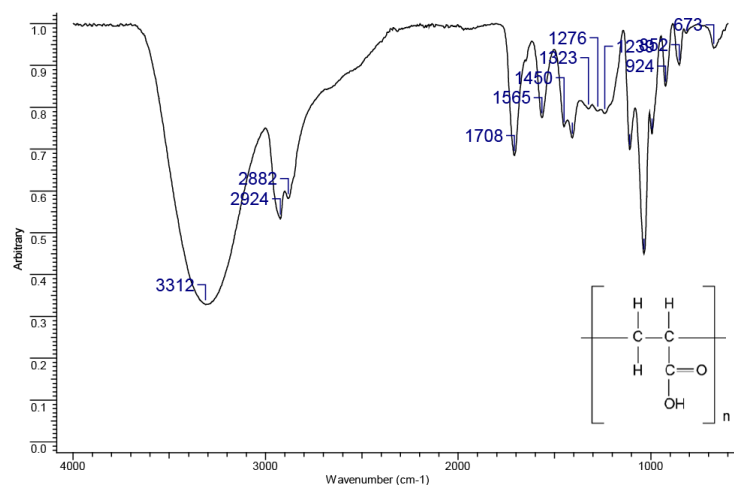


Fig. 5 – FTIR spectrum of pure Carbopol hydrogel.

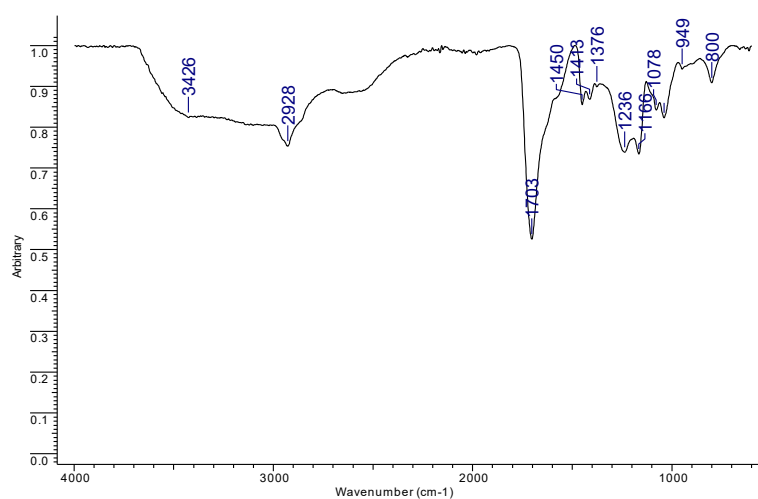


Fig. 6 – FTIR spectrum of dried hydrogel.

Figure 6 illustrates the spectrum recorded for the hyaluronic acid and Carbopol-based hydrogel sample. In contrast, the FTIR spectrum of the hyaluronic acid-Carbopol hydrogel network exhibits a well-resolved and intense profile. The broad absorption band associated with the stretching vibrations of hydroxyl (O–H) groups, originally centered at 3312 cm^{-1} in the pure Carbopol spectrum, shifts to a higher wavenumber at 3426 cm^{-1} and becomes broader in the hybrid gel. This phenomenon is attributed to the overlapping of the O–H and N–H stretching modes from both polymers, confirming an extensive, interconnected hydrogen-bonding network. Another critical structural indicator is

the behavior of the carbonyl (C=O) stretching vibration. Upon hydrogel formation and hybridization with the polysaccharide, the prominent peak originally located at 1708 cm^{-1} shifts downward to 1703 cm^{-1} . Rather than disappearing, the slight shift and persistent high intensity of this carbonyl signal provide robust evidence of molecular-level interactions, specifically the formation of strong hydrogen bonds between the -COOH functionalities of Carbopol and the glucuronic acid units of the hyaluronic acid matrix. Furthermore, the bands appearing at 1450 cm^{-1} and 1418 cm^{-1} correspond to the bending modes of -CH_2 and -OH groups, respectively, the newly emerged peak at 1236 cm^{-1} is assigned to C–N stretching vibrations, validating the structural retention of the amide linkages from the hyaluronic acid component.

Finally, the polysaccharide carbohydrate fingerprint region between 1200 cm^{-1} and 1000 cm^{-1} features well-defined peaks at 1160 and 1078 cm^{-1} , linked to C–O and C–O–C stretching. These bands, which are weakly resolved or completely absent in the pure Carbopol profile, definitively confirm the successful and homogeneous molecular integration of hyaluronic acid within the cross-linked hydrogel framework.

The absence of any extraneous baseline signals demonstrates the high purity and structural stability of the synthesized formulation.

The FTIR results are in agreement with literature data (Shahid *et al.*, 2025), with minor spectral variations being attributed to the different polymer ratio and the degree of carboxyl group deprotonation following the neutralization process.

4. Conclusions

The primary objective of the present study was the development and characterization of a hyaluronic acid and Carbopol-based hydrogel with significant potential for biomedical applications. By combining hyaluronic acid, a natural polysaccharide with a fundamental role in human tissue structure, with Carbopol, a synthetic polymer frequently utilized in pharmaceutical formulations, this study aimed to obtain a stable system with enhanced physico-chemical properties and expanded applicability.

The performed analyses (optical microscopy, Scanning Electron Microscopy, EDX spectroscopy and FTIR spectroscopy) provided an integrated perspective on the structure, composition and molecular-level chemical interactions within the hydrogel network. The results demonstrated excellent compatibility between the two components, a homogeneous distribution of elements and the presence of specific functional groups (hydroxyl, carboxyl, carbonyl and amide), which are essential for the formation of a stable network driven by hydrogen bonding.

The observed morphology, along with the physico-chemical properties and network stability, suggests that the synthesized hydrogel holds great promise

for various biomedical applications. Due to the high biocompatibility of hyaluronic acid and the superior gelling properties of Carbopol, the investigated system is highly promising in fields such as controlled delivery of active substances, topical and ophthalmic formulations, tissue engineering, wound healing systems or injectable scaffolds for cellular regeneration.

Looking forward, to further extending the applicability of this hydrogel, future research directions may focus on evaluating its swelling and degradation behavior in physiologically simulated fluids, the incorporation of specific active pharmaceutical ingredients, as well as rheological assessments. Consequently, these advancements could pave the way for innovative, tailored and minimally invasive solutions within biomedical field.

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OBȚINEREA ȘI CARACTERIZAREA FIZICO-CHIMICĂ A UNUI HIDROGEL HIBRID PE BAZĂ DE ACID HIALURONIC ȘI CARBOPOL

(Rezumat)

Combinarea polimerilor naturali cu cei sintetici reprezintă o strategie eficientă pentru a obține hidrogeluri hibride cu proprietăți îmbunătățite pentru aplicații biomedicale. În acest context, scopul acestei lucrări este obținerea și caracterizarea unui hidrogel hibrid bazat pe acid hialuronic și Carbopol. Deși acidul hialuronic oferă proprietăți biologice și regenerative superioare, utilizarea sa este limitată de proprietățile mecanice și reologice slabe. Pentru a depăși acest impediment, s-a ales combinarea acidului hialuronic cu Carbopolul, un polimer sintetic recunoscut pentru proprietățile de gelifiere și capacitatea de a forma hidrogeluri stabile. Structura și morfologia rețelei au fost investigate prin microscopie optică, SEM, EDX și FTIR. Analiza FTIR a demonstrat formarea unei rețele stabilizate prin legături de hidrogen. Microscopia optică a indicat o amestecare omogenă în stare umedă, în timp ce analiza SEM a relevat o porozitate tridimensională interconectată, cu structuri fibrilare fine. Rezultatele confirmă puritatea și stabilitatea biomaterialului obținut, demonstrând un potențial promițător pentru sistemele de eliberare controlată a medicamentelor și de regenerare tisulară.