

Synthesis and *in vitro* characterization of quercetin-loaded alginate-keratin composite hydrogels for enhanced wound healing applications

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The effective management of cutaneous wounds remains a significant clinical challenge because traditional dressings, such as cotton gauze, often fail to maintain an optimal moisture balance, leading to secondary tissue trauma and delayed healing. This study details the synthesis and characterization of a novel hybrid hydrogel, formulated as a bioactive component for a multilayered wound dressing. The preparation protocol consisted of blending a 1% (w/v) sodium alginate solution, with a series of concentrations of keratin (1%; 2%; 3%) at 45 °C, before ionic cross-linking with CaCl₂. The delivery of an anti-oxidant and anti-inflammatory agent was achieved by loading quercetin (0.1%) within the matrix. Spectroscopic analysis by FTIR showed the successful entrapping of quercetin and the molecular stability of the compound was confirmed by the presence of the characteristic aryl ether (C-O-C) peak at 1271 cm⁻¹. The swelling profile of the formulations demonstrated a maximum swelling ratio of 293% after 48 h with the optimum formulation (2%) showing an excellent rate of control over excess wound exudates. Following *in vitro* release into PBS of pH 7.4, an increase in keratin content was shown to influence the diffusion coefficient with the greatest release profile being achieved by the 3% keratin formulation. The alginate-keratin-quercetin hydrogel system presents a biocompatible, chemically stable and therapeutically active alternative for advanced wound care.

Keywords: Hydrogel, Sodium alginate, Keratin, Quercetin, Drug release, Wound care.

INTRODUCTION

Hydrogels, due to their three-dimensional network structure and high water retention capacity, are promising biomaterials for wound care applications, as they provide a moist environment and enable controlled drug delivery. Re-epithelialization is greatly accelerated by their innate capacity to provide a moist milieu at the site of injury, which also makes regulated medication administration possible [1]. Hydrogels are structurally incredibly versatile, with thicknesses varying from nanometers to centimeters. Additionally, they can conform to complex, irregular, and constrained wound geometries due to their exceptional deformability and relatively low shear modulus. Because of these special physicochemical characteristics, hydrogels are widely used as structural matrices in biomedical engineering, especially for pressure ulcers, severe burns, cutaneous blisters, and surgical wounds [2]. Hydrogels have a huge variety of structures and thicknesses, ranging from nanometers to centimeters. Their excellent deformability and relatively low shear modulus also allow them to conform to complex, irregular and constrained

wound geometries. These unique physicochemical properties have made hydrogels widely used as structural matrices in biomedical engineering, especially for pressure ulcers, severe burns, cutaneous blisters, and surgical wounds [3].

In recent years, the development of drug-eluting wound dressings has significantly improved the clinical efficiency of these matrices. These technologies offer significant advantages over traditional delivery routes by maintaining appropriate plasma medication levels within a predefined therapeutic window over extended periods of time by directly incorporating therapeutic agents into the polymeric network [4]. Microbial infection is a major impediment to the physiological cascade of healing. Hydrogel dressings are often combined with antimicrobial and anti-inflammatory drugs to eradicate microorganisms from the wound bed and to reduce delays caused by infection [3]. Besides their great biocompatibility, hydrogel-based dressings are usually transparent, which enables medical professionals to non-invasively monitor the course of wound healing in real time without the need for painful or premature dressing changes [3]. To improve biological affinity and biodegradability, present research has turned to natural polymers such

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as chitosan and alginate. For instance, recent developments disclose the development of hybrid dressings based on alginate containing green-synthesized metallic nanoparticles, including copper, in addition to bioactive plant extracts [5]. The phytochemical profiling through liquid chromatography-mass spectrometry (LC-MS/MS) reveals that the phenolics, flavonoids, and glycosidic chemicals present in these natural extracts are very effective reducing and stabilizing agents while synthesis [5]. The exploitation of the synergistic potential of biopolymer matrices and natural bioactive compounds is essential for the development of next-generation multifunctional wound treatments.

EXPERIMENTAL

Materials

The functional biomaterial matrix was synthesized using sodium alginate as a main, non-toxic polymeric backbone for the controlled therapeutic delivery.

Keratin was added to the matrix as structural additive in different percentages of 1%, 2% and 3% (w/v) to improve biocompatibility and structural integrity. Encapsulation of a potent natural antioxidant, quercetin, at a defined concentration of

0.1% (w/v) was provided with bioactive healing properties to the composite system. Then, the alginate polymer chains were ionically cross-linked by using a 0.1 M calcium chloride (CaCl_2) solution to fabricate a flexible and mechanically stable three-dimensional hydrogel scaffold.

In Fig. 1, the aforementioned composite hydrogels (1K1A, 2K1A and 3K1A) were prepared by incorporating different levels of keratin into a constant 1% (w/v) sodium alginate suspension and entrapment of 0.1% (w/v) quercetin, at optimum pH. The ultimate architectural stabilization was achieved by salt-mediated ionic cross-linking with Ca^{2+} , converting the polymeric mixture into a resilient bioactive 3D matrix. Purification steps, and final controlled thermal drying at 45 °C were performed.

The first step in the preparation of the bioactive composite hydrogels was the preparation of the main polymer components. To maintain a constant baseline concentration of 1% (w/v), sodium alginate was continuously dissolved in deionized water, resulting in a homogeneous, viscous polymeric matrix (Fig. 2a). At the same time, different concentrations of 1%, 2%, and 3% (w/v) keratin stock solutions were prepared (Fig. 2b).

Hydrogel material preparation process

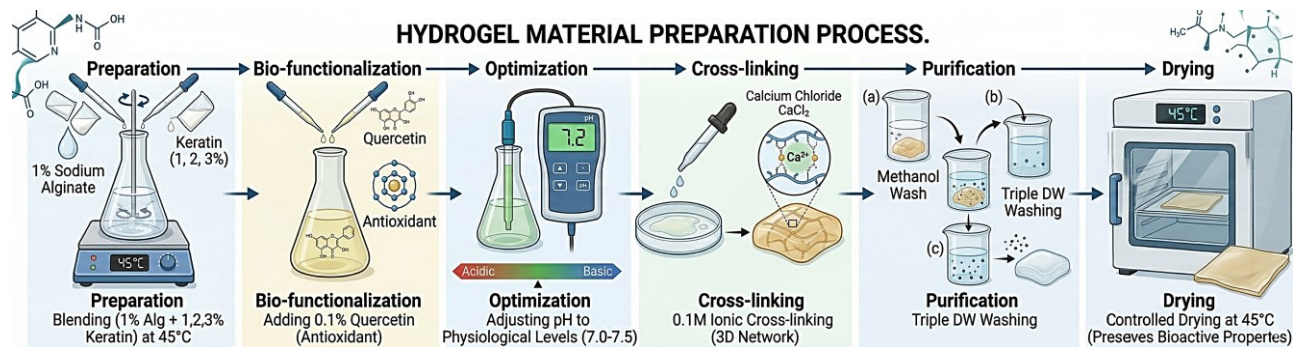


Figure 1. Hydrogel material preparation process.

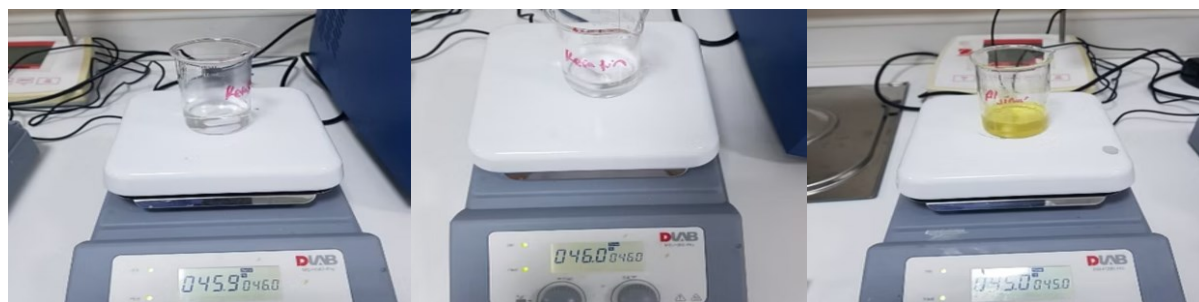


Figure 2. (a) 1% sodium alginate solution, (b) keratin solution, (c) mixture of all solutions with pH adjusted and 0.1% quercetin added

The different keratin solutions were methodically added to the fixed sodium alginate matrix under constant, regulated magnetic stirring to produce the hybrid polymeric matrices—designated as 1K1A, 2K1A, and 3K1A, respectively—to guarantee total structural homogeneity.

Quercetin was added to the composite mixes at a specific concentration of 0.1% (w/v) in order to give the scaffold medicinal effectiveness (Fig. 2c). This powerful natural polyphenol was chosen to reduce localized oxidative stress at the site of injury and quicken the physiological wound healing cascade. The dense polymer matrix in this engineered configuration performs two functions: it creates a convoluted pathway that controls the sustained, controlled release kinetics of the sensitive polyphenol while acting as a protective barrier to prevent oxidative degradation under harsh physiological conditions.

The pH of the solution was carefully optimized during the mixing process (Fig. 2c). This parameter must be controlled accordingly during the blending stage, as it is known to influence the compatibility of the blend with tissues, prevent the premature alkaline degradation of quercetin, and identify the optimal ionization of the functional groups implicated in the cross-linking process.

Standard neutralization of the blends was followed by ionic cross-linking of the liquid polymers to form a stable three-dimensional gel network. These hydrated alginate polymers can be cross-linked using divalent calcium ions. This commences with the immersion of the gels within a 0.1 M CaCl_2 clone solution whereupon the ionic calcium (Ca^{2+}) effectively forms ionic bridges between the blocks of neighboring alginate chains. The use of a 0.1 M concentration was chosen with the utmost care, as the higher concentrations (0.2 and 0.3 M) observed to lead to inhomogeneous, rapid curing of the polymer. This concentration was optimal for covalent gel formation, achieving the higher levels of cross-linking producing gel medium with an efficient quercetin encapsulation ability, while leaving the medium easily maneuvered for a prolonged, efficacious diffusion. After the cross-linking procedure, the hydrogels were filtered, treated with methanol, and then incubated for 30 min at 4 °C in a refrigerator to aid in precipitation and purification. Following the incubation period, the hydrogels were taken out of the refrigerator, washed once with methanol, and carefully cleaned three times with distilled water to get rid of any remaining ions and unreacted species. Structural scaffolds were created by drying the hydrogels in a controlled oven at 45 °C for 1 h in order to eliminate excess moisture without causing

thermal degradation of the encapsulated bioactive chemicals. The final appearance and structural morphology of the obtained hydrogels are presented in Fig. 3.

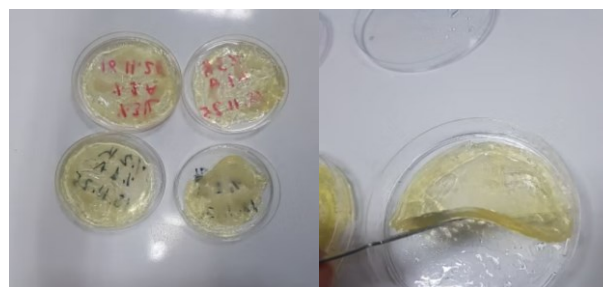


Figure 3. (a) The lower ones have 2% K%1A, and the upper ones have 3% K%1A; (b) These are hydrogels containing 1% keratin and 1% alginate.

RESULTS AND DISCUSSION

The synthesized alginate-keratin-quercetin composite hydrogels were comprehensively investigated for characterization and functional assessment to ascertain their suitability for advanced wound therapies, as schematically shown in Fig. 4. The experimental workflow includes swelling analysis, *in vitro* drug release techniques, and FTIR spectroscopy for the characterization of hydrogels.

The confirmation of the polymeric matrix at the molecular level, its macro-scale physical swelling behavior, and finally its functional drug release performance under physiologically simulated settings are all logical steps in the experimental workflow. It includes swelling analysis, *in vitro* drug release techniques, and FTIR spectroscopy for the characterization of hydrogels.

FTIR was used to determine the chemical structures and functional groups of the produced composite hydrogels.

The three critical spectral zones over which the experimental spectra were systematically analyzed were the hydroxyl area, the carbonyl/carboxylate region and the fingerprint region, all of which together provide a clear insight into the structural organization of the composite matrix.

- **Hydroxyl area ($3000\text{--}3600\text{ cm}^{-1}$)** The stretched vibrations of the hydroxyl ($-\text{OH}$) groups are illustrated by the broad absorption between 3000 and 3600 cm^{-1} , as seen in Fig. 5. As keratin content increased from 1K1A to 3K1A formulations the broad OH band is significantly increased in both size and intensity. This is indicative of the formation of a dense network of inter-molecular hydrogen bonds involving the keratin functional groups, the backbone of the sodium alginate and the phenolic hydroxyl groups of quercetin [6].

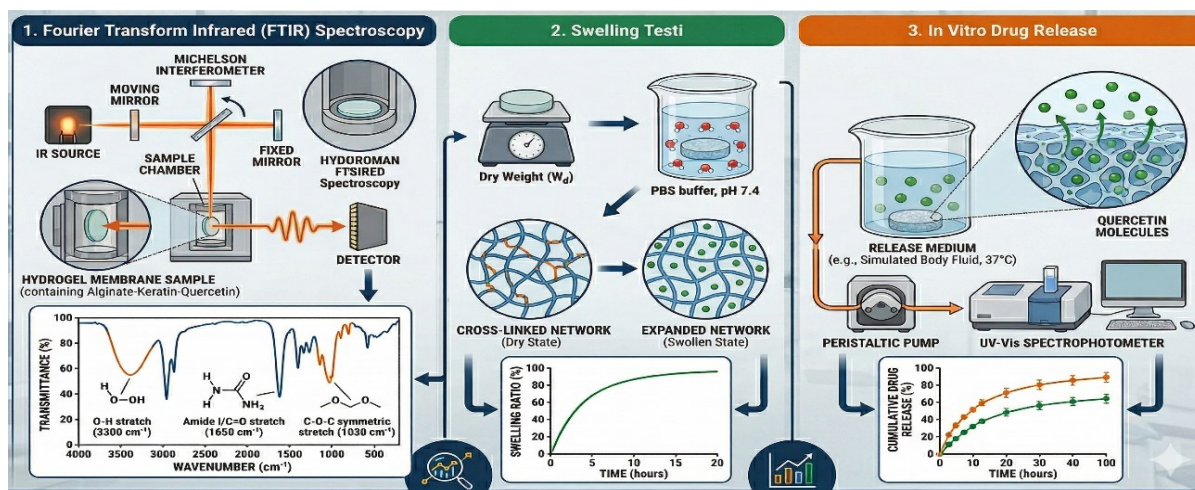


Figure 4. Characterization of hydrogels using; FTIR spectroscopy, swelling analysis, and *in vitro* drug release methods

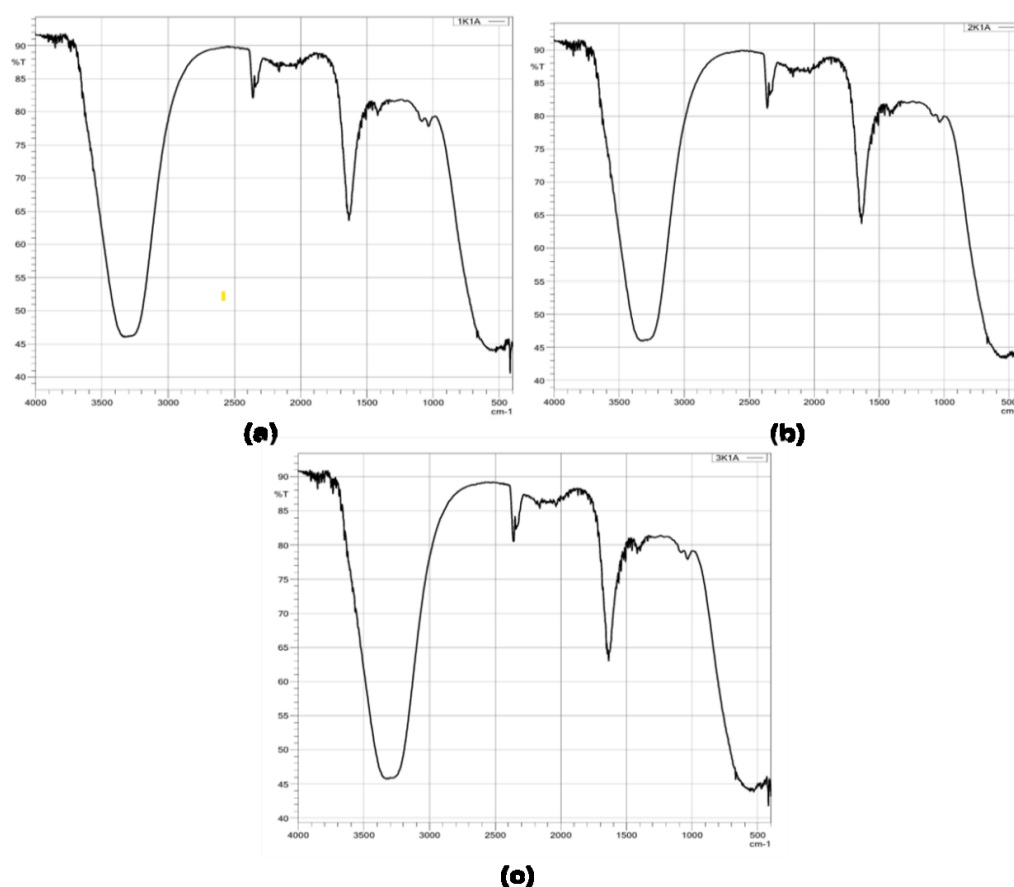


Figure 5. FTIR spectra of (a) 1K1A (1% keratin, 1% alginate), (b) 2K1A, and (c) 3K1A hydrogels.

- At the carbonyl and carboxylate regions (1500–1700 cm⁻¹) all alginate carboxylate peaks identified in these regions in Fig. 6 showed substantial displacements to higher wave numbers (blue shifts). These consistent modifications confirm the successful co-ordination and molecular mixing between biopolymer chains and consequently they are indicative of a change in the nearby ion

environment. However, the encapsulated drug exhibited an interesting behavior in this same region; it appeared that even at the highest level of polymer loading, as evidenced by the 3K1A formulation (Fig. 6 c), the crystalline peaks of pure quercetin had become masked. The polyphenol was successfully entrapped within the polymer matrix at a molecular, amorphous level.

Table 1. Swelling test of 1K1A (1% keratin, 1% alginate), 2K1A, and 3K1A hydrogels.

Time	1%			2%			3%		
	Dry Weight (W ₀) (g)	Swollen Weight (W ₁)(g)	Swelling Ratio (%)	Dry Weight (W ₀) (g)	Swollen Weight (W ₁)(g)	Swelling Ratio (%)	Dry Weight (W ₀) (g)	Swollen Weight (W ₁)(g)	Swelling Ratio (%)
30 min	0,5821	1,1970	105,6348	0,5438	1,0672	96,2486	0,5270	1,1063	109,9241
1h	0,5548	1,4700	164,9603	0,5755	1,0581	83,8575	0,3920	0,8460	115,8163
2h	0,4557	1,1235	146,5438	0,5177	1,2965	150,4346	0,4281	0,9360	118,6405
3h	0,3738	0,8075	116,0246	0,4998	1,3115	162,4050	0,3200	0,8170	155,3125
4h	0,6110	1,0967	79,4926	0,6260	1,2304	96,5495	0,3838	0,8982	134,0281
5h	0,4195	0,7558	80,1669	0,5462	1,1375	108,2570	0,4432	0,7120	60,6498
6h	0,6763	0,9075	34,1860	0,4540	1,1680	157,2687	0,2480	0,5808	134,1935
24h	0,4305	0,9603	123,0662	0,5050	1,2900	155,4455	0,5167	1,2537	142,6360
48h	0,4890	1,0675	118,3027	0,4920	1,9380	293,9024	0,4843	1,1180	130,8486

• The fingerprint region provides the strongest evidence of drug loading and chemical stability. The most direct evidence of chemical loading and stability within the polymeric matrix is a well-defined prominent peak at 1271 cm⁻¹, a characteristic aryl ether (C–O–C) stretching vibration. The similar representation of this peak in the 1K1A, 2K1A and 3K1A spectra confirms the bioactive compound maintained chemical stability inside the polymeric matrix in the course of loading and polymerization processes, since there is an absence of this particular signal within free alginate. The relative intensity of this peak increased linearly with increased drug loading, providing further confirmation of its use as a reliable indicator of drug loading and structural stability [7]. It becomes more intense as the drug concentration increases. To summarize our FTIR results, we see three major phenomena: broadening of the OH band which indicates improved physical stability, shift and masking of the drug peaks provides evidence that the drug is encapsulated in our system, and finally the baseline insertion of the 1271 cm⁻¹ peak shows that quercetin is indeed present and chemically stable [6].

Swelling ratio and water absorption capacity measurements were performed to evaluate the structural features (such as polymer network structure) of the biopolymer composite hydrogels. The dried hydrogels were weighed to determine the initial weight (W₀). The samples were then immersed in distilled water for periods ranging from 30 minutes to 48 hours, after which the swollen weight (W₁) was measured. The swelling percentage was calculated using the following formula:

$$\text{Percentage Swelling (\%)} = \left(\frac{W_1 - W_0}{W_0} \right) \times 100$$

The hydration profiles varied considerably depending on the exact keratin concentration, as

summarized in the quantitative data presented in Table 1. The changing biopolymer ratios yielded distinct performance behaviors for each formulation: 1% keratin, fluctuating swelling was observed throughout the testing time. Apart from some clinical situations which require an initial fast drug release profile upon first contact with moisture, the relatively weak network stability may impede its broad use. 2% keratin, The matrix demonstrated an optimum initial balance between overall hydrophilicity and structural elasticity, with a maximum swelling ratio of 293% at 48 h. 2% keratin matrix is the most desirable one for further application in advanced wound dressing purpose as it harbors fluid well without any compromise in the matrix structure. 3% keratin swelling behavior was very stable and well-controlled for this gel. For certain biomedical applications requiring a higher degree of mechanical strength and structural durability, the dense network structure of the macromolecules provides a great advantage by restricting rapid water transport [8].

According to the swelling experiments (Table 1), the formulation with 2% keratin is the ideal option for wound care, when fluid absorption is crucial, while the formulation with 3% keratin is the pick when some degree of structure needs to be preserved [9].

In vitro drug release, test for quercetin release once removed from the hydrogel, 0.5 g of the hydrogel was introduced to 10 mL of PBS (pH 7.4). To observe and monitor the release of quercetin, samples of 1 mL were taken at 15, 30, and 45 min, then in hours from 1 h and in half-hour time periods between 2 and 5 h. The samples were diluted with ethanol and spectrophotometric analysis was then conducted at 370 nm. All caps were covered with aluminum.

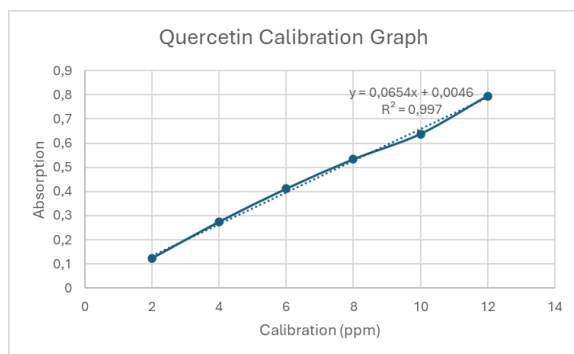


Figure 6. Quercetin calibration graph.

Table 2. Calculation of the concentration values of hydrogels containing 1% (w/v), 2% (w/v), and 3% (w/v) keratin.

Time	Absorption			Concentration (ppm)		
	1%	2%	3%	1%	2%	3%
15m	0,573	0,703	0,800	8,697	10,671	12,164
30m	0,504	0,723	0,928	7,636	10,990	14,124
45m	0,612	0,761	0,935	9,280	11,566	14,226
1h	0,573	0,711	0,930	8,693	10,798	14,154
2h	0,596	0,702	0,964	9,046	10,663	14,665
3h	0,621	0,747	0,886	9,427	11,344	13,472
4h	0,622	0,823	0,880	9,447	12,508	13,378
5h	0,595	0,604	0,759	9,034	9,157	11,534

The absorbance data demonstrated that there was a continuous release of quercetin from polymer matrix. However, fluctuations in concentration indicate quercetin's oxidative instability under physiological conditions. According to Table 2, release is time-dependent and positively correlated with keratin content ($1\% < 2\% < 3\%$), with the 3% (w/v) formulation yielding the highest release. While increasing keratin concentration enhanced the release rate, observed fluctuations highlight the drug's oxidative instability. The profile exhibited an initial burst release followed by a more controlled release phase. However, fluctuations in concentration indicate quercetin's oxidative instability under physiological conditions [10].

CONCLUSION

In this study, we successfully created a set of sodium alginate-keratin composite hydrogels that are bioactive and loaded with quercetin, specifically designed for advanced wound care. The results demonstrate that by varying the biopolymer ratios, the scaffolds' structural performance may be precisely adjusted. Interestingly, the formulation stabilized with 2% (w/v) keratin achieved an excellent swelling capacity of 293%, providing the perfect hydrophilic balance needed to control wound exudates while maintaining the proper moisture content at the injury site. As demonstrated by the masking of its distinctive spectral peaks, FTIR

analysis verified that quercetin was successfully and thoroughly entrenched inside the cross-linked networks of the 1K1A and 2K1A samples.

Importantly, the distinct presence of important phenolic groups in the spectra demonstrates that the polyphenol's strong antioxidant ability was unaffected by the encapsulation process. The unique protein-drug synergy seen when the keratin concentration was raised is one of the study's main highlights. Strong connections between the drug molecules and the protein chains in the 3K1A matrix effectively reduced the rate of diffusion. The gradual swelling and structural deterioration of the keratin network in this particular structure directly controls the therapeutic release profile, enabling a highly controlled and prolonged delivery phase. When combined, these findings show that these composite hydrogels offer a very flexible, biocompatible platform. The interaction between the proteins and the medications appears to be favored by an increase in keratin content, which effectively lowers the drug's diffusion rate. It is also this connection that makes the hydrogel more stable, which explains why the pharmaceuticals within have longer biological activity. Consequently, hydrogels with a high keratin concentration may be viable options for treating wounds.

Strong connections between the drug molecules and the protein chains in the 3K1A matrix effectively reduced the rate of diffusion.

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