

Optimization of a Carbopol 940–Agar Injectable Hydrogel for Sustained Delivery of Rebaudioside A: Physicochemical Characterization and Release profile studies”

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ABSTRACT

This study aimed to develop and optimize an injectable Carbopol 940–agar hydrogel system for sustained delivery of Rebaudioside A for cartilage-related applications. Eight formulations (F1–F8) were prepared by varying agar concentration from 0.25–2.0% w/v while maintaining constant Carbopol 940 (0.2% w/v) and Rebaudioside A (0.100 mg/mL) concentrations. The prepared hydrogels were evaluated for physicochemical characteristics, gelation behavior, structural integrity, morphology, and in vitro drug release. All formulations exhibited a smooth and homogeneous appearance with near-neutral pH values ranging from 6.72 ± 0.05 to 7.24 ± 0.06 . Gelation time decreased progressively from 168 ± 6 s to 61 ± 2 s, while gel fraction increased from $61.2 \pm 2.1\%$ to $96.1 \pm 1.0\%$ with increasing agar concentration, indicating enhanced network formation and structural integrity. In vitro drug release demonstrated an agar concentration-dependent reduction in Rebaudioside A diffusion. F1 and F2 showed relatively rapid release, whereas F7 and F8 exhibited slower release. Formulation F5 demonstrated a favorable balance between gelation behavior, matrix integrity, and controlled drug release, with $86 \pm 2.8\%$ cumulative drug release at 48 h. FESEM analysis of F5 revealed a porous and interconnected three-dimensional structure, while FTIR analysis indicated compatibility between Rebaudioside A and the Carbopol 940–agar matrix. Overall, F5 exhibited desirable physicochemical and sustained-release characteristics and may serve as a promising hydrogel platform for delivery of Rebaudioside A in cartilage-related regenerative applications. Further in vitro biological studies are required to establish its regenerative potential.

KEYWORDS

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1. INTRODUCTION

The development of effective localized drug delivery systems for cartilage-related applications remains a significant challenge due to the limited vascularization and restricted regenerative capacity of cartilage tissue. Successful regeneration requires not only bioactive stimulation but also a supportive matrix capable of maintaining hydration, ensuring structural stability, and enabling sustained release of therapeutic agents. Conventional delivery approaches often fail to achieve prolonged drug retention at the target site, resulting in reduced therapeutic efficiency.¹

Hydrogel-based systems have emerged as promising platforms for localized drug delivery due to their high water content, biocompatibility, and ability to mimic the extracellular matrix. Among these, composite hydrogels combining

synthetic and natural polymers offer improved performance by integrating mechanical strength, swelling behavior, and controlled degradation. Carbopol 940, a pH-responsive polymer, contributes to viscosity and gel formation, while agar acts as a structural reinforcement agent, enhancing gel strength and diffusional control.² Rebaudioside A, a naturally derived glycoside, possesses anti-inflammatory and regenerative potential, making it a suitable candidate for cartilage-related applications. However, its rapid diffusion and limited retention at the site of administration restrict its therapeutic effectiveness. Incorporation into a hydrogel matrix can overcome these limitations by providing sustained release and prolonged interaction with the target tissue.

The present study focuses on the systematic optimization of a Carbopol 940–agar hydrogel

system by varying agar concentration to evaluate its influence on physicochemical properties, gelation behavior, structural integrity, and drug release. The objective is to identify an optimized formulation that achieves a balance between injectability, stability, and sustained therapeutic delivery.

2. METHODOLOGY

2.1 Formulation Design

Eight hydrogel formulations (F1–F8) were developed to evaluate the effect of agar concentration on the properties of an injectable Carbopol 940/agar hydrogel for cartilage regeneration. Carbopol 940 (0.2% w/v) and Rebaudioside A (0.100 mg/mL) concentrations were kept constant, while agar content varied from 0.25–2.0% w/v. This approach enabled assessment of agar's influence on gel strength, injectability, swelling, degradation, water retention, adhesiveness, and sustained drug release without interference from other formulation variables.

Table 1. Composition of Rebaudioside-loaded Carbopol 940/Agar hydrogels

Formulation	Carbopol 940 (% w/v)	Agar (% w/v)	Rebaudioside A (mg/ mL)
F1	0.2	0.25	0.100 mg/mL
F2	0.2	0.50	0.100 mg/mL
F3	0.2	0.75	0.100 mg/mL
F4	0.2	1.0	0.100 mg/mL
F5	0.2	1.25	0.100 mg/mL
F6	0.2	1.50	0.100 mg/mL
F7	0.2	1.75	0.100mg/mL
F8	0.2	2.0	0.100 mg/mL

2.2 Preparation of Hydrogels

Hydrogels were prepared by a polymer dispersion–neutralization method. Agar was dissolved in distilled water at 85–90°C and maintained at 55–60°C. Carbopol 940 was separately dispersed and hydrated for 4–6 h, followed by incorporation of Rebaudioside A. The agar solution was blended with the Carbopol-drug dispersion, and pH was adjusted using triethanolamine to induce gelation. The resulting hydrogel was equilibrated, and stored in sterile containers for further evaluation.

2.3 Visual Appearance and Homogeneity

The prepared hydrogels were evaluated visually under ambient daylight conditions to determine their color, clarity, texture, and the presence of lumps or phase separation. Approximately 0.5 g of each formulation was placed on a clean glass slide and inspected for uniformity and the absence of particulate matter. Formulations exhibiting a smooth, consistent appearance without visible aggregates or localized polymer accumulation were considered homogeneous.

2.4 pH Determination

The pH of each hydrogel formulation was determined at room temperature using a calibrated digital pH meter. Approximately 1 g of the hydrogel was dispersed in 10 mL of distilled water and allowed to equilibrate prior to analysis. The pH electrode was then immersed in the sample dispersion, and measurements were recorded in triplicate to ensure accuracy. Formulations exhibiting a pH close to physiological neutrality were considered appropriate for local injectable use in cartilage-related applications.³

2.5 Gelation Time

Gelation time was determined by measuring the duration required for the formulation to convert from a free-flowing polymer dispersion into a stable, non-flowing gel following pH adjustment. Immediately after the addition of triethanolamine, a stopwatch was initiated, and gel formation was monitored using the vial inversion technique. The time at which the formulation ceased to exhibit visible movement upon inversion of the vial was recorded as the gelation time.⁴

2.6 Sol–Gel Fraction Analysis

A predetermined amount of hydrogel was initially dried to a constant weight and recorded as W_0 . The dried sample was subsequently immersed in distilled water for 24 h to remove the soluble components. The remaining insoluble portion was collected, dried until a constant mass was achieved, and designated as W_1 . The gel

fraction and sol fraction were then calculated to evaluate the extent of crosslinked network formation and the structural stability of the hydrogel matrix.^{5,6}

Gel fraction (%) = $(W1 / W0) \times 100$

Sol fraction (%) = $100 - \text{Gel fraction}$

2.7 In Vitro Drug Release

The in vitro drug release profile was determined using the dialysis bag diffusion technique. A specified amount of hydrogels were loaded into a pre-hydrated dialysis membrane, securely sealed, and immersed in phosphate-buffered saline (PBS, pH 7.4) maintained at $37 \pm 0.5^\circ\text{C}$ with constant stirring. At predetermined time intervals, sample aliquots were withdrawn and replaced with an equal volume of fresh release medium to preserve sink conditions. The collected samples were analyzed using UV–Visible spectrophotometry, and the cumulative percentage of drug released was subsequently calculated.^{7,8}

Cumulative drug release (%) = $(\text{Amount released} / \text{Total drug content}) \times 100$

2.8 Field Emission Scanning Electron Microscopy (FESEM)

Field Emission Scanning Electron Microscopy (FESEM) was used to examine the surface morphology and porous microstructure of the optimized Carbopol 940/agar hydrogel and Rebaudioside A -loaded Carbopol 940/agar hydrogel (F5). Lyophilized samples were fractured, mounted on aluminum stubs, sputter-coated with gold or platinum, and imaged under appropriate operating conditions at various magnifications. Surface characteristics, pore architecture, interconnectivity, and structural compactness were evaluated and compared with the blank hydrogel to assess the impact of drug incorporation on matrix morphology.^{9,10}

3. RESULTS AND DISCUSSION

All eight hydrogel formulations were successfully developed as smooth and injectable systems, with no observable drug precipitation or phase separation. Distinct formulation-dependent variations were evident as the agar concentration increased from 0.25 % to 2% w/v while maintaining a constant Carbopol 940 concentration. Higher agar levels resulted in more compact and cohesive hydrogels, enhancing structural stability, water retention, and resistance to degradation, while providing sustained drug release. However, these benefits were associated with decreased spreadability and reduced syringe extrudability. These findings highlight the inherent balance between injectability and matrix stability, a critical

consideration in the design of injectable hydrogels for regenerative medicine applications. (Table 2)

Table 2. Summary of physicochemical characterization studies

Formulation	pH ($\pm$ SD)	Gelation Time (s $\pm$ SD)	Gel Fraction (%) $\pm$ SD)	Sol Fraction (%) $\pm$ SD)
F1	6.72 $\pm$ 0.05	168 $\pm$ 6	61.2 $\pm$ 2.1	38.8 $\pm$ 2.1
F2	6.81 $\pm$ 0.05	142 $\pm$ 5	67.8 $\pm$ 2.0	32.2 $\pm$ 2.0
F3	6.90 $\pm$ 0.06	121 $\pm$ 4	74.5 $\pm$ 1.9	25.5 $\pm$ 1.9
F4	7.02 $\pm$ 0.05	102 $\pm$ 4	81.6 $\pm$ 1.7	18.4 $\pm$ 1.7
F5	7.08 $\pm$ 0.05	88 $\pm$ 3	87.9 $\pm$ 1.5	12.1 $\pm$ 1.5
F6	7.13 $\pm$ 0.06	76 $\pm$ 3	91.8 $\pm$ 1.3	8.2 $\pm$ 1.3
F7	7.19 $\pm$ 0.06	69 $\pm$ 2	94.6 $\pm$ 1.2	5.4 $\pm$ 1.2
F8	7.24 $\pm$ 0.06	61 $\pm$ 2	96.1 $\pm$ 1.0	3.9 $\pm$ 1.0

3.1 Visual Appearance and Homogeneity

Visual examination confirmed that all formulations were physically stable and showed no signs of visible incompatibility or phase separation. Formulations F1 and F2 exhibited a softer, less viscous appearance, while F7 and F8 appeared more compact, opaque, and structurally rigid. Among the formulations, F3–F6 demonstrated the most desirable pharmaceutical characteristics, exhibiting a smooth texture, uniform consistency, and adequate cohesiveness upon spreading. A gradual increase in agar concentration resulted in greater gel compactness and structural organization, suggesting enhanced reinforcement of the Carbopol-based hydrogel network.

3.2 pH Determination

The pH of formulations F1–F8 ranged between 6.72 and 7.24, falling within the acceptable physiological range for localized administration. A slight increase in pH was observed with increasing agar concentration, possibly due to changes in polymer hydration and ionic distribution following neutralization. Nevertheless, all formulations maintained near-neutral pH values, indicating effective pH adjustment and suggesting a low likelihood of pH-related tissue irritation upon administration.

3.3 Gelation Time

Gelation time showed a progressive decline from 168 s in F1 to 61 s in F8, indicating that increasing agar concentration promoted faster formation of a stable gel network following neutralization and cooling. Prolonged gelation may compromise handling and formulation stability, whereas excessively rapid gelation can hinder processing, filling, and administration. Among the formulations, F4–F6 exhibited an optimal gelation profile, offering a suitable balance between ease of handling and efficient gel formation for practical applications.

3.4 Sol–Gel Fraction Analysis

The gel fraction increased steadily from 61.2% in F1 to 96.1% in F8, demonstrating enhanced network formation and structural integrity with increasing agar concentration. Correspondingly, the sol fraction decreased, indicating greater incorporation of polymer chains into the insoluble gel matrix. These findings reflect the reinforcing effect of agar within the hydrogel system. Formulations F5–F7 showed notably high gel fractions, suggesting efficient matrix development while avoiding the excessive stiffness associated with the highest agar concentration.

3.5 In Vitro Drug Release

The drug release profiles demonstrated that increasing agar concentration progressively reduced the diffusion of Rebaudioside A from the hydrogel matrix. Formulations F1 and F2 exhibited a rapid release pattern with a pronounced initial burst, making them less suitable for sustained local delivery. In contrast, F7 and F8 showed excessively slow release, which could limit therapeutic availability within the desired treatment period. Among all formulations, F5 displayed the most favorable release behavior, characterized by a controlled initial release followed by sustained drug delivery over 48 h. Such a release profile is particularly advantageous for cartilage regeneration applications, where prolonged local exposure to the bioactive agent is preferred to maintain therapeutic activity and support continuous tissue repair (Table 3 & Figure 1).

Table 3. Cumulative drug release (%) of formulations F1–F8

Time (h)	F1	F2	F3	F4	F5	F6	F7	F8
1	22 ± 1.5	18 ± 1.3	15 ± 1.1	12 ± 0.9	10 ± 0.8	8 ± 0.7	6 ± 0.6	5 ± 0.5
2	31 ± 1.8	27 ± 1.6	22 ± 1.4	18 ± 1.2	15 ± 1.1	12 ± 0.9	10 ± 0.8	8 ± 0.7
4	44 ± 2.1	39 ± 1.9	33 ± 1.7	27 ± 1.5	23 ± 1.3	19 ± 1.2	16 ± 1.1	13 ± 1.0
8	59 ± 2.6	54 ± 2.4	47 ± 2.2	40 ± 1.9	35 ± 1.7	29 ± 1.5	24 ± 1.3	20 ± 1.2
12	71 ± 2.9	66 ± 2.7	59 ± 2.5	52 ± 2.2	46 ± 2.0	39 ± 1.8	33 ± 1.6	28 ± 1.5
24	86 ± 3.2	81 ± 3.0	74 ± 2.7	68 ± 2.5	61 ± 2.3	54 ± 2.1	48 ± 1.9	41 ± 1.8
36	94 ± 3.5	91 ± 3.3	86 ± 3.0	81 ± 2.8	75 ± 2.5	68 ± 2.3	62 ± 2.1	55 ± 1.9
48	98 ± 3.8	96 ± 3.6	93 ± 3.3	90 ± 3.0	86 ± 2.8	79 ± 2.6	73 ± 2.3	67 ± 2.1

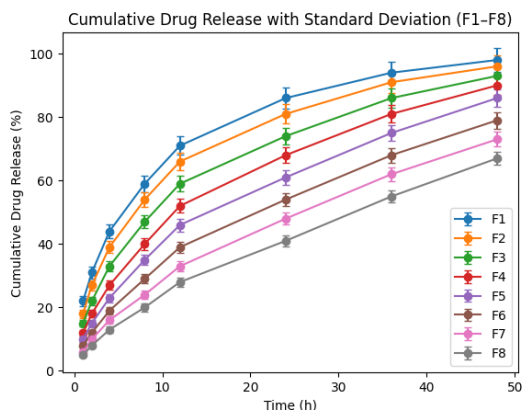


Figure 1. Cumulative Drug Release of developed hydrogel scaffolds

3.6 Field Emission Scanning Electron Microscopy (FESEM)

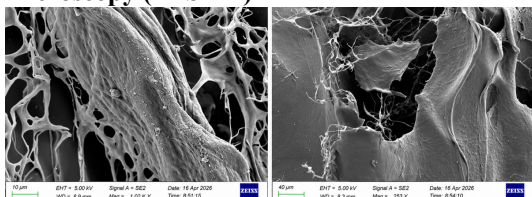


Figure 2. FESEM of Carbopol/Agar Hydrogel

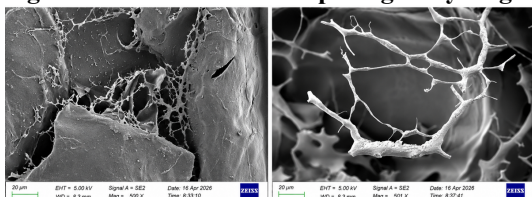


Figure 3. FESEM of Rebaudioside loaded Carbopol/Agar Hydrogel

FESEM analysis of the optimized Rebaudioside-loaded hydrogel (F5) demonstrated a porous, interconnected, sponge-like three-dimensional architecture, characteristic of hydrogels designed for regenerative applications. The micrographs revealed a smooth and continuous surface with uniformly distributed pores and internal channels, providing an optimal balance between permeability and structural integrity. Such a morphology is advantageous for facilitating water absorption, nutrient and oxygen transport, and metabolic waste exchange, while also supporting controlled drug diffusion. Compared with the blank hydrogel, the drug-loaded formulation exhibited a slightly denser and more compact network, indicating that incorporation of Rebaudioside A enhanced polymer matrix organization. Furthermore, the absence of visible drug crystals or particulate aggregates confirmed uniform drug distribution within the hydrogel and the lack of significant phase separation. These morphological observations were

consistent with the favorable swelling, degradation, and sustained-release characteristics exhibited by the optimized formulation. (Figure 2 & Figure 3.)

3.7 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of the Carbopol–Agar hydrogel shows distinct absorption peaks confirming the presence of functional groups from both polymers and their interactions.

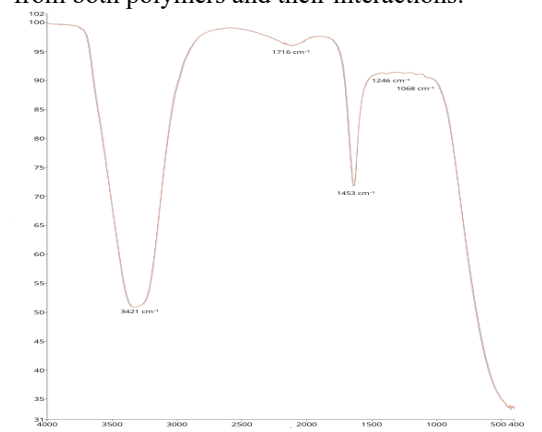


Figure 4. FTIR Spectra of Carbopol/Agar Hydrogel

The FTIR spectrum of the Carbopol–agar hydrogel exhibited characteristic absorption bands corresponding to both polymers and their intermolecular interactions. A broad peak at 3421 cm^{-1} was attributed to O–H stretching vibrations, indicating hydrogen bonding between agar hydroxyl groups and Carbopol carboxylic groups, thereby confirming hydrogel network formation. The peak at 1716 cm^{-1} represented C=O stretching of Carbopol, while the absence of significant peak shifts suggested the predominance of physical rather than chemical crosslinking. Bands at 1453, 1246, and 1068 cm^{-1} corresponded to COO^- stretching, C–O stretching, and C–O–C vibrations of agar glycosidic linkages, respectively, confirming polymer integrity.

In the Rebaudioside A-loaded hydrogel, a broadened O–H stretching band at 3418 cm^{-1} indicated enhanced hydrogen bonding involving the drug. Minor shifts in the C=O peak (1712 cm^{-1}) and related bands suggested physical interactions between Rebaudioside A and the polymer matrix. Importantly, the retention of characteristic drug peaks without the appearance of new bands confirmed successful drug incorporation and chemical compatibility within the hydrogel system.

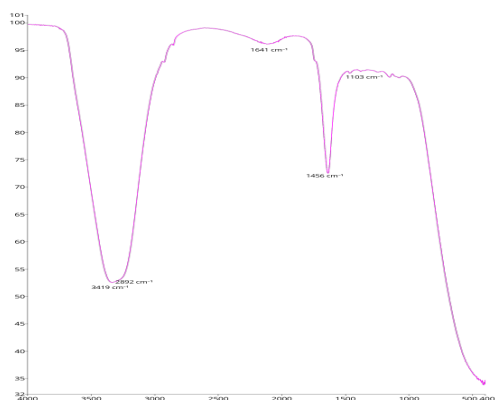


Figure 5. FTIR Spectra of Rebaudioside A loaded Carbopol/Agar Hydrogel

4. CONCLUSION

The findings of this study highlight the significant influence of agar concentration on the physicochemical properties and drug release performance of Carbopol 940-based hydrogels. Agar Concentration-dependent changes were observed in gelation time, gel fraction, and release kinetics, demonstrating the role of agar as a critical determinant of hydrogel structure and functionality. One-way ANOVA ($p < 0.05$) confirmed statistically significant differences among the formulations. Among the developed systems, formulation F5 provided the most favorable balance of injectability, matrix stability, and sustained drug release, exhibiting an optimal gelation profile, high gel fraction, and controlled release behavior. Formulations with lower agar content lacked adequate structural strength, whereas those with higher concentrations became excessively rigid and showed diminished release efficiency. Further FESEM, FTIR confirmed the hydrogel's structural integrity, chemical compatibility. Collectively, these results suggest that the optimized hydrogel is a promising candidate for localized sustained drug delivery and regenerative applications, warranting further preclinical and translational investigation.

5. CONFLICT OF INTEREST: None

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