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Development of Ionically Crosslinked Microspheres Containing Acyclovir Using Natural Polymers for Sustained Release

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Abstract: Acyclovir is a widely used antiviral agent with limited oral bioavailability, requiring frequent dosing that may lead to increased pharmaceutical burden and patient non-compliance. Developing an environmentally conscious, sustained-release delivery system using natural, biodegradable polymers can mitigate these issues while aligning with green pharmaceutical practices. This study aimed to formulate and evaluate ionically cross linked microspheres using biodegradable natural polymers, sodium alginate and gellan gum and environmentally benign ionic crosslinkers ($CaCl_2$ and $AlCl_3$) to achieve sustained release of acyclovir. Microspheres were prepared by ionotropic gelation using varying polymer and crosslinker concentrations. Six formulations (AC-M1 to AC-M6) were evaluated for particle size, yield, entrapment efficiency, swelling index, surface morphology (SEM), and compatibility (FTIR and DSC). *In vitro* drug release was assessed over 12 h and fitted to release kinetic models (Zero-order, First-order, Higuchi, Korsmeyer–Peppas). Microspheres displayed smooth, spherical morphology with sizes ranging from 540–710 µm. Entrapment efficiencies reached up to 82.1% (AC-M5), with swelling indices between 117.9% and 156.3%. FTIR and DSC confirmed chemical stability. AC-M5 showed sustained drug release over 12 hours and followed First-order kinetics ($R^2 > 0.998$) and Korsmeyer–Peppas diffusion behavior (n = 0.612–0.753).

The use of natural, biodegradable polymers in microsphere development offers a green and sustainable approach to pharmaceutical delivery, reducing dosing frequency and potential pharmaceutical waste. This system demonstrates promising potential for eco-conscious, sustained antiviral therapy.

Keywords: Acyclovir, Ionotropic Gelation, Microspheres, Sodium alginate, Gellan gum, Sustained release

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Introduction

Acyclovir is a proven antiviral that is commonly prescribed to treat herpes simplex virus (HSV) type 1 and 2 infections and herpes varicella-zoster virus (VZV)(Taylor and Gerriets, 2023). Its antiviral effect is caused by the blockage of the viral DNA polymerase, which consequently prevents the viral replication in host cells (Sarkar *et al.*, 2025). Although the clinical utility of acyclovir has been proven, this agent has drug pharmacokinetic disadvantages such as poor oral bioavailability (about 1530 per cent), low biological half-life (about 2.53 h), and frequent pharmacokinetic regimens, restrictions to clinical use (Gnann *et al.*, 1983). These restrictions highlight the need to establish alternative drug delivery systems that may lead to constant plasma concentration, low dosing rate, and improved patient compliance (Adepu and Ramakrishna, 2021).

Over recent years, Microsphere-based electronic delivery system has attracted a lot of attention due to its promise of control and sustained drug release. Microspheres are tiny spherical objects that have a diameter of between 1 and 1000 µm (Gupta *et al.*, 2017). They can entrap therapeutic agents and deliver them slowly in a long duration. These systems may be used to facilitate maintenance of therapeutic drug levels, reduction of variability in plasma levels, preventing side effects and enhance compliance of patients to treatment programs (Vora *et al.*, 2023).

Natural polymers such as sodium alginate and gellan gum have mostly been used in microsphere formulation because it is very biocompatible, biodegradable, non-toxic, and can form a gel (Kruk and Winnicka, 2022). Sodium alginate is a natural polysaccharide, which is a brown sea weed polysaccharide, which forms gels easily when in contact with the divalent cations like calcium. An extracellular polysaccharide called gellan gum produced by Sphingomonas elodea has outstanding gelation and film-forming functionality. These polymers may be combined to improve the mechanical stability of the microspheres and be able to control drug release profiles(Gomes *et al.*, 2023).

Calcium chloride ($CaCl_2$) and aluminum chloride ($AlCl_3$) are cross-linking agents, and important in the formation of microsphere. They facilitate cationic interactions with the functional groups of the polymers to give stable gel networks (Xie *et al.*, 2024). These cross-linkers vary in type and concentration and this has a direct impact on such important variables as encapsulation efficiency, drug release kinetics as well as stability of the microspheres in general (Herdiana *et al.*, 2022).

The aim of the present study was to develop and test acyclovir loaded microspheres with sodium alginate and gellan gum as polymeric carrier with calcium chloride and aluminum chloride as cross-linking reagents (Das *et al.*, 2024). It aimed at creating a system of biopolymer-based microsphere able to deliver sustained drug delivery and enhanced therapeutic outcomes. The aim of the research will be to determine optimal concentrations of polymer and cross-linker, and then, complete the physic-chemical and *in vitro* characterization of the formulations prepared to determine their synthesis as a promising controlled-release delivery system of acyclovir (Arshad *et al.*, 2023).

Materials and Methods

Acyclovir was kindly provided as a gift sample by a reputed pharmaceutical company. Sodium alginate was procured from HiMedia Laboratories Pvt. Ltd., India, while gellan gum was obtained from Sigma-Aldrich, USA. Calcium chloride ($CaCl_2$) of analytical grade, used as one of the cross-linking agents, was

purchased from Merck. Aluminum chloride ($AlCl_3$), another cross-linker used in the study, was obtained from Loba Chemie Pvt. Ltd., India. Distilled water was used throughout the formulation and evaluation procedures. All other chemicals and reagents employed were of analytical grade and used as received without further purification(Desu *et al.* 2023).

Preparation of Acyclovir Microspheres by Ionotropic Gelation Method

Acyclovir-containing microspheres were synthesized through the ionotropic gelation method,which is a common technique to encapsulate drugs using natural, biodegradable polymers (Tao *et al.*, 2009). Sodium alginate and gellan gum were the polymeric carriers used in this work because these polymers have an anionic character and possess the capability of making gels when combined with multivalent cation. The cross-linking agents were calcium chloride ($CaCl_2$) and aluminumchloride ($AlCl_3$)(Gadziński *et al.*, 2022).

400 mg of sodium alginate was dissolved in 50 ml warm distilled water through constant stirring of 50 ℃and 300 rpm on a magnetic stirrer until a clear, homogenous solution was attained (Soo *et al.*,2017). In a like fashion 400 mg of gellan gum was also dissolved into another 50 ml of warm distilled water under the same conditions. The solutions of the two polymers were then mixed properly and stirred until the mixture became uniform (Smith *et al.*, 2007).

400 mg Acyclovir was dissolved in 20 ml of warm distilled water. The drug was of low solubility and, consequently, the fine dispersion was formed and then incorporated into the polymer mixture and stirred by constant mixing to form a homogeneous drug-polymer dispersion (Choi *et al.*, 2022). It was stirred during the process of filling the polymer to allow the drug to be dispersed well through the polymer.

The cross-linking solutions were made by dissolving the necessary amount of any of the concentrations of calcium chloride or aluminum chloride in 100 ml of distilled water (Feyissa *et al.*, 2023). A dropwise addition of the drug-polymer dispersion into 70 ml of the cross-linking solution was then performed using a pipette to allow ionic cross-linking to occur and develop microspheres. After taking about 30 min, the mixture was stirred, slowly and gently, and allowed to be fully gelated (Redaelli *et al.*, 2017).

The cross-linking agents that were not used to bind the microspheres to each other after the filtration step were removed by washing the microspheres with several distilled water washes. The licular microspheres were washed and dried at room temperature overnight on a clean petri dish in the process of further characterization (Aljohani *et al.*, 2025). All the formulations are mentioned in Table 1.

Evaluation of Microspheres

Percentage Yield (%):

The production yield represents the efficiency of the microsphere formulation process. It was determined by comparing the total dried weight of the prepared microspheres with the total initial weight of the drug and polymers used in the formulation (Nataren-Rodríguez *et al.*, 2025).

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of dried microspheres}}{\text{Total weight of drug and polymers used}} \times 100$$

A higher yield indicates minimal material loss during processing and suggests that the formulation conditions were well-optimized for effective microsphere formation (Farooq *et al.*, 2014).

Particle Size Analysis:

The particle size of microspheres is an important parameter that directly influences drug release behavior and overall bioavailability (Mok, 2024). The average particle size was determined by optical microscopy. Approximately 100 micro-spheres from each formulation batch were randomly selected and measured to ensure statistical reliability. A narrow size distribution

Table 1: Composition of Acyclovir Microsphere Formulations

Formulation Code	Sodium Alginate (% w/v)	Gellan Gum (% w/v)	Acyclovir (% w/v)	Calcium Chloride (% w/v)	Aluminium Chloride (% w/v)
AC-M1	1.0	0.5	0.5	1.0	–
AC-M2	1.5	0.5	0.5	1.0	1.0
AC-M3	2.0	0.5	0.5	–	1.0
AC-M4	1.5	1.0	0.5	1.0	–
AC-M5	2.0	1.0	0.5	1.0	1.0
AC-M6	1.0	1.0	0.5	–	1.0

The six formulations were designed to systematically investigate the effects of polymer concentration and cross-linker type on microsphere performance. Sodium alginate varied from 1.0% to 2.0% w/v and gellan gum from 0.5% to 1.0% w/v to adjust solution viscosity, which directly influences microsphere size and drug loading efficiency higher polymer concentrations enhance structural integrity and entrapment, as supported by studies reporting increases in encapsulation efficiency and particle size with elevated alginate levels. Cross-linking agents included Ca^{2+} and Al^{3+} ions, both individually and in combination.

and consistent particle size indicate uniform emulsification and efficient cross-linking during microsphere formation (Vladisavljević and Schubert, 2003).

Drug Entrapment Efficiency (*EE%*):

Entrapment efficiency reflects the percentage of drug successfully incorporated into the microspheres compared to the total amount initially added. For this determination, an accurately weighed quantity of crushed microspheres was dissolved in phosphate buffer (pH 7.4), filtered, and analyzed spectrophotometrically at 254 nm (Patel and Patel, 2014).

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount of drug encapsulated}}{\text{Theoretical drug content}} \times 100$$

A high entrapment efficiency signifies strong interaction between the drug and polymer matrix, influenced by the concentration of polymers and the type of cross-linking agent used.

Swelling Index (*%*):

The swelling behavior of microspheres determines their capacity to absorb fluid, which plays a crucial role in controlling the rate and mechanism of drug release. Dried microspheres were placed in phosphate buffer (pH 7.4) maintained at 37 °C, and their weights were recorded at regular time intervals (Martinac *et al.*,2005). The swelling index was calculated using the formula:

$$\text{Swelling Index (\%)} = \frac{W_t - W_0}{W_0} \times 100$$

Where, W_t is the swollen weight of microspheres at a given time and W_0 is the initial dry weight. A balanced swelling profile ensures sustained and controlled drug release.

Surface Morphology (*SEM Analysis*):

The surface characteristics of the microspheres were examined using Scanning Electron Microscopy (SEM). Samples were coated with a thin layer of gold under vacuum before analysis (Heu *et al.*, 2019). SEM images were captured at different magnifications to observe surface texture, shape, and particle integrity. Spherical particles with smooth and uniform surfaces indicated efficient cross-linking and well-controlled formulation conditions (Hejmady *et al.*, 2021).

Differential Scanning Calorimetry (*DSC*):

Differential Scanning Calorimetry was carried out

to study the thermal properties of the microspheres and to detect any possible drug–polymer interactions. Pure acyclovir, the individual polymers, and the formulated microspheres were analyzed for thermal transitions such as melting and crystallization. The disappearance or shift in the characteristic melting peak of acyclovir within the microsphere formulation suggested successful drug encapsulation and possible conversion of the drug into an amorphous state (Liu *et al.*, 2014).

Fourier Transform Infrared Spectroscopy (*FTIR*):

FTIR spectroscopy was performed to investigate potential chemical interactions between acyclovir and polymers. The spectra of pure drug, individual polymers, physical mixtures, and final microsphere formulations were compared. Any noticeable shifts in peak positions or changes in intensity indicated possible hydrogen bonding or ionic interactions, confirming compatibility and stable encapsulation within the polymer matrix (Lim *et al.*, 2007).

In Vitro Drug Release Study:

The *in vitro* release of acyclovir from the microspheres was evaluated using a USP dissolution apparatus containing phosphate buffer (pH 7.4) maintained at 37 ± 0.5 °C. Microspheres equivalent to a known amount of drug were placed in the dissolution medium, and samples were withdrawn at predetermined intervals (Kulkarni *et al.*, 2021). Each sample was filtered, analyzed spectrophotometrically, and replaced with an equal volume of fresh buffer to maintain sink conditions. The cumulative percentage of drug released was plotted against time to obtain the release profile (Pilicheva *et al.*, 2014).

Drug Release Kinetics:

To elucidate the mechanism of drug release, the *in vitro* release data were fitted into various kinetic models, including Zero-order, First-order, Higuchi, and Korsmeyer–Peppas models. The model showing the highest correlation coefficient (R^2) was considered the best fit for describing the release mechanism (Wójcik-Pastuszka *et al.*, 2019).

- Zero-order model ($Qt = Q_0 + k_0t$): Indicates a constant release rate independent of drug concentration (Borowy and Ashurst, 2022).
- First-order model ($\log Qt = \log Q_0 - (k_1/2.303)\ t$): Suggests a release rate proportional to the remaining drug concentration (Iftime *et al.*, 2020).
- Higuchi model (Qt = kHt^0.5): Describes diffusion-controlled drug release from the matrix (Petropoulos *et al.*,2012).
- Korsmeyer–Peppas model ($Qt/Q\infty = kKt^n$): Explains the release mechanism based on the release exponent (*n*), distinguishing between Fickian diffusion and anomalous transport (Petropoulos *et al.*, 2012).

Results and Discussion

Percentage Yield (*%*):

The percentage yield serves as an important measure of how efficiently the microsphere formulation process converts raw materials into the final product. It provides a direct indication of how effectively the initial quantities of drug and polymers were utilized during microsphere formation.

In this study, acyclovir-loaded microspheres were successfully prepared using the ionotropic gelation method a gentle, solvent-free technique particularly suitable for thermolabile drugs. Accurately weighed quantities of sodium alginate and gellan gum were dissolved in warm distilled water under continuous magnetic stirring until clear solutions were obtained. Acyclovir was then uniformly dispersed within the polymer blend to ensure homogenous distribution of the drug.

The resulting drug–polymer mixture was carefully added dropwise into an aqueous solution containing ionic cross-linking agents, specifically calcium chloride and/or aluminum chloride, under gentle agitation. The interaction between the negatively charged polymer chains and the

Table 2: Particle Size and Estimated Volume of Acyclovir-Loaded Microsphere Formulations

Formulation Code	Mean Diameter (µm)	Mean Radius (µm)	Estimated Volume (µm³)	Volume (×10⁶ µm³)
AC-M1	540	270	82,446,000	82.45
AC-M2	610	305	119,115,000	119.12
AC-M3	670	335	157,314,000	157.31
AC-M4	695	347.5	175,589,000	175.59
AC-M5	710	355	187,034,000	187.03
AC-M6	555	277.5	89,423,000	89.42

The analysis shows that a small increase in microsphere diameter leads to a disproportionately large increase in internal volume. For instance, AC-M5 (710 µm) has more than double the volume of AC-M1 (540 µm), despite only a 170 µm increase in diameter. This has significant implications for drug loading capacity and controlled drug release behavior, as larger microspheres offer greater internal space for encapsulating the active pharmaceutical ingredient (API) and can exhibit more prolonged release profiles due to reduced surface area-to-volume ratio.

multivalent cations initiated gelation, leading to the formation of discrete, spherical microspheres. The formed microspheres were allowed to harden for a predetermined time, then washed repeatedly with distilled water to remove any residual cross-linking agents. Finally, the washed microspheres were air-dried at room temperature for 24 h.

Once dried, the percentage yield for each batch was determined using the following formula:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of dried microspheres}}{\text{Total weight of drug and polymers used}} \times 100$$

The percentage yields of the prepared formulations ranged from 85.1 ± 1.6% to 92.2 ± 3.0%, depending on the polymer-to-cross-linker ratio. The highest yield was observed in formulations that utilized both calcium and aluminum ions, suggesting that dual cross-linking produced a more cohesive and mechanically stable polymer matrix. This enhanced structural integrity likely reduced material loss during washing and drying. Conversely, slightly lower yields in batches prepared with a single cross-linker may be attributed to weaker gel formation and partial breakdown during processing.

These observations align well with previous reports on alginate-based microsphere systems, which have demonstrated similar yield ranges. The consistently high yields obtained in this work emphasize the reproducibility, robustness, and practicality of the ionotropic gelation technique for developing sustained-release formulations of acyclovir.

Particle Size and Volume Analysis:

The particle size and volume of microspheres are critical parameters influencing drug encapsulation efficiency, release kinetics, and ultimately, bioavailability. In this study, six different formulations of acyclovir-loaded microspheres (coded AC-M1A to AC-M6F) were analyzed to determine their average particle diameter using optical microscopy (Table 2). For each batch, approximately 100 microspheres were randomly selected and measured using a calibrated eyepiece micrometer to ensure reliable statistical representation.

The average particle diameters ranged between 540 µm and 710 µm, demonstrating consistent spherical morphology across all batches. The variation in size could be attributed to differences in polymer concentration and the

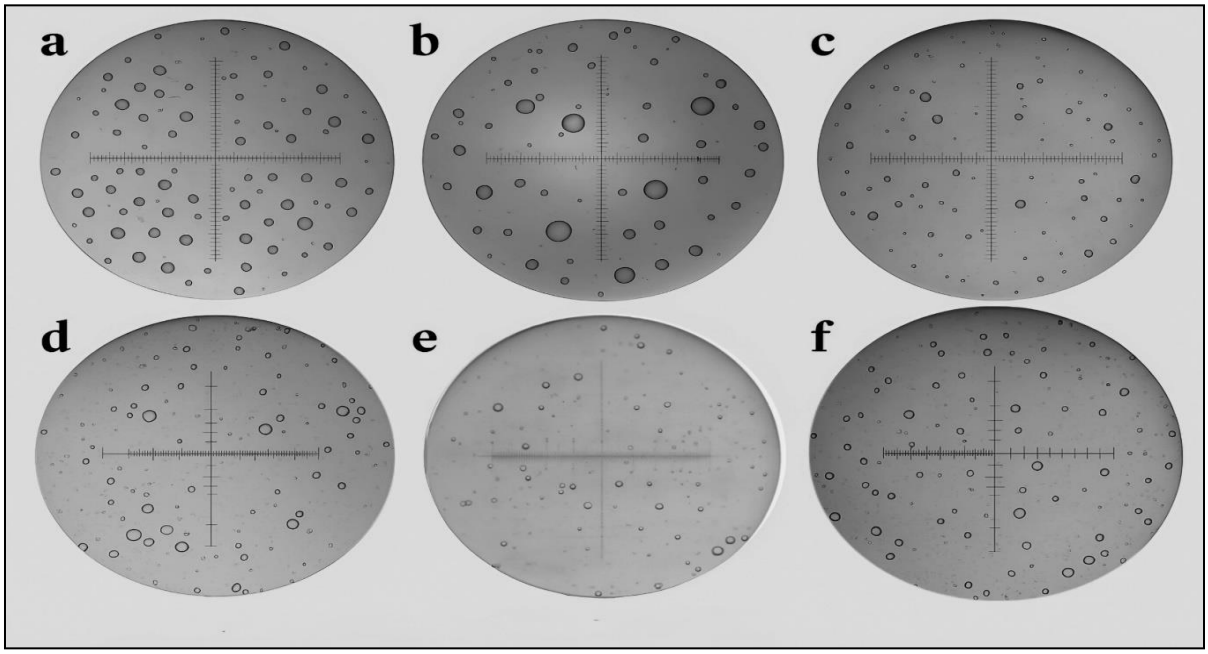

Fig. 1: Photomicrographs of acyclovir-loaded microspheres (AC-M1A to AC-M6F) observed under optical microscopy at 10× magnification. The images represent: (a) AC-M1A, (b) AC-M2, (c) AC-M3, (d) AC-M4, (e) AC-M5, and (f) AC-M6F. Each formulation was prepared using varying concentrations of sodium alginate, gellan gum, and cross-linking agents (calcium chloride and/or aluminium chloride). The images highlight differences in particle size distribution, morphology, and uniformity among the formulations.

type of cross-linking agent employed, as shown in Figure 1. Generally, higher polymer and cross-linker concentrations tended to produce larger microspheres, likely due to increased viscosity and stronger gel network formation during ionotropic cross-linking.

To estimate the internal volume and understand the structural potential for drug loading, the volume of the microspheres was calculated using the geometric formula for the volume of a sphere:

$$V = \frac{4}{3}\pi r^3$$

Where V represents the microsphere volume and r is the radius (in μm).

This theoretical model assumes ideal sphericity and helps illustrate that even a modest increase in diameter results in a significant increase in internal volume due to the cubic relationship between radius and volume. This relationship is particularly relevant for optimizing drug loading and release characteristics in sustained-release microsphere systems.

Drug Entrapment Efficiency (EE%):

A higher drug entrapment efficiency (EE%) (Table 3) generally indicates stronger drug–polymer interactions and better matrix formation. This efficiency largely depends on the type and concentration of polymer used, as well as the nature of the ionic cross-linkers.

The entrapment efficiency results for all six formulations (AC-M1 to AC-M6) are presented in Table 3. Among them, formulation AC-M1 showed the lowest EE% (65.2%). This lower efficiency could be due to its reduced polymer concentration and the use of only calcium ions as cross-linkers, which likely produced a weaker, more porous matrix. In contrast, formulation AC-M5 achieved the highest EE% (82.1%). The higher efficiency here can be explained by the increased polymer content and the combined use of calcium and aluminium ions, which together formed a denser

Table 3: Entrapment Efficiency of Acyclovir Microsphere Formulations

Formulation Code	Entrapment Efficiency (EE%) ± SD
AC-M1	65.2 ± 1.8%
AC-M2	72.6 ± 2.1%
AC-M3	68.4 ± 1.6%
AC-M4	75.9 ± 2.4%
AC-M5	82.1 ± 1.9%
AC-M6	70.3 ± 2.0%

Table 4: Swelling Index of Acyclovir Microsphere Formulations at 4 h

Formulation Code	Swelling Index (%) ± SD
AC-M1	124.5 ± 3.2
AC-M2	138.7 ± 2.8
AC-M3	117.9 ± 3.1
AC-M4	142.6 ± 3.0
AC-M5	156.3 ± 2.5
AC-M6	130.8 ± 2.9

and more stable polymer network.

Overall, formulations that incorporated both polymers and dual cross-linkers (AC-M2, AC-M4, and AC-M5) consistently exhibited better entrapment efficiency. This suggests that the synergy between different cross-linkers and the polymer blend plays an important role in improving drug encapsulation and matrix integrity.

Swelling Index (%):

As shown in Table 4, the swelling index of the microsphere formulations varied between 117.9% and 156.3% after 4 h of immersion in phosphate buffer (pH 7.4). Among all the formulations, AC-M5 exhibited the highest swelling index (156.3%). This can be attributed to its higher polymer concentration (2% sodium alginate and 1% gellan gum) along with the combined presence of calcium and aluminium ions. These factors likely promoted greater water absorption by creating a porous yet structurally stable polymer network.

On the other hand, formulation AC-M3 showed the lowest swelling index (117.9%). This reduced swelling behavior may be due to the absence of calcium chloride, which could have led to a lower cross-linking density and, consequently, a decreased ability of the microspheres to retain water.

Overall, the results suggest that both polymer concentration and the type of cross-linking ions play a crucial role in determining the swelling behavior of microspheres.

Moderate swelling values were observed in AC-M2, AC-M4, and AC-M6, suggesting that a combination of moderate polymer content and single or dual crosslinkers yields a balanced swelling behavior suitable for sustained release.

Surface Morphology (SEM Analysis):

The surface characteristics of the acyclovir-loaded microspheres were examined using scanning electron microscopy (SEM), as shown in Figure 2. At lower magnification (1000×), the microspheres appeared mostly spherical with smooth surfaces and uniform size distribution. Their diameters ranged from about 295 µm to 541 µm, suggesting that the emulsification process and ionic cross

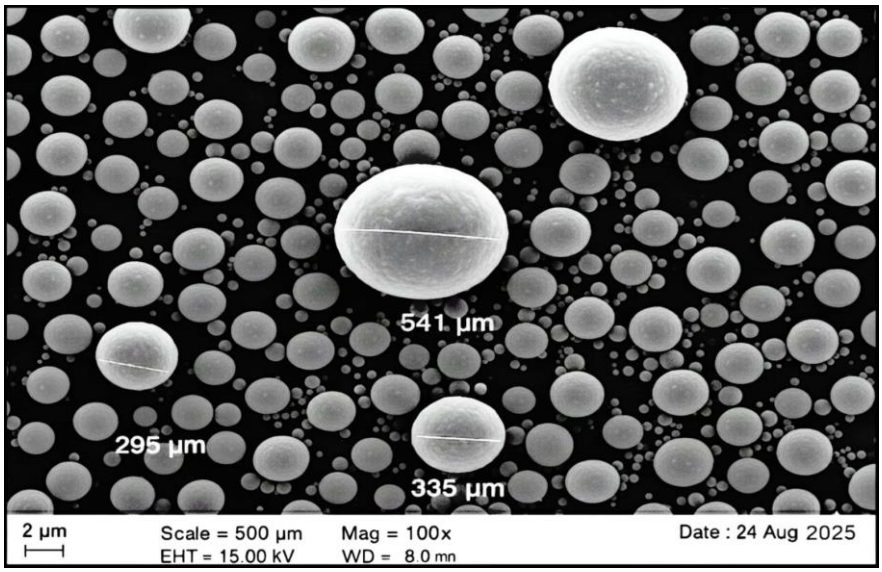

Fig. 2: SEM images of acyclovir-loaded microspheres at two different magnifications. The left image shows spherical particles with smooth surfaces and uniform distribution (1000×), while the right image (high magnification) reveals surface texture and possible polymer-drug interactions. Scale bars and size annotations indicate particle uniformity and surface characteristics.

linking were well-controlled during preparation. The overall spherical shape and compact structure indicate strong interactions between sodium alginate, gellan gum, and the crosslinking agents used.

At higher magnification, the images revealed additional surface details. Some microspheres showed slight roughness and minor irregularities, which could be due to a small amount of drug diffusing to the surface or uneven polymer solidification. A few particles also appeared slightly deformed or fused together, possibly as a result of compression or drying effects. These surface variations might increase the available surface area, influencing both the initial burst release and water uptake behavior of the microspheres.

Taken together, the SEM images confirm that the ionotropic gelation process produced microspheres with good structural integrity and suitable morphology for sustained-release drug delivery.

Fourier Transform Infrared Spectroscopy (*FTIR*):

The FTIR spectra of pure acyclovir and its ionically crosslinked microsphere formulation (prepared using natural polymers) clearly highlight the molecular interactions that play a key role in achieving sustained drug release. As shown in Figure 3 , the spectrum of pure acyclovir displays distinct and well-defined peaks most notably the N–H stretching vibration around 3450 cm^{-1}, the C=O stretching at 1684 cm^{-1}, and the C–N stretching near 1420 cm^{-1}. These characteristic peaks confirm the integrity of the functional groups present in the drug.

In contrast, the FTIR spectrum of microsphere formulation shows noticeable changes, including slight shifts and broadening of specific bands. The broadening of the N–H and O–H stretching bands around 3420–3400 cm^{-1} suggests the formation of hydrogen bonds between acyclovir and the hydroxyl groups of the natural polymers, such as chitosan and alginate. Similarly, the C=O band shows a minor shift toward 1690–1700 cm^{-1}, indicating molecular-level interactions and successful encapsulation of the drug within the polymer matrix. Additional peaks observed in the 3200–3400 cm^{-1} region correspond to polymeric –OH and –COO stretching vibrations, further

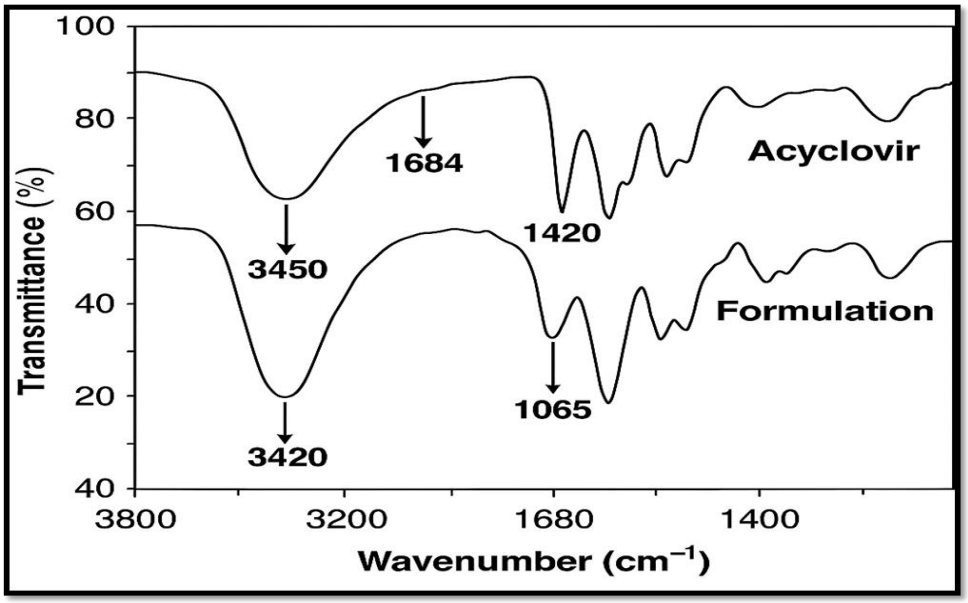

Fig. 3: FTIR spectra of pure Acyclovir and ironically cross-linked microspheres.

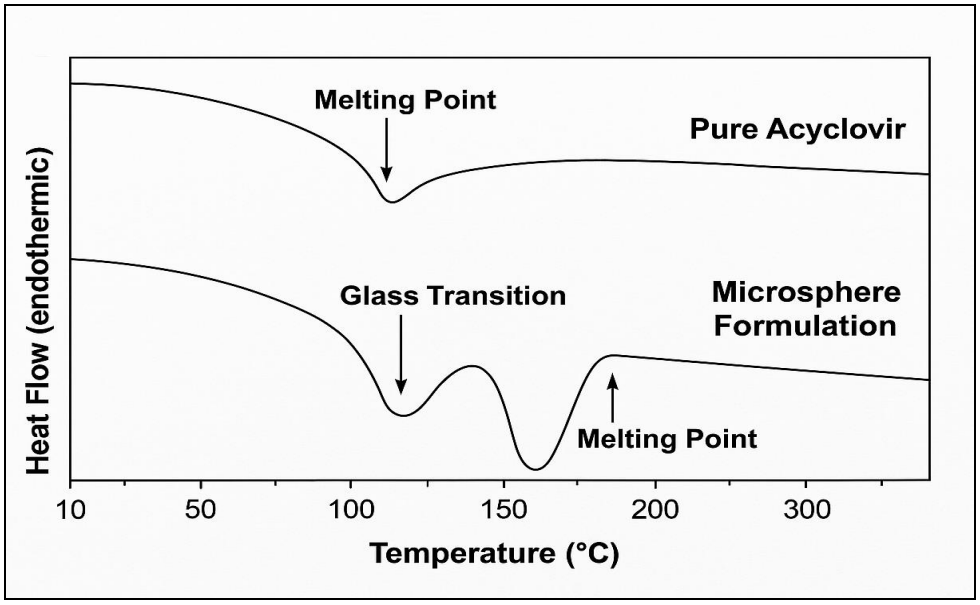

Fig. 4: DSC thermograms of pure acyclovir and the acyclovir-loaded microsphere formulation.

supporting the formation of a stable crosslinked structure.

Overall, these spectral changes confirm that acyclovir has been effectively incorporated into the polymer network without any chemical incompatibility. The FTIR results thus validate the stability, compatibility, and successful entrapment of the drug within the natural polymer system, an essential feature for sustained-release formulations.

Differential Scanning Calorimetry (*DSC*):

Differential Scanning Calorimetry (DSC) was performed to examine the thermal behavior of pure acyclovir and the acyclovir-loaded microsphere formulation, with the aim of understanding the drug's physical state, crystallinity, and compatibility with the polymers used. The obtained thermograms are presented in Figure 4.

Table 5: Time-Dependent In-Vitro Cumulative Drug Release (%) of Acyclovir-Loaded Microsphere Formulations

Time (h)	AC-M1	AC-M2	AC-M3	AC-M4	AC-M5	AC-M6
1	15.2	12.4	10.8	9.3	8.6	14.5
2	28.9	24.6	21.3	18.4	16.9	26.8
3	39.8	34.5	30.1	25.9	23.4	36.7
4	50.5	44.7	39.6	33.7	30.5	47.5
5	61.3	53.1	48.2	40.9	36.4	58.2
6	70.4	60.9	55.6	47.1	42.8	66.3
7	76.2	67.3	61.2	52.6	47.5	72.9
8	80.9	72.8	66.5	57.4	52.3	78.1
9	85.1	76.9	70.8	61.7	56.8	83.4
10	88.3	80.5	74.3	65.5	60.5	86.9
11	90.5	82.8	76.6	68.2	63.2	88.7
12	92.4	84.7	78.2	70.9	68.4	89.5

The DSC thermogram of pure acyclovir exhibited a sharp, well-defined endothermic peak at around 256 °C, which corresponds to its melting point and confirms its crystalline nature. This distinct thermal transition reflects the drug's purity and inherent thermal stability.

In contrast, the thermogram of the microsphere formulation revealed noticeable changes in the thermal pattern. A broad endothermic band was observed between 80 °C and 110 °C, representing the glass transition temperature (Tg) of the polymeric matrix composed of sodium alginate and gellan gum. This Tg indicates the amorphous character of the polymer system. Additionally, the sharp melting peak of acyclovir seen in the pure drug was considerably reduced in intensity and shifted to a lower temperature, appearing as a broad endothermic signal around 180 °C.

This shift and broadening of the melting peak suggest that acyclovir no longer exists in its original crystalline form but is molecularly dispersed or partially converted to an amorphous or semi-crystalline state within the polymeric network. The observed thermal behavior also points to possible intermolecular interactions such as hydrogen bonding or ionic complexation between the drug and the polymer chains, facilitated by multivalent ions (Ca^{2+} and Al^{3+}) during crosslinking.

Importantly, no additional peaks were detected in the thermogram of the formulation, confirming the absence of degradation or any incompatibility between the drug and excipients.

Overall, the DSC findings indicate that the ionic crosslinking process effectively encapsulated acyclovir within the polymeric matrix and induced a transition from crystalline to amorphous form. This transformation is advantageous, as it typically enhances solubility and supports a more controlled, sustained release of the drug from the microspheres.

In Vitro Drug Release Study:

The *in vitro* release behavior of acyclovir from the prepared microsphere formulations (AC-M1 to AC-M6) was investigated over a period of 12 h in phosphate buffer (pH 7.4) to simulate physiological conditions. The cumulative percentage release (CPR) values recorded at different time intervals are summarized in Table 5.

All six formulations exhibited a sustained and controlled release pattern, with the percentage of drug released gradually increasing with time.

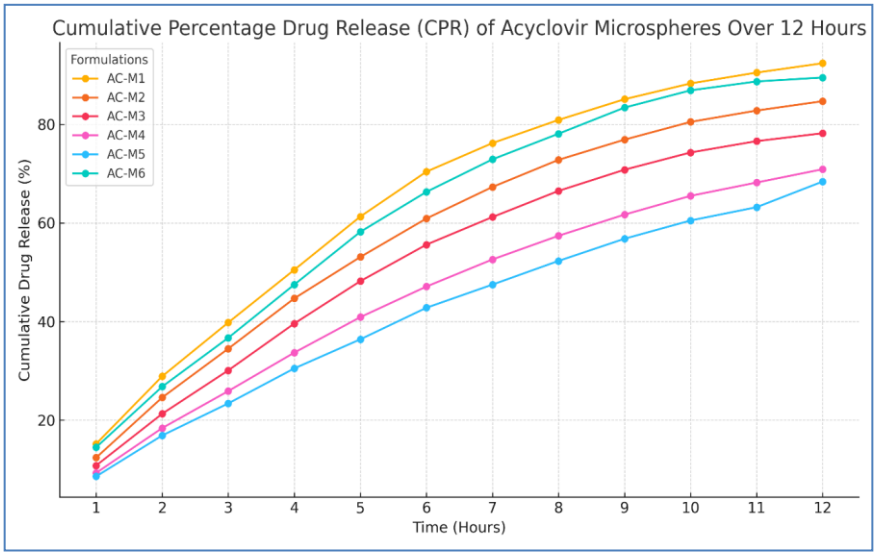

Fig. 5: Cumulative percentage drug release (CPR) profiles of acyclovir-loaded microsphere formulations.

After 12 h, the total drug release ranged from 68.4 % for formulation AC-M5 to 92.4 % for AC-M1. The variation in release rates among the formulations highlights the significant role of formulation parameters particularly polymer concentration, cross-linking density, and particle size in governing the diffusion of the drug through the polymeric matrix.

Formulations such as AC-M4 and AC-M5, which contained a higher proportion of cross-linking agents and relatively larger microspheres, displayed a slower release rate. This can be attributed to the formation of a denser, more compact gel network that restricts water penetration and drug diffusion. Conversely, formulations with lower cross-linking levels, such as AC-M1 and AC-M6, allowed faster drug diffusion through a more porous structure, resulting in higher CPR values.

Overall, the sustained release profiles observed confirm the effectiveness of the ionotropic gelation process in achieving controlled release behavior. The obtained CPR data, graphically represented in Figure 5, provide a strong basis for subsequent kinetic modeling to better understand the mechanisms involved in drug release whether governed predominantly by diffusion, matrix erosion, or a combination of both.

Drug Release Kinetics Study:

To understand the mechanism governing drug release from the prepared acyclovir-loaded microspheres, the *in vitro* release data were analyzed using different kinetic models, namely Zero-order, First-order, Higuchi, and Korsmeyer–Peppas equations. The best-fit model for each formulation was identified based on the correlation coefficient (R^2) obtained from linear regression analysis. The calculated kinetic parameters for all six formulations (AC-M1 to AC-M6) are presented in Table 6. Among the evaluated models, the First-order kinetic model showed the highest correlation for all formulations, with R^2 values consistently above 0.998. This strong linearity indicates that the drug release rate is concentration-dependent meaning that as the amount of drug remaining in the microsphere matrix decreases, the release rate gradually slows.

The Higuchi model also showed excellent correlation (R^2 > 0.99 for all formulations), confirming that diffusion is the predominant mechanism of drug release. Such diffusion-controlled release is typical for hydrophilic polymer matrices like sodium alginate and gellan gum, where the drug diffuses through a hydrated, porous structure.

Although the Zero-order model produced slightly lower R^2 values than the First order and

Table 6: Drug Release Kinetic Parameters of acyclovir-loaded microspheres

Formulation	Zero-order (R^2)	rst-order (R^2	Higuchi (R^2)	Korsmeyer–Peppas (R	(release exponent)
AC-M1	0.9629	0.9986	0.9910	0.9866	0.612
AC-M2	0.9721	0.9994	0.9950	0.9904	0.653
AC-M3	0.9748	0.9989	0.9954	0.9906	0.681
AC-M4	0.9847	0.9997	0.9985	0.9953	0.718
AC-M5	0.9914	0.9996	0.9989	0.9980	0.753
AC-M6	0.9685	0.9980	0.9926	0.9881	0.640

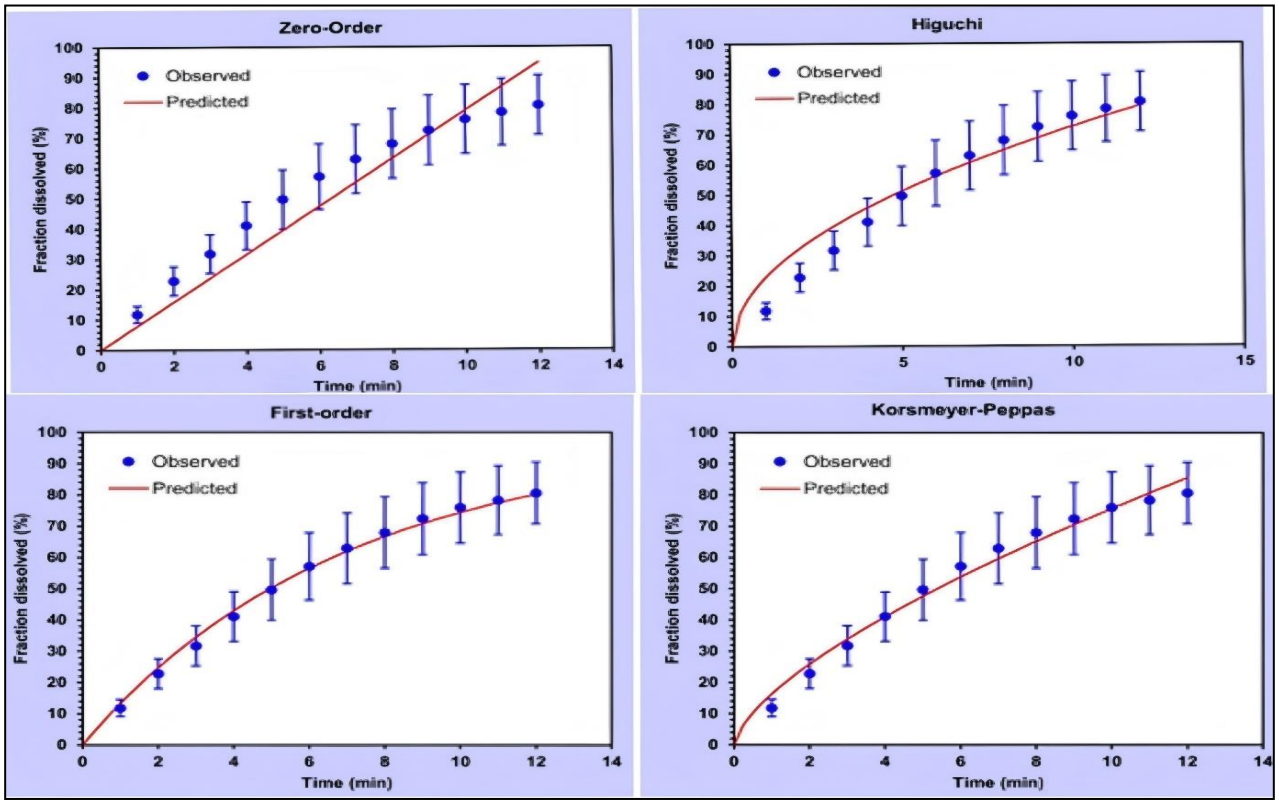

Fig. 6: Drug Release Profile Graphs.

Higuchi models, it still demonstrated good linearity, particularly for formulation AC-M5 (R^2 = 0.9914). This suggests that AC-M5 possesses the potential to deliver the drug at a relatively constant rate over an extended period. The average Zero-order release profile for all formulations is depicted in Figure 6, showing a near-linear and predictable release behavior an important feature for sustained-release systems.

Further interpretation using the Korsmeyer–Peppas model revealed release exponent (n) values between 0.61 and 0.75 for all formulations. These values indicate an anomalous or non-Fickian transport mechanism, where both diffusion of the drug and relaxation or swelling of the polymer matrix contribute to the overall release process.

In summary, the kinetic modeling results

demonstrate that drug release from the acyclovir microspheres follows a combined mechanism involving both diffusion and polymer relaxation. Among the tested batches, formulation AC-M5 exhibited the most favorable characteristics for sustained and controlled drug delivery.

The present investigation aimed to develop and evaluate ionically crosslinked microspheres of acyclovir using natural polymers to achieve a sustained drug release profile. Sodium alginate and gellan gum were specifically selected as matrix-forming agents due to their excellent biocompatibility, biodegradability, and ability to form gels upon interaction with multivalent cations such as calcium (Ca^{2+}) and aluminum (Al^{3+}). The ionotropic gelation method employed in this study provided mild preparation conditions, thereby minimizing the risk of drug degradation and maintaining acyclovir's physicochemical stability.

The results from the physical characterization studies highlighted the influence of polymer and crosslinker concentration on microsphere properties. An increase in polymer concentration and dual crosslinking resulted in larger particle sizes and improved sphericity, particularly observed in formulation AC-M5. The improved structural uniformity and surface integrity contributed to a reduction in the initial burst release and supported a more prolonged and controlled drug diffusion. The higher entrapment efficiency noted for dual-ion crosslinked formulations suggested that the combination of Ca^{2+} and Al^{3+} ions produced a denser and more compact polymeric network, which effectively restricted drug loss during the gelation process.

Thermal characterization through DSC confirmed partial amorphization of acyclovir within the polymeric system, signifying molecular-level dispersion in the matrix. FTIR analysis revealed no major alterations in the characteristic peaks of the drug, confirming the absence of any significant chemical interaction between the drug and the excipients. Swelling studies further emphasized the hydrophilic nature of the polymers, where gradual water uptake led to polymer relaxation and formation of a gel barrier that moderated the drug release rate over time.

The *in vitro* release studies provided a clear indication of sustained drug release behavior. Among all formulations, AC-M5 exhibited the most desirable performance, showing approximately 56.8% release within 9 h compared to the faster 85.1% release from AC-M1. The release kinetics data best fitted the First-order model ($R^2 > 0.998$), indicating that the drug release rate was concentration-dependent. A strong correlation was also observed with the Higuchi model ($R^2 > 0.99$), suggesting that diffusion played a dominant role in drug transport through the hydrated polymeric matrix. Moreover, the Korsmeyer–Peppas model showed release exponent (n) values between 0.61 and 0.75 for all formulations, confirming an anomalous (non-Fickian) diffusion mechanism. This implies that both drug diffusion and polymer relaxation contributed synergistically to the overall release process.

Taken together, the findings confirm that microspheres prepared using sodium alginate and gellan gum with dual ionic crosslinking form a stable and efficient delivery system capable of maintaining sustained release of acyclovir over an extended duration. Such a formulation approach is particularly advantageous for drugs like acyclovir, which possess a short half-life and low bioavailability, as it can help maintain therapeutic plasma concentrations for longer periods and improve patient adherence to therapy.

Conclusion

The developed ionically crosslinked microspheres demonstrated strong potential as a sustained release delivery system for acyclovir. The use of natural polymers and the ionotropic gelation technique enabled successful drug encapsulation under mild processing conditions, ensuring stability and reproducibility. All formulations exhibited satisfactory production yield, appropriate particle size distribution, and uniform morphology. DSC and FTIR analyses confirmed the

absence of drug–polymer incompatibility, ensuring formulation stability.

Among the various formulations, AC-M5 stood out as the most optimized system, exhibiting high entrapment efficiency, controlled swelling, and an extended drug release pattern lasting up to 12 h. The kinetic analysis revealed that the release followed First order and Higuchi models, while the Korsmeyer–Peppas exponent (n) values indicated a combined mechanism of diffusion and polymer relaxation.

Overall, this study demonstrates that naturally derived polymers such as sodium alginate and gellan gum can be effectively utilized to design biocompatible, eco-friendly, and efficient sustained release microspheres. The approach provides a promising alternative for enhancing the bioavailability of acyclovir and other drugs with similar pharmacokinetic limitations, potentially improving therapeutic outcomes and patient compliance by reducing dosing frequency.

Ethical Statement

No animal or microbes have been used or sacrificed for this study, hence Ethical approval not required.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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