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Research article

Formulation and Evaluation of Antiviral Agent Loaded Polymeric Nanoparticles

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ABSTRACT

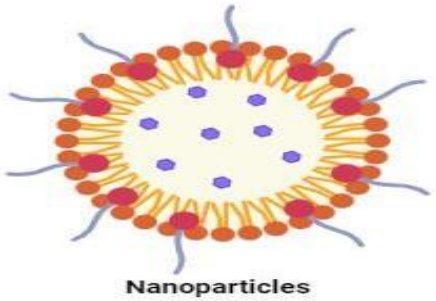
The present study focuses on the development of polymeric gelatin nano-particulates of acyclovir sodium with the goal of improving solubility and bioavailability through controlled release. Acyclovir sodium is an antiviral agent with a short half-life and oral bioavailability of 2.5–3.3 hours (15–30%), respectively, and is used to treat a variety of herpes infections. The purpose of this study was to prepare, evaluate, and in vitro release the characterization of acyclovir sodium-loaded polymeric nanoparticles. We fabricated the polymeric nanoparticles of Acyclovir sodium using varying concentrations of gelatin, a biodegradable polymer, acetone as a desolating agent, and glutaraldehyde as the cross-linking agent. Currently, biodegradable polymeric nanoparticles stand out significantly as they offer an enhanced release system due to their biodegradability, biocompatibility, low cost, and versatility in various formulations. We fabricated Acyclovir sodium-loaded polymeric gelatin nanoparticles using a two-step desolation technique. The Nano formulations showed that the entrapment efficiency ranges from 74% to 87%. The optimized formulation's SEM image revealed the nanoparticles' almost spherical surface. The DSC thermograph showed the molecular range of acyclovir dispersion in the polymeric nanoparticle. FTIR studies reported no acyclovir-gelatin interaction. Prepared nano-formulations (AGP1-AGP6) demonstrate a controlled initial burst release. The optimized formulation (AGP3) showed significant drug release in comparison to the marketed product.

Keywords: Biodegradable polymer, Acyclovir sodium, Nanoparticles.

INTRODUCTION

Today, herpes simplex virus infections are a major global health problem, affecting one-third of the world. People frequently use Acyclovir sodium, a powerful antiviral medication, to treat herpes simplex viral infections, chickenpox, and cytomegalovirus infections. The current treatment options for acyclovir sodium formulation include oral, topical, and parental administration. 4. Due to its high solubility and low permeability, acyclovir sodium falls under class 3 according to the Biopharmaceutical Classification System (BCS). Currently, numerous techniques exist to enhance the bioavailability of low-water-soluble drugs, such as the addition of ionized salts, the solid dispersion method, micronization techniques, and self-gel technology.

Figure 1: Nanoparticles



All of these methods have their own drawbacks, including drug loading capacity, biodegradability, dosing capacity, and environmental factors. In the past few years, nanotechnology has gained attention by limiting its drawbacks.

Nanoparticles are very influential as a delivery system for variant bioactive molecules. These colloidal nanoparticles encapsulate or absorb drugs and biological compounds. We prepare nanoparticles using various types of polymers, such as natural protein components, polysaccharides, or synthetic polymers. Biodegradable natural polymers gain more consideration in drug delivery applications. Since gelatin is a naturally biodegradable and biocompatible polymer, administering these polymeric nanoparticles to the human body does not cause any harmful reactions. Leaching, rapture, erosion, or degradation of the polymer may contribute to the drug-releasing character of gelatin nanoparticles. Nanoparticles offer novel physico-chemical properties for effective drug delivery, which makes them an ideal tool for viral treatment. Antiviral agents use various nano-materials such as nanoparticles, liposomes, nanogels, nanospheres, nanosuspensions, and nano emulsions for their drug delivery. We characterize nanoparticles to determine their shape, size, and specific area. Polymeric nanoparticles exhibit two distinct characteristics. The initial classification is the determination of the following properties of nanoparticles: shape, size, crystal structure, or monodispersed. The second classification entails the assessment of chemical properties, such as the existence of conjugated molecules and zeta potential. We determine the physical properties using techniques such as FESEM, UV spectroscopy, x-ray diffraction, and dynamic light scattering.This study looked at how evolving biodegradable polymeric nanoparticles of acyclovir sodium might help make the drug more bioavailable. The aim of this research is to create and assess acyclovir sodium-gelatin polymeric nanoparticles that exhibit superior bioavailability, comparable to that of pure drugs. [1-3]

MATERIAL AND METHODS

Micro Lab (Baddi, Himanchal Pradesh) generously provided Acyclovir sodium as a gift. We procured gelatin type a (180 bloom) from Sigma Aldric. We purchased a 25% v/v aqueous solution of glutaraldehyde from Molychem (Mumbai) and potassium di-hydrogen phosphate from S.D. Fine Chem. Ltd. (Mumbai, India). We purchased analytical-reagent grade acetone and sodium hydroxide from C.D.H. Pt. Ltd. The other chemicals and solvents used were analytical grade.

Designing and formulation of nanoparticles

Coester et al. advanced this method. We decomposed gelatin (type A) in 25 ml of pure distilled water at 500 °C in a water bath, and then added 25 ml of acetone at 250 °C to achieve gelatin desolvation. We removed the supernatant, which contained low-molecular-weight gelatin, from the solution. We dissolved the high molecular weight gelatin sediment in 25 ml of distilled water at 500 °C and combined it with an antiviral agent. We set the pH of the polymeric solution at 2.5 using 2 M HCL. We desolvated the drug-loaded polymeric nanoparticle system once more by adding 75 ml of acetone drop-wise while vigorously stirring. We mixed 25% v/v aqueous glutaraldehyde to crosslink the nanoparticles after 10 minutes and kept them at a low temperature (40 °C) for 12 hours. We stopped the phase cross-linking by adding 5 ml of 12% w/v aqueous sodium meta bisulphite. We acquired the prepared drug-loaded nanoparticles by centrifuging and suspending them twice in acetone. We dried and stored the final polymeric nanoparticle product at room temperature [4].

Evaluation of drug-loaded gelatin nanoparticles

Assessment of entrapment efficiency (%EE)

We calculated the entrapment efficiency (% EE) of acyclovir sodium-loaded polymeric nanoparticles with different formulations by centrifuging the prepared formulation at 10000 rpm for at least 30 min using a centrifuge (REMI, India). We assessed the amount of segregated acyclovir sodium from the supernatant liquid solution at 252 nm using a UV spectrophotometer. Then, this calculated amount is subtracted from the total amount of acyclovir sodium that is in the polymeric formulation. This gives us the amount of acyclovir that is trapped in the polymeric formulations.

$$\% \text{ Entrapment Efficiency (\%EE)} = \frac{\text{Total acyclovir added in nanoparticles- Free or non-entrapped acyclovir}}{\text{Total acyclovir added in nanoparticles}} \times 100$$

Fourier transform infrared spectroscopy analysis

FTIR spectrum of acyclovir sodium, gelatin, solid mixture of acyclovir sodium with gelatin, and prepared nanoparticles were recorded to check the compatibility or any sort of interaction by using FTIR spectrophotometer, Pekin Elmer spectrum. The % transmittance (%T) was sketched in spectral region of 4000-650cm^{-1}.

Scanning electron microscopy of prepared polymeric nano-formulation (SEM)

The morphological features of the acyclovir sodium loaded polymeric nanoparticles were analyzed by using SEM. The prepared formulation was fixed on aluminum stub, and slaked with gold for the testing [5].

Differential scanning calorimetry study of optimized formulation (DSC)

We obtained a DSC thermogram of the sample using SDTQ 600. We maintained the DSC of the sample at both the acyclovir sodium melting point and the polymer's glass transition temperature. We explored the thermal nature of the acyclovir in the polymeric nanoparticles by heating the formulation under an inert gas at a temperature range of 0-6000 °C, with a heating scanning rate of 100 °C/min [6-7].

In-vitro drug release of Acyclovir from the prepared nanoparticles

We dispersed the prepared polymeric nanoparticles, equivalent to 2 mg of acyclovir sodium nanoparticles, once more into 10 ml of phosphate buffer (pH 7.4). We then encased them in a dialysis bag, serving as a donor compartment, secured it, and placed it into a 10 ml buffer solution, serving as a receptor compartment. We assembled the beaker on a magnetic stirrer and equilibrated the medium at a sustained temperature of 37 oC with continuous stirring. At specific intervals, we removed 1 ml of aliquots and added 1 ml of fresh phosphate buffer (pH 7.4) solutions to the donor compartment. An extent of acyclovir sodium in the release medium was considered with the help of a UV-visible spectrophotometer at 252 nm. All calculations were performed in triplicate.We compared the drug release from the anti-viral agent-loaded gelatin nanoparticles (AGP3) with the acyclovir-containing marketed tablet (Zovirax). We analysed the drug release from the marketed tablet using a dissolution apparatus type II (paddle). We placed the tablet in a 1000-ml beaker, which held 900 ml of phosphate buffer (pH 7.4) at 370 °C. We withdraw 5 ml of the sample and checked it on a UV Spectrophotometer at 252 nm. We substituted the excluded sample volume with 5 ml of a fresh pH 7.4 buffer solution [8].

Study of acyclovir sodium release kinetics

To consider the release kinetics and acyclovir release behavior, the calculated data of in- vitro acyclovir release from Acyclovir sodium loaded polymeric nanoparticles was included to five firstly used mathematical models(r) (stated below) for the calculation of drug release kinetics of Acyclovir sodium from the prepared polymeric gelatin nanoparticulate system [9].

Zero order model	$D_t = D_0 + k_0 t$
First order model	$D_t = \ln D_0 + k_1 t$
Higuchi square root model	$D_t = k_H t^{1/2}$
Korsmeyer –Peppas model	$D_t / D_\infty = k_P t^n$

D_t is the amt of drug release at time t, D_0 is the initial amount of drug released, D_t / D_∞ is the fraction of drug released at time t, k_0 is the zero order reaction rate constant, k_1 is the First order release constant, k_H is the Higuchi release constant, k is the Peppas release constant, and n is the release exponent respectively [10].

RESULT AND DISCUSSION

Determination of Entrapment efficiency (%EE)

It was showed as the percentage of drug (acyclovir sodium) entrapped in these fabricated gelatin nanoparticles holding acyclovir sodium compared with the initially added amount of acyclovir sodium incorporated in the prepared formulations. The fabricated formulation showed the drug entrapment in the range of 74.8- 86.57% (Table.1).The fabricated formulation was optimized on the basis of % drug entrapped in the polymeric nanoparticles. Formulation (AGP_3) showed best drug entrapment which is 87.46±0.67.

Table-1: Formulation and % entrapment efficiency of the Acyclovir sodium loaded Gelatin nanoparticles

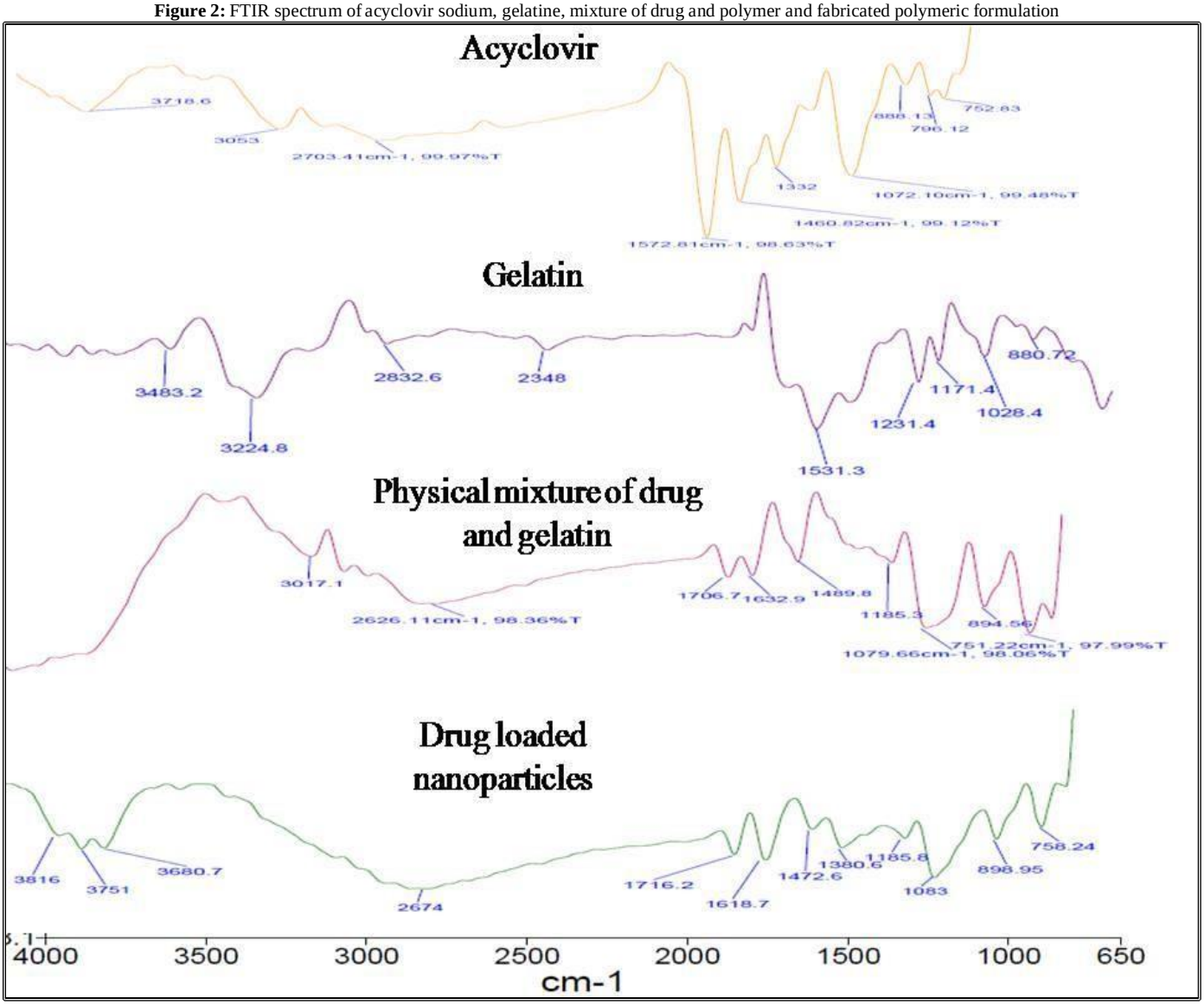
Formulation code	% (w/v) Gelatin	% (w/v) Drug	Glutaraldehyde(µg/ml)	% Entrapment efficiency
AGP_1	0.5	0.5	100	86.57±1.45
AGP_2	1.0	1.0	150	83.2±0.41
AGP_3	1.5	1.5	200	87.46±0.67
AGP_4	2.0	2.0	250	81.4±1.05
AGP_5	2.5	2.5	300	78.7±0.53
AGP_6	3	3	350	74.8±0.57

Fourier transform infrared spectroscopy

Figure 1 presents the FTIR spectra of acyclovir sodium, gelatin, a mixture of acyclovir sodium and gelatin type-A, and acyclovir sodium-loaded gelatin nanoparticles. When you look at acyclovir sodium, its main peaks are at 3053 cm1 for NH stretching, 1572 cm1 for N-H, C-C bending, 1460 cm1 for O-H bending, 1332 cm1 for C-N aromatic amines, and 888 cm1 for C-C stretching. Certain IR frequencies are unique to type a gelatin. These include 3483 cm1 for N-H stretching amide a, 3224 cm1 for N-H and O-H stretching, 2832 cm1 for O-H hydrogen bond, 1531 cm1 for N-H deformation, and 1171 cm1 for C-N stretch. Acyclovir sodium-loaded polymeric nanoparticles showed the gelatin absorption peaks the most, with some of the acyclovir sodium

peaks overlapping. The interaction of drugs with polymers leads to identifiable changes in the IR pattern. The FTIR spectra of the drug and polymer suggested no possible interaction of acyclovir sodium with gelatin, as there were no distinctive changes in the spectra

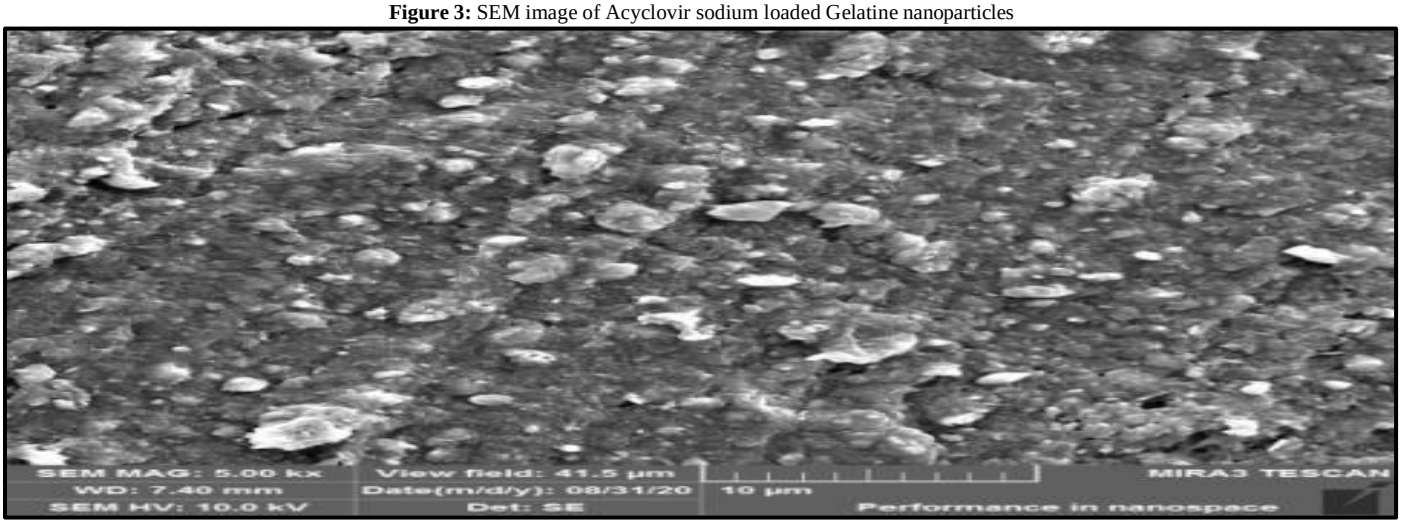
Figure 2: FTIR spectrum of acyclovir sodium, gelatine, mixture of drug and polymer and fabricated polymeric formulation



Scanning electron microscopy

Figure 2 displays the morphological characteristics of the fabricated acyclovir sodium-loaded gelatin nanoparticles. Acyclovir-sodium-loaded gelatin nanoparticles showed a smooth, spherical surface. The study's findings are based on the ratio of polymers and cross-linking agents used in the formulation. The prepared polymeric nanoparticles were mostly nanoscale.

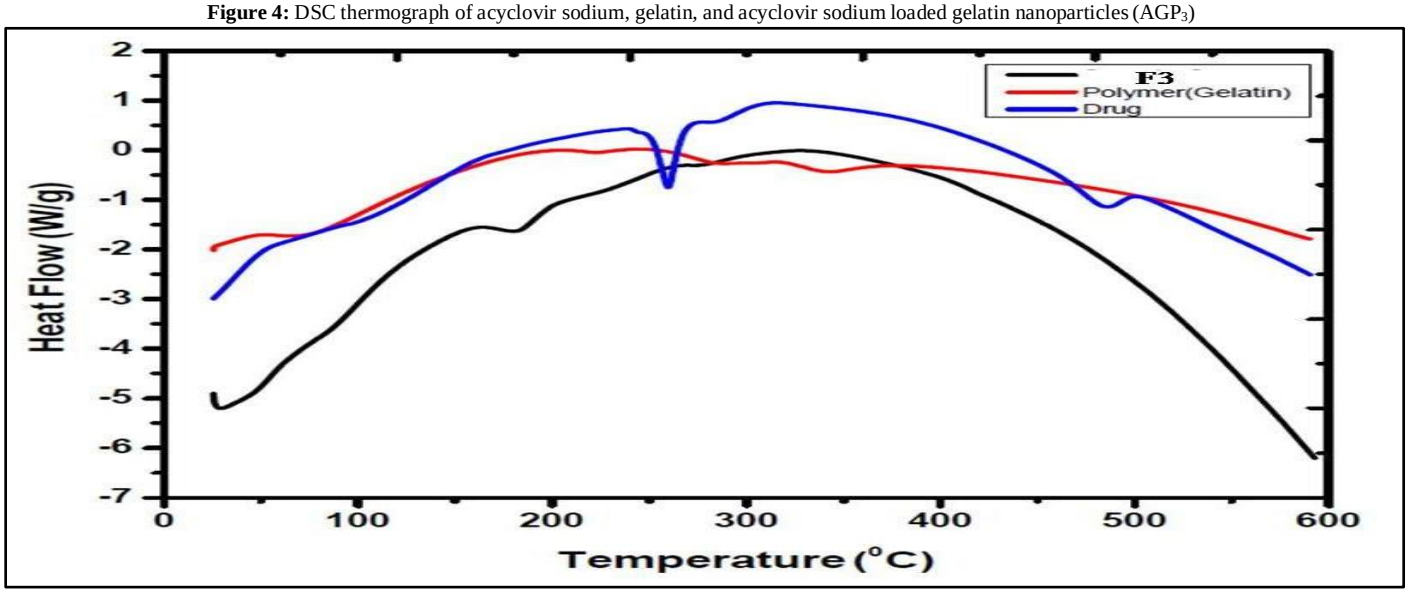
Figure 3: SEM image of Acyclovir sodium loaded Gelatine nanoparticles



Differential scanning calorimetry (DSC)

The DSC curve of acyclovir sodium shows endothermic peaks at 2550 °C, which represent its crystalline nature. According to the literature, the DSC thermogram of gelatin exhibits a fairly flat thermal skiagram, indicative of the amorphous nature of the polymer. The DSC thermogram of drug-loaded gelatin nanoparticles (Fig. 3) shows no distinctive peaks that suggest decreasing the crystallinity of the drug in nanoparticles.

Figure 4: DSC thermograph of acyclovir sodium, gelatin, and acyclovir sodium loaded gelatin nanoparticles (AGP_3)



***In-vitro* drug release**

The drug release from the fabricated formulation showed a burst release pattern during the initial hour and then followed a controlled release method for 12 hours. It is distinctly revealed from the results that drug release from the gelatin nanoparticles successively decreased as the amount of the crosslinker increased (initial burst release was 20.744% for batch AGP1 and 10.234% for batch AGP6 within the first hour and increased in a controlled manner till 12 hours; 84.674% and 68.242% for batch AGP1 and AGP6, respectively), where the ratio of drug, polymer, and crosslinker was 0.5: 0.5: 100 and 3: 3: 350 for batch AGP1 and AGP6.

Drug entrapment in the prepared nanoparticles and the hydrophilic nature of the gelatin, which absorbed water from the buffer media in a controlled release pattern for the next 12 hours, are responsible for the burst release.

We compared the in vitro drug release of the optimised formulation (AGP3) with the marketed tablet of acyclovir containing zovirax for 12 hours. The in-vitro release of AGP3 displayed a more controlled release pattern in comparison to the marketed tablet. Fig. 4 displays the cumulative drug release patterns for the formulations over time. Fig. 5 shows the comparative drug release pattern between acyclovir sodium, marketed tablets, and prepared formulations.

Figure 5: In-vitro Release of Acyclovir sodium from formulations prepared by two step desolvation method

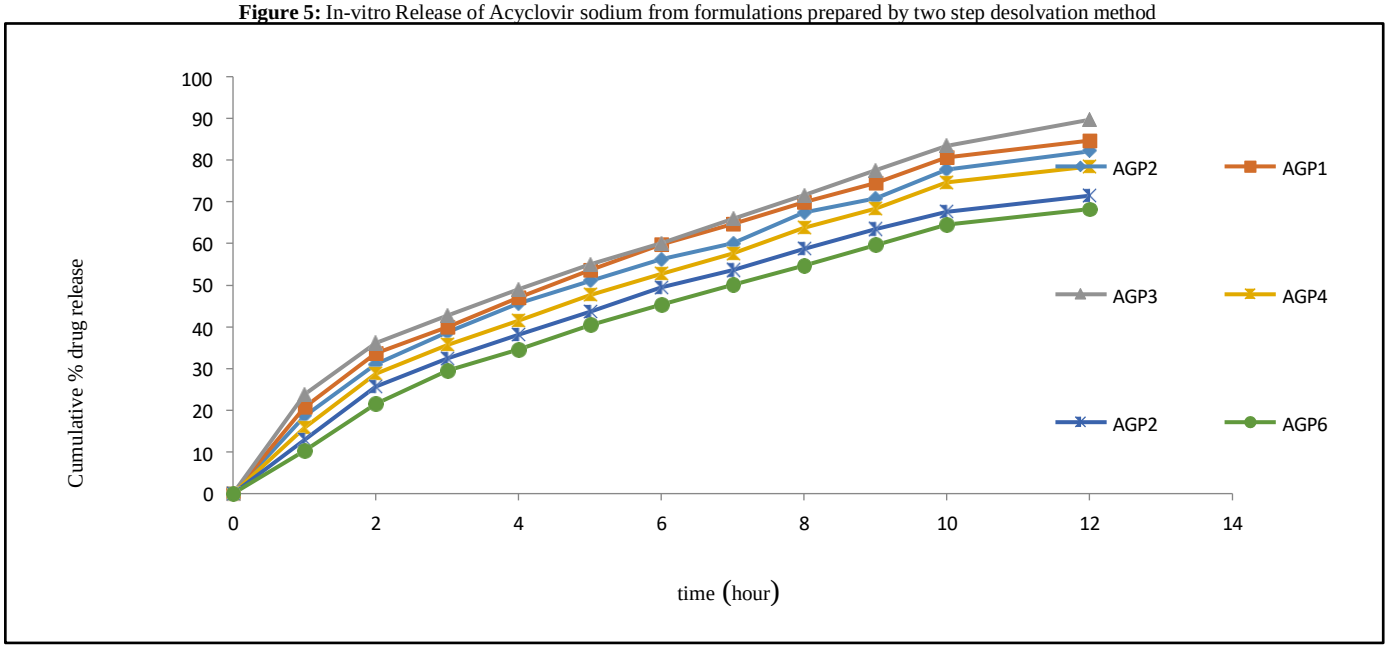
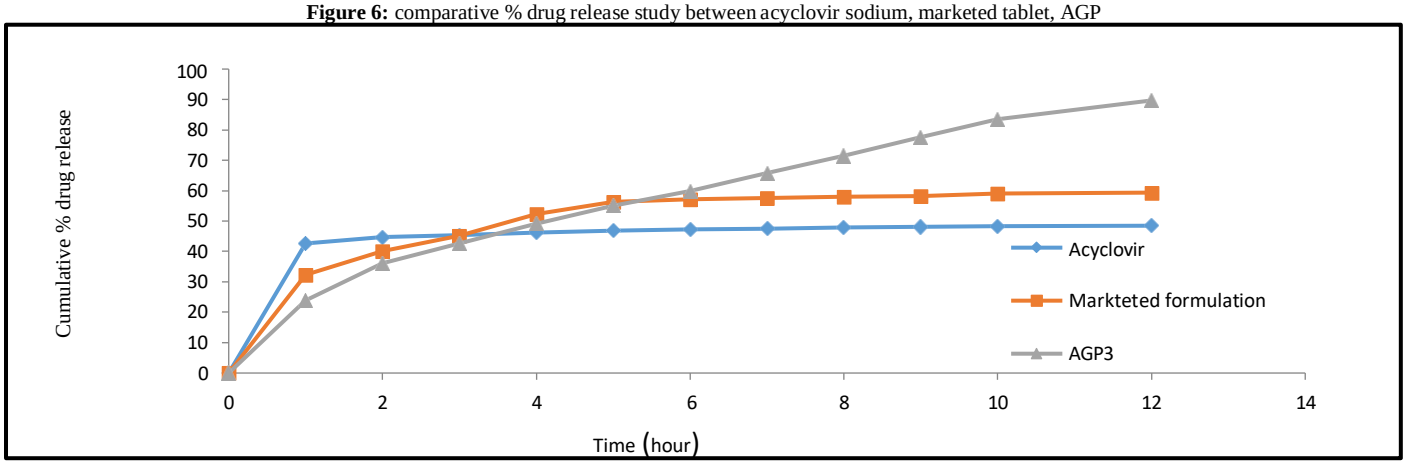


Figure 6: comparative % drug release study between acyclovir sodium, marketed tablet, AGP



Estimation of drug release kinetics

It is important to fit these nanoparticles containing acyclovir sodium into a good mathematical model so that we can predict and connect how they will release the drug in vitro. The mechWe deduced the mechanism of drug release by fitting the in-vitro drug release data into various models, including zero-order, first-order, Higuchi, and Korsmeyer-We analysed the R2 data of these mathematical models to determine their accuracy. Table 2 presents the study's results. The Korsmeyer peppas showed better linearity (R2 = 0.996). We can conclude from the release exponent (n = 0.508) that multiple processes, including diffusion and erosion, control the release of drugs from nanoparticles. We have also discussed the benefits of the release exponent in comprehending the anomalous release mechanism, as evidenced by the data n ranging from 0.43 to 0.85.

Table.2: Data of curve fitting of the in-vitro drug release profile of prepared acyclovir sodium loaded polymeric nanoparticles.

Formulation	R^2			Korsmayer	
	Zero order	First order	Higuchi model	R^2	N
AGP_1	0.933	0.991	0.990	0.996	0.576
AGP_2	0.939	0.989	0.986	0.995	0.596
AGP_3	0.936	0.967	0.992	0.996	0.508
AGP_4	0.949	0.991	0.974	0.988	0.646
AGP_5	0.946	0.995	0.970	0.986	0.696
AGP_6	0.958	0.995	0.949	0.984	0.741

CONCLUSION

We prepared acyclovir-sodium-loaded polymeric nanoparticles using a two-step desolation method. The drug entrapment efficiency of fabricated nanoparticles ranges from 74 to 86%. The AGP3 batch had the best entrapment efficiency (87.56%), which was made with 200µg/ml of cross-linking agent and a ratio of 1.5:1.5 w/v for acyclovir sodium and polymer. We analysed the morphological characteristics, stability, and physical state of the acyclovir-loaded polymeric nanoparticles using SEM, FTIR, and DSC testing. In the phosphate buffer, pH 7.4, Acyclovir sodium-loaded gelatin nanoparticles showed controlled drug release. The formulation (AGP3) exhibited a more controlled drug release compared to the acyclovir-containing marketed tablet ($p > 0.05$). Overall, all these results demonstrated that drug-Loaded polymeric nanoparticles could be impressive in controlled drug release.

Data availability statement

All of the data supporting the findings of the presented study are available for corresponding author on request.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

All authors Participate Equally.

ACKNOWLEDGMENTS

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Ethical approval

The manuscript has not been published or submitted to another journal, nor is it under review.

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