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ACYCLOVIR LOADED GELATIN BIODEGRADABLE NANOPARTICLES: FORMULATION, OPTIMIZATION, CHARACTERIZATION, AND *IN VIVO* EVALUATION

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Keywords:

Gelatin nanoparticles, Acyclovir, Glutaraldehyde, Optimization, Pharmacokinetic study

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ABSTRACT: Acyclovir has a ratified antiretroviral effect for Herpes simplex virus type 1 (HSV-1), HSV-2, Varicella zoster virus (VZV), Herpesvirus simiae and to a minor extent, Epstein-Barr virus (EBV). The oral bioavailability of acyclovir is indigent, with only 15%–30% of the oral formulations' vitality absorbed. To baffle the issue of insignificant bioavailability, acyclovir was formulated into biodegradable gelatin nanoparticles through a double desolvation method adopting gelatin, acetone as desolvating agent, and glutaraldehyde as a cross-linking agent. Optimization was imposed through design expert software whereby the aftermath of gelatin polymer concentration (X_1) and glutaraldehyde-crosslinking agent (X_2) was studied on particle size (Y_1), zeta potential (Y_2) and entrapment efficiency (Y_3). The optimized formulation (F_9) demonstrated a particle size, zeta-potential and maximum entrapment efficiency of 139.87 nm -32.67mv and 91.23%, respectively. The rate of drug release from acyclovir-loaded gelatin nanoparticles ensued from first-order kinetics, and Korsmeyer-peppas plots established the mechanism of drug release from nanoparticles. The release exponent (n) value indicating that drug release embellished by quasi-fickian diffusion transport. The *in-vivo* pharmacokinetic parameters such as maximum plasma concentration (Cmax) (4.6 ng/ml), time for peak plasma concentration (tmax) (60 min), plasma half life ($t_{1/2}$) (508 min) mean residence time (MRT)(479.8 min), area under curve (AUC0-α) (941.93 ng/ml.min) and AUMC(451931.6921) of optimized formulation showed better results than pure drug and marketed formulation. The relative bioavailability of acyclovir was increased about three-fold after gelatin nanoparticles administration as compared to pure drugs.

INTRODUCTION: The invention of useful antiviral agents has been expedited with the aid of advances within the area of molecular biology and virology.

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In a pre-antiviral generation, the extensively adhered notion was that some therapeutically meaningful obstruction with viral replication could damage the host cells, leading to which viral replication became dependent.

Developing knowledge of host cell virus relations and viral replication has led to the development of secure and powerful antivirals. Those dealers act by impeding access of viruses into host cells; interfering with the viral meeting, launch, or de-aggregation, forbidding transcription or replication

of the viral genome; or averting viral protein synthesis [1]. Antiviral drugs can be passed down to deal with an ailment as a therapeutic approach, save from contamination as a prophylactic strategy, or save from disorder as a preemptive approach. The oral bioavailability of acyclovir is terrible, with only 15%–30% of the oral formulations being absorbed [2, 3].

Nanoparticles have currently emerged as of extra specialty within the biomedical industry because of their sizable wonderful residences. Essentially, they have a substantial surface-area-to-volume ratio, which is extremely useful in a drug shipping context because it means that the drug is more likely to interact with the target region and achieve its desired effect [4].

Gelatin is a natural biopolymer with a huge array of biomedical applications in diverse industries and drug transport and gene therapy. It's far derived from collagen through a hydrolysis response, which is normally sourced from animals [5]. A critical benefit of making use of gelatin NPs is the reality that they are substantially biocompatible. This is critical for biomaterials because it approaches that they may elicit minimal immune reaction from the body; accordingly, there's a lower threat of rejection. In addition to this, gelatin is biodegradable, possesses proper adhesive abilities, is effortlessly and effectively to be had in abundance, and is particularly cheap [6].

Moreover, the surface of gelatin NPs can be functionalized, which enables the promotion of drug transport profiles to precise sites within the body and with modifiable launch charges. Gelatin is also widely regarded as secure to be used for medical packages. Because of being denatured, it has very low antigenicity. They may be derived from collagen and hence do not produce any harmful by-products after they degrade [7, 8]. The general technique for fabricating the small length (<100nm) of gelatin NPs involved dissolving and rapidly decreasing the temperature of a gelatin method to compress the gelatin molecules (and subsequently reduce their size), accompanied by cross-linking. Moreover, the drug release profile can be efficaciously altered by enhancing the concentration of the drug. The fabricated <100nm gelatin NPs have splendid potential in drug shipping and possess the benefits of any gelatin-based scientific device while overcoming the weaknesses of standard gelatin NPs [9].

MATERIALS AND METHODS:

Materials: Acyclovir was obtained as gift samples from Micro Labs Ltd.; Gelatin (Type A) was obtained from Sigma-Aldrich Chemicals Private Limited, Bangalore; Glutaraldehyde was obtained from Molychem, Mumbai. All other materials used were of analytical grade. Pharmacokinetics studies were carried out in Natreon Inc, Kolkata.

Methods:

Formulation of Acyclovir Loaded Biodegradable Gelatin Nanoparticles: A double-step desolvation approach was used to form gelatin nanoparticles, hitherto described by Coester *et al.* in 2000 [10.] Different formulations (F_1 to F_{13}) were prepared, and calculated amounts of gelatin (Type A) (0.5 to 1.1% w/v) was dissolved in 25 ml distilled water in steady heating at 37°C. After the solution was clear, the 25 ml of the desolvating agent was combined to precipitate the gelatin. The supernatant was thrown away, and the gelatin was again mixed with 25 ml distilled water containing acyclovir (1%) and the pH of the solution was corrected to values 2.5 by using 2M HCL. The solution was heated to 37°C and swirled at 600 rpm using a magnetic stirrer. During a second desolvation phase, drop-wise inclusion of around 75 ml of acetone with constant stirring turned out gelatin nanoparticles with a narrow size range. Later 10 minutes, variable amounts of 25% v/v aqueous glutaraldehyde solution (100 to 400 µl) were mixed with cross-linking the nanoparticles, and after half an hour the cross-linking process was interrupted by the addition of 5 ml of 12% w/v aqueous sodium meta-bisulfite solution. The gelatin nanoparticles dispersion was then mixed at 10,000 g for 30 minutes before being rinsed three times through water to discard adherent-free drug from the nanoparticles' outer surface. The lyophilized powder was then kept at room temperature in impenetrable glass containers until required.

Optimization of Acyclovir Loaded Biodegradable Gelatin Nanoparticles: Formulation was optimized by Design-Expert software (File version: 13.0.8.0), through randomized response surface quadratic modeling

where two independent variables were motley at higher levels (+1) and lower levels (-1). The independent variables were gelatin concentration (X_1) and glutaraldehyde amount (X_2) as shown in **Table 1**. The levels of independent variables were settled from preparatory trials. While, Particle size (Y_1), Zeta potential (Y_2) and entrapment efficiency (Y_3) were selected as dependent variables.

TABLE 1: INDEPENDENT VARIABLES AND THEIR LEVELS

S. no.	Independent variables	Lower levels (-1)	Higher levels (+1)
a.	Gelatin Concentration (%w/v) (X_1)	0.5	1.1
b.	Volume of glutaraldehyde (cross linking agent) (µl) (X_2)	100	400

Characterization of Acyclovir Loaded Biodegradable Gelatin Nanoparticles:

Drug Encapsulation Efficiency: The capacity of manufacturing technique and components to incorporate or accomplice the drug correctly in nanoparticles is represented as drug entrapment performance (EE), which may be quite simply decided by reading the loose drug entrapped drug or overall drug [11].

The drug encapsulation performance of acyclovir-charged gelatin nanoparticles was decided by using the ultracentrifugation method for setting apart the non-entrapped drug. Parallel to this method, 1 ml aliquot of acyclovir loaded gelatin nanoparticles dispersion changed into ultra-centrifuged at 10,000 rpm for 1 h.

The supernatant solution becomes separated. The nanoparticles were then suspended in PBS at pH 7.4 and centrifuged yet again. The cleansing method was repeated twice to ensure no free drug remained in the empty area between the nanoparticles. Every time the supernatant becomes separated and drug content is evaluated, the usage of cm $^{-1}$ quartz cells and UV–visible spectrophotometry (Shimadzu 1700) at 252 nm as opposed to phosphate buffer (pH 7.4) as a clean.

Via subtracting the quantity of loose drug from the entire drug incorporated, the load of entrapped drug turned into anticipated. The experiments had been duplicated three times in general. The usage of the components under the drug entrapment efficiency (percentage) was computed [12]. (Eq.1)

% Entrapment efficiency = Total amount of drug – Amount of free drug / Total amount of drug × 100 …….. (1)

Determination of Zeta Potential: Nanoparticles with a more zeta potential (positive or negative) are electrically stable, while those with a low zeta potential coagulate or flocculate.

A zetasizer was used to evaluate the zeta potential of the acyclovir-loaded gelatin nanoparticles (Malvern units, zeta analyzer). Setting diluted samples (with ultra-purified water) within the capillary dimension cell and editing the mobile position, the measurements have been carried out in an automated mode using an aqueous dip cellular [13].

Determination of Particle Size: The particle measurement and size division of the acyclovir-loaded gelatin nanoparticles was distinct by photon correlation spectroscopy using a Zetasizer 2000 Malvern apparatus, UK. Nanosuspension was diluted with strained (0.22µm) ultra-pure water and analyzed using Zeta-sizer [14].

Morphological Examination of Acyclovir Loaded Gelatin Nanoparticles: Surface morphology of acyclovir-loaded gelatin nanoparticles (F_9) was resolved by scanning electron microscope (SEM). The sample resided in an aluminium sample holder, which was enclosed through double-sided carbon tape (Ted Pella Inc., California, US).

The assembly was placed in the SEM chamber, which was managed in a low vacuum mode and put up at a steady pressure of 80 Pa. To establish the surface view and form of the samples, photographs were taken [14].

***In-vitro* Drug Release Study:** Acyclovir containing gelatin nanoparticles was explored for *in-vitro* release in impalement. For the calculations, a sealed dialysis bag (MWCO, 12–14 kDa; pore size 2.4 nm), acyclovir loaded GNPs equivalent to 20 mg acyclovir was entirely afloat in 50 ml drug release media (PBS containing 0.1% w/v Tween 80, pH 7.4). The temperature of the media was sustained at 37 ± 0.5ºC and the media was stirred at 50 rpm using a magnetic bead.

The drug release media were absolutely replaced at predetermined time intervals to the keep sink conditions.

Cumulative release of acyclovir in sample solution was investigated by UV spectrophotometer at 252 nm [15, 16].

Drug Release Kinetics: To know the order of kinetics, the collected drug release data from the optimized formulation (F_9) was disposed to zero-order and first-order kinetic design, as well as Higuchi's and Korsmeyer-Peppas plots to identify the mechanism of drug release from the gelatin nanoparticles containing acyclovir [17, 18, 19].

ANOVA Studies: The mean ± standard deviation is utilized to display the accumulated experimental data (Mean ± SD).

The outcome of particle size, zeta-potential, and entrapment efficiency were enforced on ANOVA modules to learn whether the selected variables had significant control or not. The ANOVA function was exercised by Design-Expert software version: 13.0.8.0 [20].

Pharmacokinetic Study: The rabbits used were nine albino adult male rabbits weighing 1.4–2.0 kg. The rabbits were housed in individual cages and given a diet and water *ad libitum*.

An oral pharmacokinetic study was conducted with three separate treatment groups, each of which included four animals.

Treatment groups were designated as: Group A (control group) - treated with acyclovir alone (100 mg/kg, acyclovir); Group B - treated with acyclovir-marketed formulation-Zovirax 200mg tablets (100 mg/kg) and Group C- treated with optimized acyclovir gelatin formulation (F_9) (100 mg/kg) [21].

RESULTS AND DISCUSSION:

Optimization of Acyclovir Loaded Bio-degradable Gelatin Nanoparticles: Chosen variables confirmed a statistically significant impact on particle size, zeta potential and entrapment performance **Table 2.**

Established assessment of statistical parameters provided by design expert software through quadratic equations indicating key results and interplay outcomes were diagnosed. Statistical validation of quadratic equations was mounted through ANOVA. In **Fig. 1A-C**, response surface graphs illustrate the effects of decisive variables on the particle size, zeta potential, and entrapment performance of acyclovir-loaded biodegradable gelatin nanoparticles.

TABLE 2: RESULTS OF PARTICLE SIZE, ZETA POTENTIAL AND ENTRAPMENT EFFICIENCY OF ACYCLOVIR LOADED GELATIN NANOPARTICLES OF ALL FORMULATIONS

		Factor 1	Factor 2	Response 1	Response 2	Response 3
Code	Run	A: Gelatin Conc. X_1 (% w/v)	B:Cross linking agent X_2 (µl)	Particle Size Y_1(nm) Mean ± SD (n = 3)	Zeta Potential Y_2 (-mv) Mean ± SD (n = 3)	Entrapment Efficiency Y_3 (%) Mean ± SD (n = 3)
F_1	1	0.8	250	138.24 ± 2.27	33.23 ± 1.22	86.29 ± 1.84
F_2	2	0.8	462.132	104.23 ± 1.21	41.29 ± 1.45	77.13 ± 1.29
F_3	3	0.5	400	109.23 ± 1.30	38.39 ± 2.09	59.39 ± 2.29
F_4	4	0.8	250	144.76 ± 0.97	32.56 ± 0.93	88.63 ± 2.07
F_5	5	0.8	250	141.32 ± 1.26	34.39 ± 0.76	89.37 ± 0.97
F_6	6	1.1	100	313.71 ± 1.27	44.27 ± 0.91	84.15 ± 1.25
F_7	7	1.22426	250	370.83 ± 0.91	45.13 ± 1.12	82.36 ± 1.86
F_8	8	0.8	37.868	144.21 ± 1.25	39.11 ± 0.86	89.85 ± 1.41
F_9	9	0.8	250	139.87 ± 1.06	32.67 ± 0.97	91.23 ± 1.01
F_{10}	10	1.1	400	286.12 ± 1.24	40.16 ± 1.29	85.67 ± 2.67
F_{11}	11	0.37574	250	114.17 ± 1.45	31.39 ± 2.93	57.59 ± 1.87
F_{12}	12	0.5	100	118.84 ± 1.86	32.15 ± 1.42	69.17 ± 0.91
F_{13}	13	0.8	250	141.34 ± 1.09	31.93 ± 1.97	87.32 ± 2.88

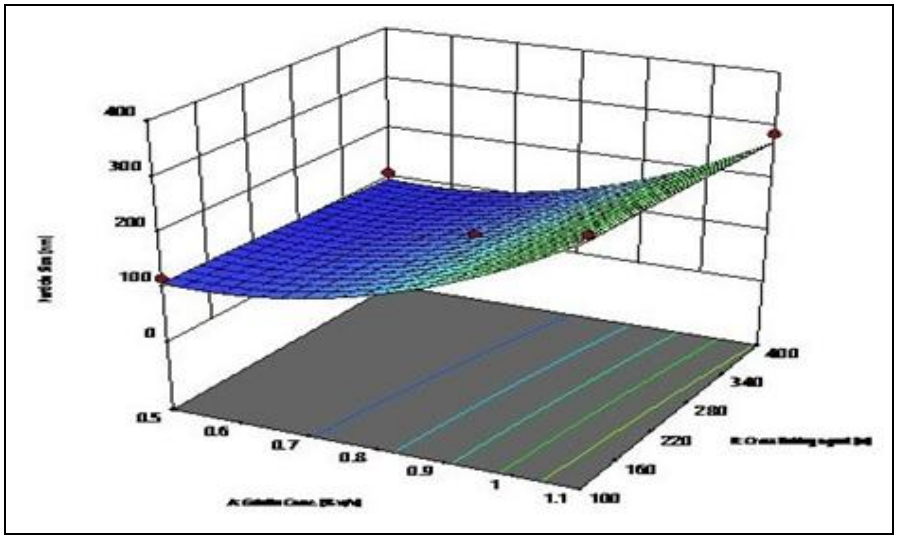

FIG. 1A: SURFACE PLOT OF PARTICLE SIZE

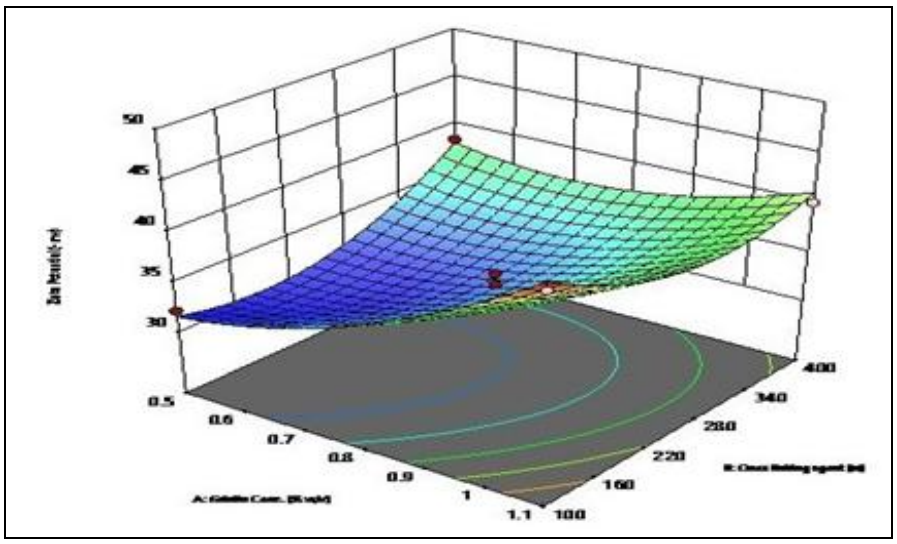

FIG. 1B: SURFACE PLOT OF ZETA POTENTIAL

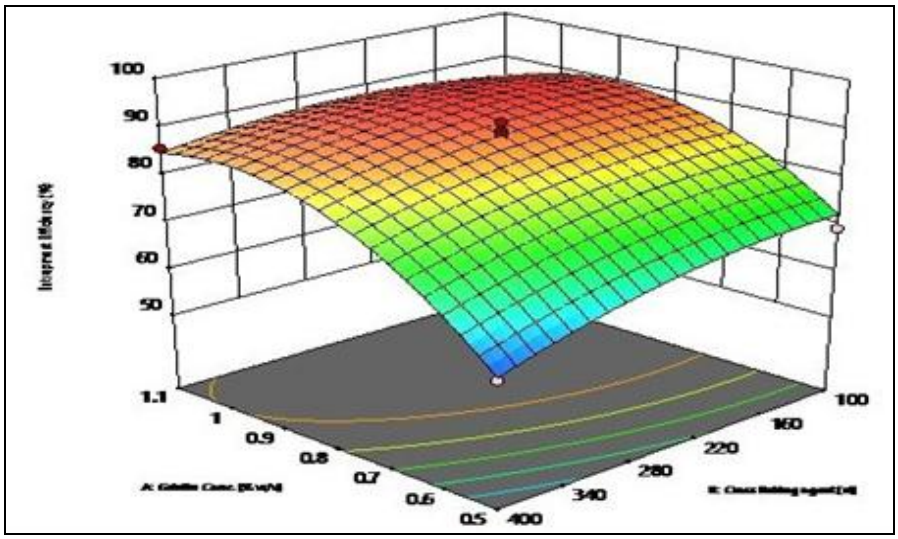

FIG. 1 C: SURFACE PLOT OF ENTRAPMENT EFFICIENCY

FIG. 1: A) RESPONSE SURFACE PLOT SHOWING EFFECT OF GELATIN CONCENTRATION (X1) AND CROSS-LINKING AGENT (X2) ON PARTICLE SIZE; B) RESPONSE SURFACE PLOT SHOWING EFFECT OF GELATIN CONCENTRATION (X1) AND CROSS-LINKING AGENT (X2) ON ZETA POTENTIAL; C) RESPONSE SURFACE PLOT SHOWING EFFECT OF GELATIN CONCENTRATION (X1) AND CROSS-LINKING AGENT (X2) ON ENTRAPMENT EFFICIENCY

Effects on Particle Size (Y1): As demonstrated in **Table 2** and **Fig. 1** (a), particle sizes of different formulations were found between 104.23nm (run1) and 370.83 nm (run 7); this chosen sensitivity of critical variables selected for the study. Experiments conceded out at the center points (runs 1, 4, 5, 9, and 13; *n* = 5) of the design show reproducibility of the experiment as the coefficient of variation (CV) is a lesser amount of than 8%. Independent factors influencing particle size can be explained by the following quadratic equation 2.

Particle Size Y_1 = +291.18751- 675.12498 (X_1) + 0.058222 (X_2) - 0.099889 (X_1X_2) + 628.89722 (X_1^2) - 0.000113 (X_2^2)……2

A regression coefficient (r^2) of 0.9871 for the equation designated a higher correlation among experimental responses and chosen significant variables.

Effects on Zeta Potential (Y_2): As revealed in **Table 2** and **Fig. 1B**, the **Zeta potential** of formulations ranged between -31.39 mv (run 11) and -45.13mv (run 7); this chosen sensitivity of critical variables selected for the study. Experiments conceded out at the center points (runs 1, 4, 5, 9, and 13; *n* = 5) of the design show reproducibility of the experiment as the coefficient of variation (CV) is a lesser amount of than 3%. Independent factors influencing Zeta potential can be explained by the following quadratic equation 3.

Zeta potential Y_2 = +36.97743 - 16.72113 (X_1) - 0.027436 (X_2) - 0.057500 (X_1X_2) +28.11250 (X_1^2) + 0.000156 (X_2^2)……..3

A regression coefficient (r^2) of 0.9732 for the equation designated a higher correlation between experimental responses and chosen significant variables.

Effects on Entrapment Efficiency (EE, Y_3): As exhibited in **Table 2** and **Fig. 1C**, EE varied between 57.59 %(run 11) to 91.23% (run 9), which displays that the response was inclined towards chosen factors. Experiments completed at the center points of the design (runs 1, 4, 5, 9, and 13; *n* = 5) set that the experimental method was highly reproducible (CV < 3%). From the data conferred in **Table 1**, it is obvious that independent factors affecting EE were; the concentration of gelatin (X_1) and the amount of cross-linking agent (X_2). Independent factors affecting entrapment efficiency can be interpreted by following quadratic equation 4.

Entrapment efficiency Y_3 = +2.71643 +190.86420 (X_1) - 0.003799 (X_2) + 0.062778 (X_1X_2) -109.23194 (X_1^2) - 0.000137 (X_2^2)……4

A regression coefficient (r^2) of 0.9745 designated a higher correlation among experimental responses and chosen significant variables.

Drug Encapsulation Efficiency: It was observed that the entrapment efficacy of acyclovir-loaded GNPs formulations was found between 57-91 %. **Table 2** Electrostatic attraction, Physical entrapments, and chemical bonding were the approaches used to load drugs into GNPs [24].

The F_9 formulation revealed superior entrapment efficacy (91 %) than other formulations ($p<0.05$) due to their lesser size, which has superior surface area for consistent absorption and ionic relations between the drug and matrices of gelatin nanoparticles to increase the drug loading [22].

Particle Size: Crosslinking of gelatin nanoparticles is vital to offer stability, spherical shape, and enhanced *in-vivo* circulation of nanoparticles within the anatomy [23].

Glutaraldehyde is a crosslinker that cross-link by free amine groups of lysine or hydroxyl lysine residues of gelatin molecules. A smaller amount of glutaraldehyde is not adequate to cross-link the gelatin nanoparticles as rapid particle size increases due to swelling of gelatin in aqua media after the organic solvent is dissipated.

Intensifying glutaraldehyde concentration justifications solidifying particles prevailing to considerable decrease in size as of cross-linking of free amino groups at the gelatin nanoparticles surface. Still, a further glutaraldehyde concentration causes additional concentration groups to be cross-linked, starting to aggregation and lift of particle size **Table 2**.

Zeta Potential: The zeta potential of nanoparticles in general to conclude particle surface charge and, consequently, dispersion stability. Tremendously high or negative zeta potential values affect more repulsive forces, but repulsion among particles with

equivalent electric charges lowers particle aggregation and allows for efficient redispersion.

A large zeta potential value is advantageous; generally, a zeta potential value of less than 30 is considered stable in dispersion **Table 2**.

Morphological Examination of Acyclovir Loaded Gelatin Nanoparticles: The surface morphology of optimized drug-loaded gelatin nanoparticles (F_9) (shown in **Fig. 2**) was smooth, and uniform because of the texture of the gelatin employed.

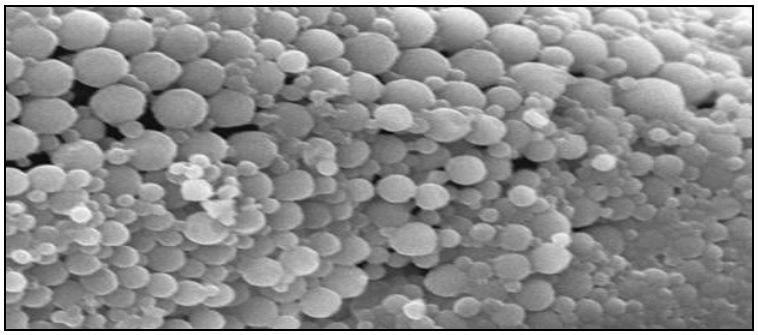

FIG. 2: SEM IMAGES OF ACYCLOVIR LOADED GELATIN NANOPARTICLES (OPTIMIZED F9 FORMULATION)

***In-vitro* Drug Release Study:** From the *in-vitro* release profile **Fig. 3(a),** it was found that optimized formulation (F_9) depicted significantly higher dissolution than a pure drug in the dissolution media (pH 7.4) due to gelatin nanoparticles cross-linked by glutaraldehyde resulted in a biodegradable carrier, in which the key part of acyclovir was controlled to the protein matrix *via* glutaraldehyde, composing a drug-conjugate to enhance the *in vitro* release activity [24]. Therefore prepared gelatin nanoparticles formulations exhibited significantly higher dissolution efficiency than pure acyclovir in dissolution media.

Kinetics of Drug Release: The kinetic description used to indicate the acyclovir release from various gelatin nanoparticle formulations is displayed in **Table 3**. The drug release from optimized F_9 formulations pursued first-order kinetics, with fickian diffusion as the drug release mechanism, as demonstrated by the regression coefficient (R^2) value and Korsmeyer-Peppas 'n' value. **Fig. 3B, Fig. 3C Table 4.**

The release mechanism of acyclovir-loaded GNPs was analyzed by using zero-order, first-order, Higuchi order, and Korsmeyer-Peppas (KP) models. The regression coefficient (R^2) values confirmed that the *in-vitro* release of optimized formulation (F_9) best fit the first-order kinetic model compared to other formulations **Table 4.**

TABLE 3: KINETIC MODELS USED TO DEPICT ACYCLOVIR RELEASE FROM FORMULATIONS OBTAINED

Formulation	Zero-order kinetics	First-order kinetics	Higuchi model	Korsmeyer–Peppas model		Type of transport
	Regression coefficient (R^2)				'n' (Release Exponent)	
F_1	0.796	0.995	0.961	0.995	0.312	quasi-Fickian diffusion
F_2	0.743	0.995	0.934	0.998	0.271	quasi-Fickian diffusion
F_3	0.787	0.997	0.952	0.985	0.289	quasi-Fickian diffusion
F_4	0.803	0.996	0.964	0.996	0.316	quasi-Fickian diffusion
F_5	0.778	0.996	0.954	0.995	0.305	quasi-Fickian diffusion
F_6	0.742	0.966	0.937	0.994	0.290	quasi-Fickian diffusion
F_7	0.725	0.957	0.927	0.993	0.280	quasi-Fickian diffusion
F_8	0.776	0.985	0.949	0.993	0.291	quasi-Fickian diffusion
F_9	0.810	0.992	0.967	0.992	0.323	quasi-Fickian diffusion
F_{10}	0.819	0.997	0.970	0.992	0.327	quasi-Fickian diffusion
F_{11}	0.716	0.995	0.917	0.994	0.249	quasi-Fickian diffusion
F_{12}	0.756	0.995	0.941	0.996	0.280	quasi-Fickian diffusion
F_{13}	0.790	0.991	0.954	0.987	0.294	quasi-Fickian diffusion

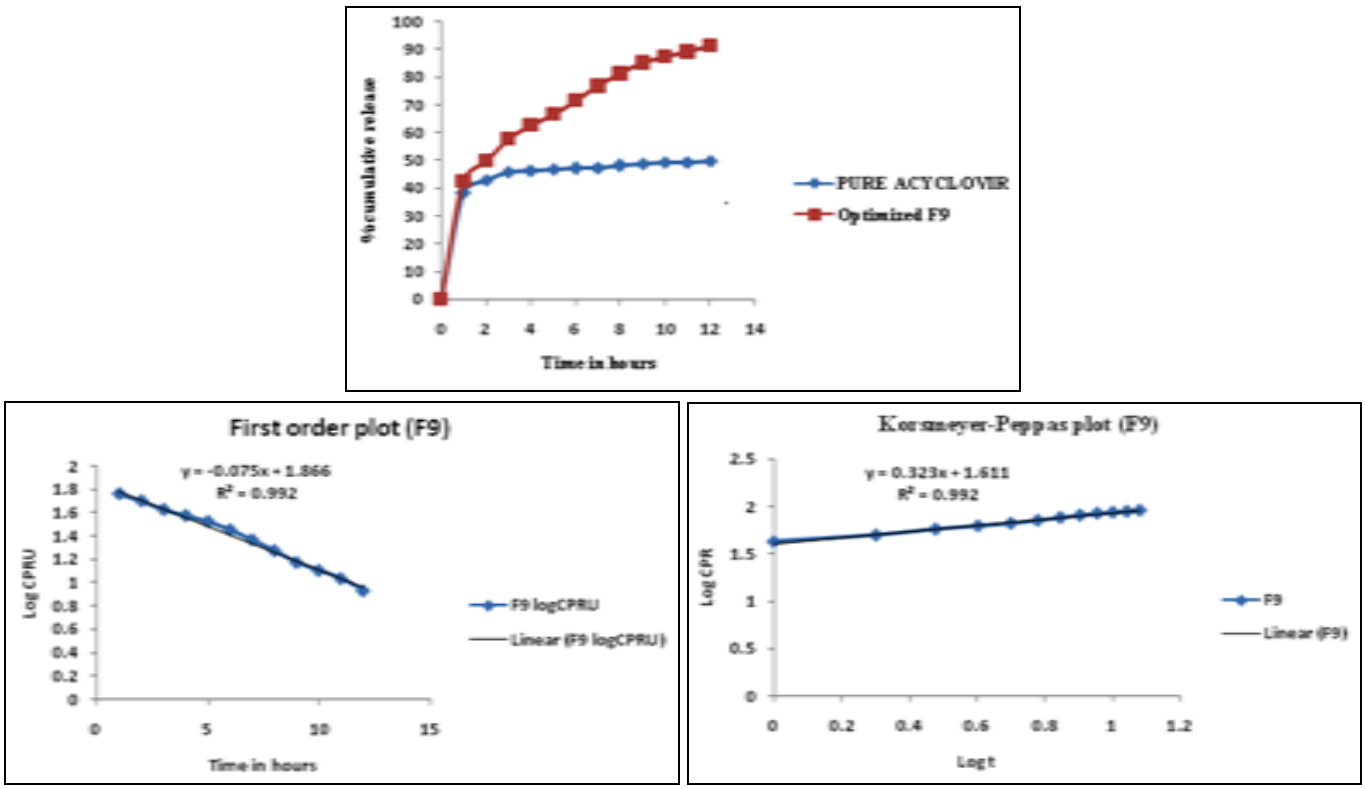

FIG. 3: (A) DRUG RELEASE PROFILE OF OPTIMIZED ACYCLOVIR LOADED GELATIN NANOPARTICLES F9 FORMULATION AND PURE ACYCLOVIR B) FIRST-ORDER PLOTS OF OPTIMIZED ACYCLOVIR LOADED GELATIN NANOPARTICLES F9 FORMULATION AND C) KORSMEYER-PEPPAS PLOT OF OPTIMIZED ACYCLOVIR LOADED GELATIN NANOPARTICLES F9 FORMULATION

ANOVA Studies: The ANOVA studies (shown in Table 3) indicated that the whole experiment involved two independent variables that were significant with respective to their control against particle size, zeta potential, and entrapment efficiency. As shown in **Table 4**, The Model F-value of 107.37, 50.76, and 53.52 mention the model is considerable. There was only a 0.01% probability that an F-value this large could occur considering noise. Model terms with P-values less than 0.0500 are considerable. A, B, A^2, and B^2 were important model terms in that scenario. As shown in **Table 4**, The Lack of Fit F-value of 3.36, 1.72, and 2.38 mention that the Lack of Fit is not considerable compared to the pure error. There was a 21.04%, 29.95%, and 21.08% probability that a Lack of Fit F-value this outsized could occur considering noise. Non-significant lack of fit was superior for the model to be robust.

TABLE 4: RESULTS OF ANOVA STUDIES FOR PARTICLE SIZE, ZETA POTENTIAL, AND ENTRAPMENT EFFICIENCY

Source of variation	F-value			P-value			
	Particle size	Zeta potential	Entrapment efficiency	Particle size	Zeta potential	Entrapment efficiency	
Model	107.37	50.76	53.52	< 0.0001	< 0.0001	< 0.0001	significant
A-Gelatin Conc.	395.29	123.76	126.37	< 0.0001	< 0.0001	< 0.0001	
B-Cross linking agent	6.43	3.03	14.96	0.0389	0.1253	0.0061	
AB	0.4734	23.88	5.54	0.5136	0.0018	0.0507	
A^2	130.55	39.71	116.78	< 0.0001	0.0004	< 0.0001	
B^2	0.2628	76.00	11.41	0.6240	< 0.0001	0.0118	
Lack of Fit	3.36	1.72	2.38	0.2104	0.2995	0.2108	not significant

Pharmacokinetic Study: *In-vivo* pharmacokinetic studies of pure acyclovir, Marketed formulation and optimized Optimized acyclovir loaded gelatin nanoparticles were performed in rabbits. The evaluated pharmacokinetic parameters are listed in **Table 6;** drug concentrations in plasma following

administration of the pure acyclovir, Marketed Formulation and optimized acyclovir loaded GNPs were plotted against time **Fig. 4.** The study exhibited more T_{max} value for Optimized acyclovir loaded gelatin nanoparticles than pure acyclovir but less than Marketed formulation, while C_{max} value for pure acyclovir were higher than Marketed Formulation and Optimized acyclovir loaded GNPs. However, $T_{1/2,}$ MRT, $AUC_{0\text{-}12h}$, $AUMC_{0\text{-}12h}$ and V_d values were higher in the case of Optimized acyclovir loaded GNPs than pure acyclovir and Marketed Formulation. The relative bioavailability of acyclovir was increased about three-fold after gelatin nanoparticles administration as compared to pure drug **Table 5.**

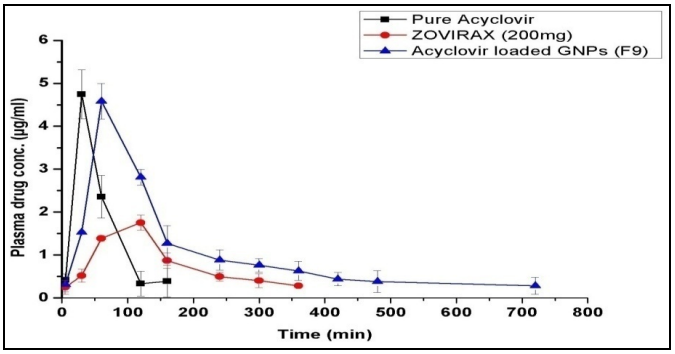

FIG. 4: PLASMA CONCENTRATIONS TIME PROFILE OF ACYCLOVIR AFTER A SINGLE ORAL DOSE OF PURE ACYCLOVIR (GROUP 1), MARKETED FORMULATION) (ZOVIRAX 200 MG) (GROUP 2) AND OPTIMIZED ACYCLOVIR LOADED GNPS (GROUP 3) (P > 0.05).

TABLE 5: PHARMACOKINETIC PARAMETERS OF PURE ACYCLOVIR, MARKETED FORMULATION AND OPTIMIZED ACYCLOVIR LOADED GELATIN NANOPARTICLES (MEAN ± SD, N = 4)

Pharmacokinetic parameters	Group 1 (Pure acyclovir)	Group 2 (Marketed Formulation) (Zovirax 200 mg)	Group 3 (Optimized acyclovir loaded GNPs)
C_{max} (ng/ml)	4.74± 0.51	1.75± 0.85	4.58± 0.91
T_{max} ((min)	30	120	60
$T_{1/2}$ (min)	32.62	126.87	508.57
MRT(min)	60.87± 1.53	205.61± 1.71	479.79± 1.05
AUC (ng/ml*min)	285.84± 2.71	339.75± 3.61	941.93± 2.49
AUMC	17399.29± 2.54	69854.89± 2.49	451931.69± 3.01
Vd (mg)/(µg/ml)	134.69± 1.21	134.69± 1.05	194.74± 2.01

CONCLUSIONS: The double step desolvation method successfully prepared acyclovir-loaded gelatin nanoparticles with varying compositions. It was concluded that 0.8 % gelatin solution, at 37º C temperature, pH 2.5, and 250 µl glutaraldehyde cross-linking agent are suitable for free-flowing, homogenous, smooth, and spherical preparation with desired size (104-370 nm) gelatin nanoparticles. The surfaces of gelatin nanoparticles observed by SEM are found to be smooth in nature. The optimized formulation has the smallest particle size, zeta potential, and maximum entrapment efficiency of 139.87 nm, -32.67mv, and 91.23%, respectively, and *in-vitro* release of 91% at 12 h in pH 7.4 dissolution medium indicated that gelatin nanocarrier: A future of controlled drug release delivery system. From pharmacokinetic studies, it was observed that the C_{max} and T_{max} values of pure drug, marketed formulation, and optimized formulation (F_9) were found to be 4.7 ng/ml, 1.7 ng/ml and 4.6 ng/ml, while Tmax values of pure drug, marketed formulation and optimized formulation (F_9) and were found to be 30 min, 120 and 60 min respectively in rabbit plasma. It was also found that the $AUC_{0\text{-}12h}$ of optimized formulation F_9 was about three times higher than that of the marketed formulation (Zovirax), which may be due to lower absorption from the commercially marketed product. Thus, the gelatin nanocarrier-based acyclovir nanoparticles formulation is a promising controlled release for antiviral remedy through oral administration.

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