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DESIGN, DEVELOPMENT AND STATISTICAL OPTIMIZATION OF ACYCLOVIR-LOADED PROTEIN NANOPARTICLES

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Gelatin, Nanoparticles, Acyclovir, Glutaraldehyde, Optimization, Zeta potential

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ABSTRACT: To solve the problem of low drug availability in the body, acyclovir was fabricated into biodegradable gelatin nanoparticles by step-wise two-times desolvation method where gelatin was used as biodegradable polymer and glutaraldehyde was utilized as a cross-linking agent. Optimization was conceded by design expert computational application whereby the outcome of gelatin polymer concentration (X1) and glutaraldehyde-crosslinking agent (X2) were studied on particle size (Y1), zeta potential (Y2), and entrapment efficiency (Y3). The drug-loaded gelatin nanoparticle formulations were characterized by particle size, surface charge, and entrapment efficiency. ANOVA studies also evaluated drug-loaded gelatin nanoparticles. The optimized formulation (F9) of 0.8% gelatin polymer (X1) and 250 µl of glutaraldehyde-crosslinking agent (X2) containing acyclovir: gelatin ratio of 1:8, which showed a particle size, zeta potential and maximum entrapment efficiency of 139.87 nm, -32.67mv and 91.23% respectively.

INTRODUCTION: Antiviral drugs can be passed down to deal with an ailment as a therapeutic approach, to save from contamination as a prophylactic strategy, or to save from disorder as a preemptive approach [1]. The oral drug availability of acyclovir is terrible in the body, with only 15%–30% of the oral formulations being absorbed [2]. Nanoparticles have emerged as an extra specialty within the biopharmaceuticals industry because of their sizable wonderful residences [3]. 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].

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Natural biopolymer, that is gelatin, has a huge array of capability biopharmaceutical applications in diverse industries, together with drug transport and gene therapy [5]. It's far derived from collagen through a hydrolysis response, which is normally sourced from animals [6]. A critical benefit of using gelatin NPs is that they are substantially biocompatible [7, 8]. This is critical for biomaterials because it approaches that they may elicit a 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 [9, 10].

Moreover, the surface of gelatin NPs can be functionalized, promoting caused drug transport profiles to precise sites within the body and with modifiable launch charges [11]. Gelatin is also widely regarded as secure to be used for medical packages. Because of being denatured, it has a very low antigen city because they may be derived from

collagen and hence do not produce any harmful by-products after they degrade [12, 13]. The general technique for fabricating the small length (<100nm) gelatin NPs involved dissolving and rapidly decreasing the temperature of a gelatin method to compress the gelatin molecules (and subsequently reduce their size), accompanied through cross-linking.

Moreover, the drug release profile can be efficaciously altered by enhancing the drug's amount [14]. 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 [15, 16].

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 experimental materials used were of analytical grade.

Methods:

Formulation of Acyclovir Loaded Biodegradable Gelatin Nanoparticles: Coester *et al.* 2000 explained gelatin nanoparticle preparation by two step-wise desolvation approach. (Coester *et al.*, 2000) Different formulations (F_1 to F_{13}) were prepared, and calculated amounts of gelatin (Type A) (0.5 to 1.1% w/v) were dissolved in 25 ml distilled water in steady heating at 37 °C. After the solution was clear, a desolvating agent was combined to precipitate the gelatin. The buoyant was thrown away, the gelatin was again mixed with summing distilled water containing acyclovir (1%), and the solution pH was corrected to value 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 min, 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 min before being rinsed through water to discard free drug adherents from the nanoparticles' extraneous surface. The lyophilized powder was then kept at room temperature in impenetrable glass containers until required [17, 18].

Optimization of Acyclovir Loaded Bio-degradable Gelatin Nanoparticles: Formulation was optimized by factorial design using Design-Expert software, File version: 13.0.8.0, Study type: Response Surface, Subtype: Randomized, Design Model: Quadratic. The Independent two variables were altered at the higher horizon (+1) and horizon (-1).

Gelatin concentration (X_1) and glutaraldehyde amount (X_2) were two independent variables shown in **Table 1**. The order of the independent two variables was decided from preparatory bathes. In contrast, particle size (Y_1), zeta-potential (Y_2), and entrapment efficiency (Y_3) were chosen as dependent variables.

TABLE 1: OPTIMIZATION STUDY TABLE WITH LEVELS OF FACTORS

S. no.	Factors	Lower levels	Higher levels
1	Gelatin Conc. (%w/v) (X_1)	0.5	1.1
2	Amount of glutaraldehyde (cross-linking agent) (µl) (X_2)	100	400

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 to ANOVA modules to learn whether the selected variables had significant control or not [19, 20]. The ANOVA function was exercised by Design-Expert software version: 13.0.8.0

RESULTS AND DISCUSSION:

Optimization of Acyclovir Loaded Biodegradable Gelatin Nanoparticles: Chosen variables confirmed statistically significant impact on impotent parameters of gelatin nanoparticles **Table 2.** The essential results and interplay outcomes were diagnosed using an established assessment of statistical parameters provided by

design expert software using quadratic mathematical statements. ANOVA was used to perform statistical acceptance of quadratic mathematical statements. In **Fig. 1A-C,** different 3D response surface graphs illustrating the effects of decisive variables at the particle size, zeta potential, and entrapment performance of acyclovir loaded biodegradable gelatin nanoparticles are presented. Design expert software diagnosed key results and interplay outcomes through quadratic mathematical statements indicating an established assessment of statistical parameters provided.

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

Response on Particle size (Y1): The following chosen sensitivity of critical variables selected for study, as demonstrated in **Table 2** and **Fig. 1(A),** particle size of different formulations were found between 104.23nm (run1) and 370.83 nm (run 7).

Statistics conducted at the design's centre points (1, 4, 5, 9, and 13; n = 5) demonstrate Statistics acceptance, with a coefficient of less than 8% variation. The quadratic mathematical statement equation 1 can be used to explain independent factors that influence particle size.

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) …….(1)

The equation had a regression coefficient (r^2) of 0.9871, indicating a higher connection between the experimental response and the selected important factors.

Response on Zeta Potential (Y_2): The following chosen sensitivity of critical variables selected for study as demonstrated in **Table 2** and **Fig 1(B),** Zeta potential of formulations ranged between -31.39 mv (run11) and -45.13mv (run 7). Statistics conducted at the design's center points (1, 4, 5, 9, and 13; n = 5) demonstrate Statistics acceptance, with a coefficient of less than 3% variation. The quadratic mathematical statement equation 2 can be used to explain independent factors that influence zeta potential.

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)…….. (2)

The equation had a regression coefficient (r^2) of 0.9732, indicating a higher connection between the experimental response and the selected important factors.

Response on Entrapment Efficiency (Y_3): The following chosen sensitivity of critical variables selected for study, as demonstrated in **Table 2** and **Fig 1(C)**, EE varied between 57.59% (run 11) to 91.23% (run 9), which displays that the return was inclined towards chosen factors. Statistics conducted at the design's centre points (1, 4, 5, 9, and 13; n = 5) demonstrate Statistics acceptance, with a coefficient of variation (CV) of less than 3%. From the data conferred in **Table 1**, it is obvious that sovereign factors affecting EE were gelatin (X_1) and cross-linking agent (X_2). The quadratic mathematical statement equation 3 can be

used to explain independent factors that influence entrapment efficiency.

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) …… (3)

The equation had a regression coefficient (r^2) of 0.9745, indicating a higher connection between the experimental response and the selected important factors.

ANOVA Studies: The results of the ANOVA studies (shown in **Table 3**) indicated that the whole experiment involved two independent variables that were significant concerning their control against different nanoparticle characterizations.

As shown in **Table 3**, the Model F-values of 107.37, 50.76, and 53.52 mentions the model is considerable. There was barely a probability of 0.01% that an F-value this outsized could occur considering noise. Model specifications with P-values < 0.0500 are considerable. A, B, A^2 and B^2 were important model specifications in that scenario. As shown in **Table 3**, the F-values of 3.36, 1.72, and 2.38 mention 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 F-values were superior for the model to robust.

TABLE 3: RESULTS OF ANOVA STUDIES

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

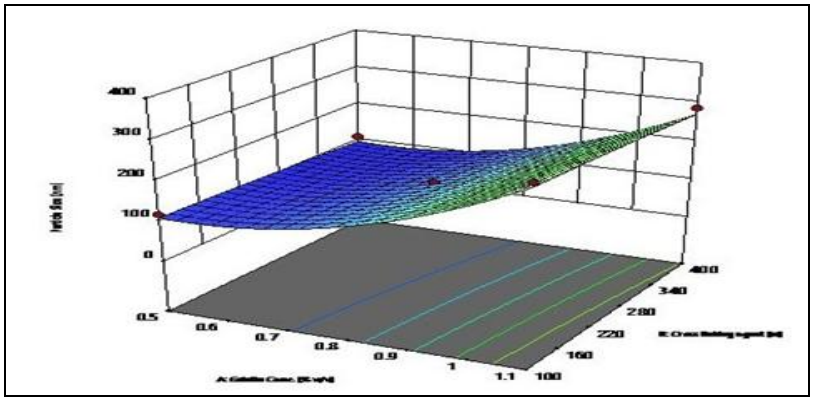

FIG. 1(A): 3D SURFACE PLOT OF PARTICLE SIZE

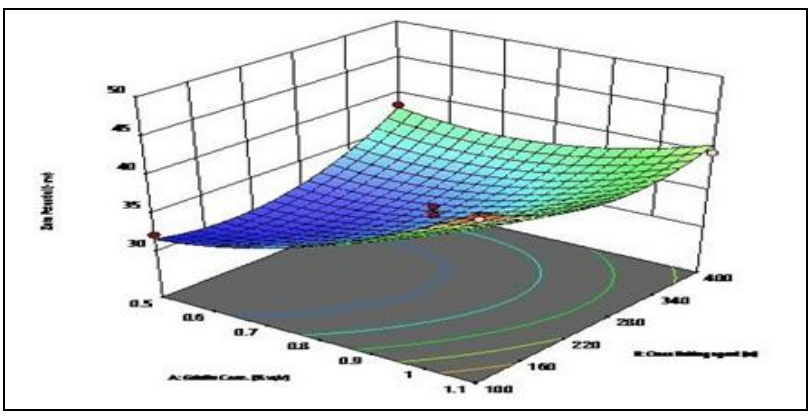

FIG. 1 (B): 3D SURFACE PLOT OF ZETA POTENTIAL

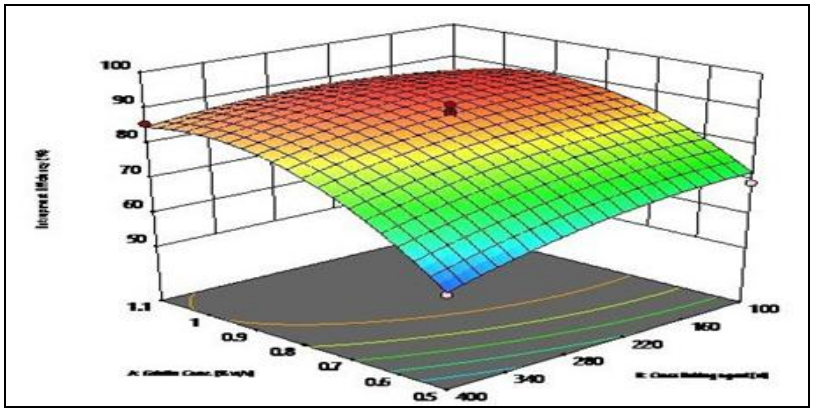

FIG. 1 (C): 3D SURFACE PLOT OF ENTRAPMENT EFFICIENCY

FIG. 1: A) THE INFLUENCE OF GELATIN CONCENTRATION (X1) AND CROSS-LINKING AGENT (X2) ON PARTICLE SIZE IS SHOWN IN A 3D SURFACE PLOT; B) THE INFLUENCE OF GELATIN CONCENTRATION (X1) AND CROSS-LINKING AGENT (X2) ON ZETA POTENTIAL IS SHOWN IN A 3D SURFACE PLOT.; C) THE INFLUENCE OF GELATIN CONCENTRATION (X1) AND CROSS-LINKING AGENT (X2) ON ENTRAPMENT EFFICIENCY IS SHOWN IN A 3D SURFACE PLOT

CONCLUSIONS: Acyclovir-loaded gelatin nanoparticles were well prepared by a step-wise two-times desolvation method with varying gelatin and glutaraldehyde. It was concluded that 0.8 % gelatin solution (pH 2.5) at 37º C temperature and 250 µl glutaraldehyde cross-linking agent is suitable for preparing free-flowing, homogenous, smooth, and spherical with particle size (139.87 nm) for acyclovir-loaded gelatin nanoparticles. The surfaces of gelatin nanoparticles were found to be smooth in nature.

The optimized (F_9) formulation has the smallest particle size, zeta potential and maximum entrapment efficiency of 139.87 nm, -32.67mv and 91.23%, respectively, indicating that gelatin nanocarrier: A future of controlled drug release delivery system. Thus, the gelatin nano carrier-based acyclovir nanoparticles formulation is a promising controlled release for antiviral remedies through oral administration.

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