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Research Article

Formulation Development and Optimization of Biodegradable Acyclovir Loaded Gelatin Nanoparticles

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ABSTRACT

To solve the problem of low drug availability in the body, acyclovir was fabricated into biodegradable gelatin nanoparticles by stepwise 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 nanoparticles formulations were characterized by particle size, surface charge, and entrapment efficiency. Drug-loaded gelatin nanoparticles were also evaluated by ANOVA studies. 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 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 currently emerged as of 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 have interaction with the target region, and for this reason achieve its desired effect [4].

Natural biopolymer, that is gelatin, has a huge array of capability biopharmaceuticals applications in diverse industries, together with drug transport and gene therapy [5].

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It's far derived from collagen through a hydrolysis response, which is normally sourced from animals [6].

A critical benefit of making use of gelatin NPs is the reality that they are substantially biocompatible [7, 8]. 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 [9, 10].

Moreover, the surface of gelatin NPs can be functionalized which enables to promote 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 very low antigenicity on account that they may be derived from collagen, and hence do not produce any harmful by way of-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 crosslinking. Moreover, the drug release profile can be efficaciously altered with enhancing the amount of the drug [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.* in 2000 explained gelatin nanoparticles preparation by two times 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) was 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 and the gelatin was again mixed with summing distilled water containing acyclovir (1%) and the solution pH 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 to crosslink the nanoparticles and after half an hour the cross-linking process was interrupted by addition of 5 ml of 12% w/v aqueous sodium meta-bisulphite solution. The gelatin nanoparticles dispersion was then mixed at 10,000 g for 30 minutes before being rinsed many times 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 Biodegradable 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 higher horizon (+1) and horizon (-1). Gelatin concentration (X_1) and glutaraldehyde amount (X_2), which were two independent variables shown in Table 1. The order of independent two variables was decided from preparatory bathes. While, 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
a.	Gelatin Conc. (%w/v) (X_1)	0.5	1.1
b.	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 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. 1 (a-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.

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

Table 3: Results of ANOVA studies

Sou rce 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**

Key results and interplay outcomes were diagnosed by design expert software through quadratic mathematical statement indicating established assessment of statistical parameters provided.

Response on Particle Size (Y_1)

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 variation of less than 8%. 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 centre points (1, 4, 5, 9, and 13; n = 5) demonstrate Statistics acceptance, with a coefficient of variation of less than 3%. 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 crosslinking 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 were significant with respect to their control against different nanoparticles 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 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.

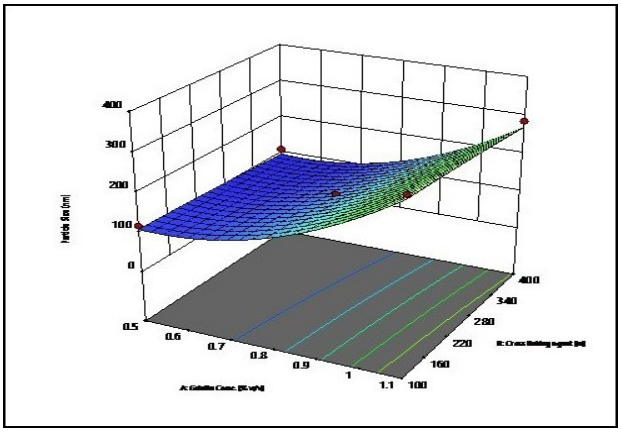

Figure 1(a): 3D Surface plot of Particle size

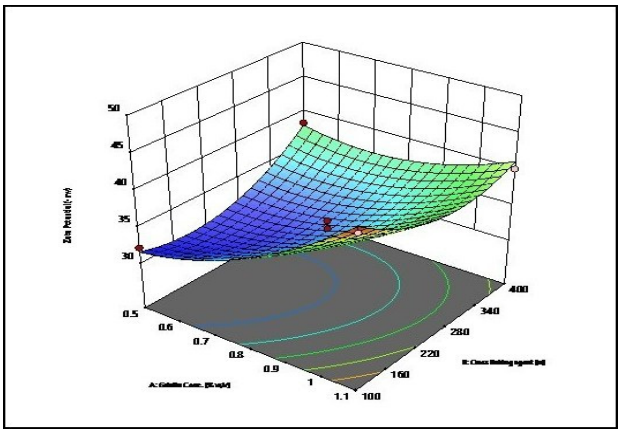

Figure 1 (b): 3D Surface plot of Zeta potential

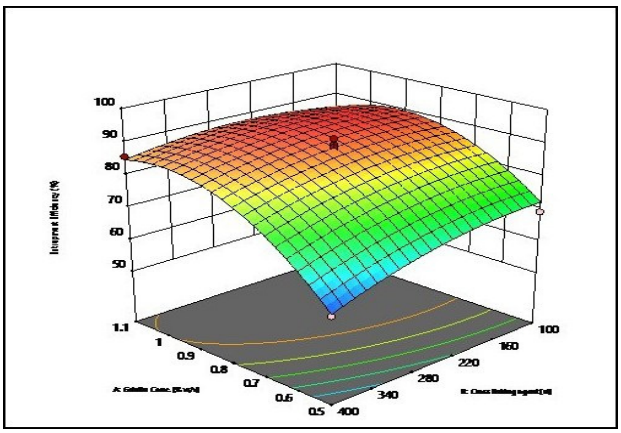

Figure 1 (c): 3D Surface plot of Entrapment Efficiency

Figure 1: a) The influence of gelatin concentration (X1) and crosslinking agent (X2) on particle size is shown in a 3D surface plot; b) The influence of gelatin concentration (X1) and crosslinking agent (X2) on zeta potential is shown in a 3D surface plot.; c) The influence of gelatin concentration (X1) and crosslinking agent (X2) on entrapment efficiency is shown in a 3D surface plot.

As shown in Table 3, the **F-values** of 3.36, 1.72 and 2.38 mention the Lack of Fit is not considerable comparative 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 model to robust.

CONCLUSIONS

Acyclovir loaded gelatin nanoparticles were well prepared by stepwise two-times desolvation method with varying gelatin and glutaraldehyde. It was concluded that 0.8 % gelatin solution (pH 2.5) at 37°C temperature, and 250 µL glutaraldehyde crosslinking agent are suitable for preparation of 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 smallest particle size, zeta potential and maximum entrapment efficiency of 139.87 nm, -32.67mv and 91.23% respectively, indicated that gelatin nanocarrier: A future of controlled drug release delivery system. Thus, the gelatin nanocarrier-based acyclovir nanoparticles formulation is a promising controlled release for antiviral remedies through oral administration.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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