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Developing and optimizing a formulation for biodegradable gelatin nanoparticles loaded with acyclovir

Anjaneyulu vinukonda[1], Gurinderdeep Singh[2], J. K. Shyamala[3], Sudhir S Hunge[4], Anil Kumar Dindigala[5], Arvind R. Bhagat Patil[6], Pankaj Bhatt[7] and Sreenivas Pippalla[8*]

[1]Scientist- formulation and process development, Strides Pharma Inc New York -USA-10977.

[2]Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala.

[3]Department of Pharmaceutics, G.R.D college of pharmacy, The Tamilnadu Dr MGR medical university, Chennai.

[4]Chintamani College of Science Pombhurna Dist Chandrapur, Gondwana University, Gadchiroli.

[5]R&D Scientist III, Thermofisher scientific, Greenville, NC, USA 27858.

[6]Yeshwantrao Chavan College of Engineering, Nagpur.

[7]R&D Scientist, Piramal pharma Limited, Navi Mumbai, Maharashtra.

[8]Sikkim Professional University, Gangtok, Sikkim- 737102.

***Correspondence:**

Dr. Sreenivas Pippalla,

Sikkim Professional University, Gangtok, Sikkim- 737102.

Email: sreenivas.pippala@gmail.com

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Abstract:

Using a two-step melting process, the drug Acyclovir was put inside biodegradable gelatin nanoparticles. Glutaraldehyde was used as a cross-linking agent, and gelatin was used as a biodegradable material in this process. The goal was to aid the body in getting the medicine. The study used the Design Expert computer program to look at what happened when the amounts of gelatin polymer (A1) and the glutaraldehyde-crosslinking agent (A2) were changed. They looked at how particle size (B1), zeta potential (B2), and trapping efficiency (B3) changed. We looked at the drug-loaded gelatin nanoparticle mixtures by measuring how well they trapped the drug, how charged the surface was, and how big the particles were. ANOVA tests were used to look at the gelatin nanoparticles that had drugs in them. One part acyclovir to eight parts gelatin made up the improved version (F5). It had 0.8% gelatin polymer (A1) and 250 µL of glutaraldehyde (A2), a bonding agent. The best trapping rate for this mixture was 91.00%, the zeta potential was -29.88mv, and the particle size was 140.01 nm.

Keywords: Biodegradable, gelatin, nanoparticles, acyclovir.

Introduction:

It is possible to treat illnesses, put an end to infections, and treat diseases with the help of antiviral medications. Acyclovirs that are taken orally have a low absorption rate, which ranges between 15 and 30 percent. This indicates that the total amount of the medication that the body receives is lower than it may be (Kharia and Singhai, 2015; Tiwari *et al.,* 2021). Nanoparticles have gained popularity in the realm of biopharmaceuticals due to the fact that they possess enormous and fascinating molecular structures. For the purpose of medication delivery, the fact that their surface area to volume ratio is significantly higher than the average is advantageous since it improves the likelihood that the drug will interact with the appropriate location and provide the intended effect. The natural biopolymer gelatin is utilized for a variety of purposes within the realm of biopharmaceuticals, including gene therapy and drug delivery, amongst others (Joshy *et al.,* 2017; Kharia *et al.,* 2012; Sowmya *et al.,* 2012).

A hydrolysis process takes collagen from animal skin. One big benefit of using gelatin nanoparticles is that they are very compatible with living things. This discovery is very important for biomaterials because it suggests that the body may react less strongly to them, making rejection less likely (Ahire *et al.,* 2020; Nippani *et al.,* 2016). Additionally, gelatin is inexpensive, doesn't harm the earth, and has the right adhesive properties. Additionally, it is

easy and quick to get large amounts of it. Gelatin nanoparticles' surfaces can also be changed, which makes it easier for drugs to get to specific parts of the body and lets release rates be changed. Additionally, gelatin is known to be safe enough to be used in medical packaging (Kharia and Singhai, 2013; Dindigala, and Kodam, 2023).

The destruction of these organisms is the source of their reduced antigenicity. They can be manufactured from collagen, but when they decompose, they do not produce any waste that could be harmful to the environment. Dissolving the gelatin, rapidly lowering the temperature, and finally crosslinking the gelatin are the typical steps involved in the process of producing tiny gelatin nanoparticles (Ahire *et al.,* 2018; Singh *et al*., 2021). Because of this, the gelatin molecules become more compact. In addition, increasing the quantity of the medication could have a significant impact on the manner in which it is delivered. Nanoparticles of gelatin that are smaller than 100 nanometres have been created, and they show a great deal of promise for the delivery of drugs. In addition to this, they combine the advantages of gelatin-based scientific equipment with those of gelatin nanoparticles in their regular form (Coester *et al.,* 2023; Khulbe *et al.,* 2023; Kattamuri *et al.,* 2012). The aim of this study was to develop formulation for biodegradable gelatin nanoparticles loaded with acyclovir.

Materials and methods:

Materials:

An Indian company sent us free samples of acyclovir. Sigma-Aldrich Chemicals Private Limited, which is based in Mumbai, provided the gelatin. Molychem, a company based in Mumbai, sold us glutaraldehyde. The materials used in all the other tests were analytical grade.

Methods acyclovir-loaded biodegradable gelatin nanoparticle formulation

Twenty-five milliliters of distilled water were mixed with gelatin, and the mixtures (F1 to F9) were slowly cooked up to 37 degrees Celsius. When the solution became clear, a desiccating product was added to make the gelatin precipitate. After the airy stuff was taken out, the gelatin was mixed again with pure water that had 1% acyclovir added to it. After that, 2M hydrochloric acid (HCl) was added to the mixture to make it less acidic, bringing its pH to 2.5. A magnetic mixer was used to stir the mixture at a speed of 600 revolutions per minute (rpm) while it was heated to 37°C. In the year 2000, Coester *et al.* described a two-step, sequential desolvation

method for making gelatin nanoparticles (Coester *et al.,* 2000). In the year 2000, Coester and two other people. A small range of sizes of gelatin nanoparticles were made by adding about 75 mL of acetone slowly while shaking all the time during the desolvation phase that followed (Khambete *et al.,* 2016; 5. Panditi and Vinukonda, 2011). With changes to the amounts of a 25% v/v glutaraldehyde solution in water, the nanoparticles were chemically joined together after 10 minutes. After 30 minutes, 5 ml of a 12% w/v water solution of sodium meta-bisulphite was added to stop the crosslinking process. After the gelatin nanoparticles were mixed with a 10,000-g rotating force for 30 minutes, the nanoparticles' outside was washed with water several times to get rid of any drug particles that might have stuck to it. After that, the freeze-dried powder was kept in sealed glass cases that were left at room temperature until they were needed (Tiwari *et al.,* 2021; Putikam *et al.,* 2012).

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Table 1: Various factor levels

Sr. No.	Factors	Low	High
a.	Polymer Conc. (A1)	0.5	1.2
b.	Amount of glutaraldehyde (A2)	100	400

Through factorial design, Design Expert software was used to make the method better. Not sure what to call it make it Quadratic is a model. When looked at higher horizons, especially at +1 and -1, both the independent and dependent variables changed. There are two separate factors in Table 1, which are the amount of glutaraldehyde (A2) and the quantity of gelatin (A1). To find out the order of the two different factors, preparatory baths were used. The factors that were used as dependents were particle size (B1), entrapment efficiency (B3), and zeta-potential (B2) (Ahirrao *et al.,* 2023; Tiwari *et al.,* 2021; Panditi *et al.,* 2011).

Statistical Studies

For the purpose of displaying the findings of the experiment, the mean plus or minus the standard deviation is utilized. For the purpose of determining whether or not the components

that we selected were adequately controlled, we utilized ANOVA modules to examine the outcomes of particle size, zeta potential, and entrapment efficiency. For the purpose of executing the ANOVA function, the Design Expert program, namely version 13.0.8.0, was utilized.

RESULT AND DISCUSSION

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According to the findings shown in Table 2, the selected parameters were found to have a significant influence on the impotence-related features of gelatin nanoparticles. The significant findings and interaction consequences were discovered by the utilization of quadratic mathematical expressions and statistical parameter assessment using design expert software. The statistical validity of assertions based on quadratic math was examined with the use of analysis of variance (ANOVA). Figures 1 and 2 present three-dimensional response surface graphs that illustrate the ways in which various parameters influence the zeta potential, trapping performance, and particle size of biodegradable gelatin nanoparticles that contain acyclovir (Pathan *et al.*, 2023; Vinukonda, *et al.*, 2023).

Table 2: Particle size, zeta potential, and entrapment efficiency of all acyclovir-loaded gelatin nanoparticle formulations

Runs	Batch	Factor 1	Factor 2	Response 1	Response 2	Response 3
		Polymer conc. (A1)	Cross linking agent (A2)	PS	ZP	EE
				B1(nm) Mean ± SD	B2 (-mv) Mean ± SD	B3 (%) Mean ± SD
1	F1	0.6	240	139.35 ± 1.66	34.47 ± 1.35	85.35 ± 1.95
2	F2	0.6	459.0	105.38 ± 1.77	40.33 ± 1.51	78.56 ± 1.44
3	F3	0.4	401	108.44 ± 1.22	39.55 ± 1.21	60.25 ± 1.33
4	F4	0.6	253	143.23 ± 0.88	31.35 ± 1.82	87.55 ± 1.97
5	F5	0.7	250	141.01 ± 1.26	-29.88 ± 0.75	91.00 ± 2.17
6	F6	1.2	105	309.82 ± 1.33	43.32 ± 0.82	85.26 ± 1.33
7	F7	1.5	256	369.34 ± 0.83	44.32 ± 1.44	83.27 ± 1.28
8	F8	1.0	38.15	145.32 ± 1.32	40.69 ± 0.36	90.96 ± 1.33

9	F9	0.8	258	140.22 ± 1.12	33.22 ± 0.89	88.44 ± 0.87

The software known as Design Expert makes use of a mathematical equation that is quadratic in order to provide a trustworthy evaluation of the statistical components. This evaluation assists in the diagnosis of significant results and interactions.

Table 3: Findings from ANOVA study

variation	P. Size	ZP	EE%	P. Size	ZP	EE%
Model	108.44	51.25	52.93	< 0.0003	< 0.0004	< 0.0002
Polymer Conc.	396.12	122.88	125.46	< 0.0004	< 0.0002	< 0.0003
Cross linking agent	7.38	4.28	15.84	0.0298	0.1344	0.0052
AB	0.3625	24.74	6.61	0.6212	0.0024	0.0608
A^2	131.44	40.65	116.61	< 0.0002	0.0013	< 0.0012
B^2	0.3131	75.01	12.52	0.5354	< 0.0003	0.0217
Lack of Fit	4.45	2.81	3.47	0.3203	0.3894	0.3005

Particle Size (B1)

The particle sizes of the different mixes were between 104.23 nm and 370.83 nm, as shown in Table 2 and Figure 1. The sensitivity of key factors that have been identified has been chosen to be looked at. With a coefficient of variation of less than 8%, the statistics done at the design's center points show that it is statistically acceptable. The separate factors that affect particle size can be shown by the quadratic equation 1 (Tiwari *et al.,* 2021; Rajora *et al.,* 2024; Rani *et al.,* 2023).

Zeta Potential (B2)

As shown in Table 2 and Figure 1, the formulas had zeta potentials that ranged from -31.39 mV to -45.13 mV. The sensitivity values for the following variables show which ones were found

to be important. With a coefficient of variation of less than 3%, the statistics done at the designs center points show that it is statistically acceptable. If you want to talk about the factors that don't depend on zeta potential, you can use the quadratic equation 2 (Tiwari *et al.*, 2021).

Entrapment Efficiency (B3)

Table 2 and Figure 2 show that the chosen key factors were sensitive between 57.59% and 91.23% of the time. It looks like the return was skewed toward the parts that were picked for the study. A coefficient of variation below 3% shows that the plan is accepted when statistics are done at the central points of it. The binding agent (A2) and the gelatin (A1) were clearly the main things that affected the EE, as shown in Table 1. It is possible to use the quadratic equation 3 to show the separate factors that affect how well trapping works (Noomwong *et al.*, 2011; Vyshnavi *et al.*, 2023).

Statistical Studies

The ANOVA study results (shown in Table 3) showed that both separate factors had a big effect on many properties of the nanoparticles, which made the experiment as a whole important. The Model F-values of 107.37, 50.76, and 53.52 in Table 3 show that the model is very important. Because there was so much noise, there was a 0.01% chance that an F-value this big would happen. It is thought that model specifications with a P value less than 0.0500 are important.

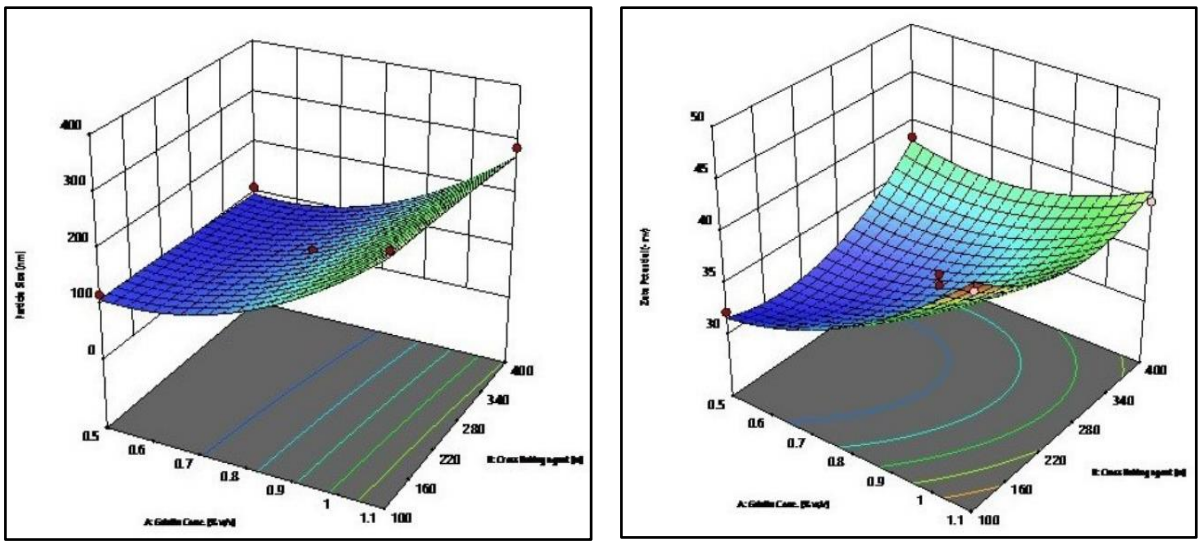

Figure 1(a): Particle size 3D surface plot; Figure 1(b): Zeta potential 3D surface plot

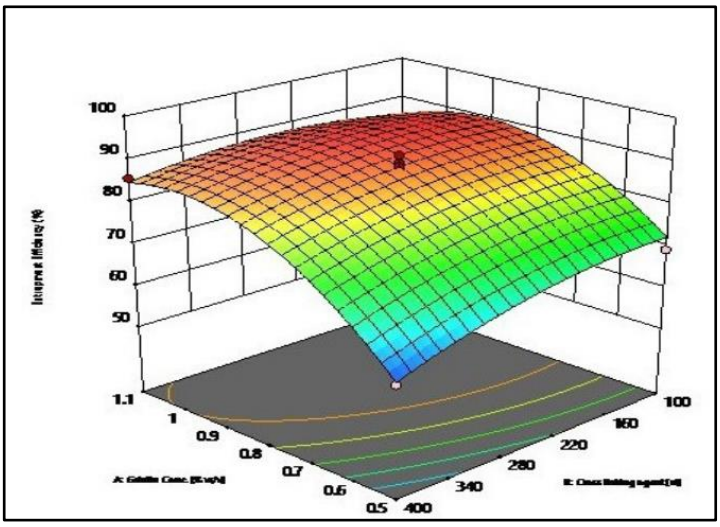

Figure 2: Entrapment Efficiency's 3D Surface Plot

A 3D surface map shows how the amount of gelatin (A1) and the bond agent (A2) affect the size of the particles. A 3D surface map shows how the amount of gelatin (A1) and the binding agent (A2) affect the zeta potential. A two-dimensional surface map shows how the quantity of gelatin (A1) and the crosslinking agent (A2) affect how well the particles are trapped. There is no statistically significant difference between the pure error and the Lack of Fit, as shown in Table 3. The F-values of 3.36, 1.72, and 2.38 show this. Because of the noise, there was a 21.04%, 29.95%, and 21.08% chance that an extremely high Lack of Fit F-value would happen. The model's F-values showed a higher amount of resilience, even though they were not statistically significant (Vadlapudi *et al.*, 2014).

Conclusion:

In this process, gelatin and glutaraldehyde were dissolved in two different stages. This made it possible to make well-prepared gelatin nanoparticles that were loaded with acyclovir. The study found that a 0.8% gelatin solution with a pH of 2.5 can be used to make gelatin nanoparticles that are loaded with acyclovir. This can be done by mixing it with 250 µL of glutaraldehyde, which is a binding agent. With a particle size of 139.87 nm, these nanoparticles are free-flowing, uniform, smooth, and spherical, all of which are good qualities. It was found that the surfaces of gelatin nanoparticles are naturally smooth. The improved (F5) formulation has shown that gelatin nanocarrier is a promising controlled drug release system. It has the smallest particle size (140.01 nm), the lowest zeta potential (-29.88mv), and the highest trapping efficiency

(91.00%). So, the creation of acyclovir nanoparticles using gelatin nanocarriers is a promising way to make oral antiviral drugs work more slowly.

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None

Conflict of Interest

None

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