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Design And Optimization of Nanosponge

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Abstract
Nanosponges are a novel class of hyper-cross linked polymer based colloidal structures consisting of solid nanoparticles with colloidal sizes and nanosized cavities. These nano-sized colloidal carriers have been recently developed and proposed for drug delivery, since their use can solubilize poorly water-soluble drugs and provide prolonged release. The present work developednanosponges containing Puerarin using the emulsion solvent evaporation technique. Puerarin (20 mg) was dispersed in dichloromethane (DCM) containing ethyl cellulose. The formulations were optimized using a Box-Behnken design (BBD) to examine the effects of ethyl cellulose, PVA, and stirring time on particle size and entrapment efficiency. Further, a gel was prepared using NP9-loaded nanosponges and its physical properties such as texture, color, and homogeneity were analyzed. The particle size and entrapment efficiency were determined for each formulation, with NP9 showing the optimal results. NP9 exhibited a particle size of 252.1 nm, an entrapment efficiency of 84.6%, and a high percentage yield of 87%. The optimized formulation (NP9) also demonstrated a zeta potential of -23.2 mV, indicating good stability of the nanosponges. Scanning electron microscopy (SEM) revealed spherical particles, confirming the successful formation of nanosponges. The gel exhibited excellent spreadability (26.22 ± 0.024) and viscosity (5421 cP ±0.016). The in vitro drug release profile showed sustained release over 8 hours, with 86.24% of the drug released. Kinetic release studies revealed that the release followed a zero-order kinetic model, indicating a controlled release mechanism. The formulation of Puerarin-loaded nanosponges using the emulsion solvent evaporation technique was successfully optimized. The results suggest that NP9 formulation holds great potential for future therapeutic applications.

1. Introduction

A nanosponge is a contemporary substance composed of minuscule particles with a small, nanometer-wide hollow. The ability of these tiny particles to contain both hydrophilic and lipophilic medicinal molecules allows these narrow spaces to be filled with a variety of other compounds **(Bhowmik et al., 2018).**Release the drug at specific target site instead of circulates through the body it will more effective for particular given dosage **(Krishnamoorthy et al., 2012).**The development of nanosponges has been a major step in resolving the complexity of the recently emerging systems. Nanosponges' small size and porous structure allow them to bind poorlywatersoluble medications within the matrix, increasing their solubility and bioavailability **(Darandale et al., 2013**).Nanosponges' internal hydrophobic chambers and exterior hydrophilic branching allow them to entrap both hydrophilic and lipophilic medicinal molecules, providing an unmatched level of versatility. A nanosponge made from several organic and inorganic materials with a suitable cross-linking agent. These formulations are suitable with most vehicles and components and are stable over a broad pH range in GI fluids as well as over 130 °C. decreasing the frequency of dosages while improving patient comfort and compliance. This medication delivery technology reduces adverse effects, improves stability, adds elegance, and allows for more formulation flexibility by entrapping a wide range of components **(Pentewar et al., 2014).** The effort to improve dissolution and solubility of poorly and practically water insoluble drugs remains one of the most challenging tasks in drug development. Several methods have been introduced to increase dissolution rate and thereby oral absorption and bioavailability of such drugs. Among various approaches, Nanosponges has shown promising results in improving solubility, wettability, dissolution rate of drug and subsequently its bioavailability. Puerarin or 4,7-dihydroxy-8β-d-glucosyl isoflavone is practically water insoluble and is abundant in the traditional Chinese medicine kudzuvine root *Pueraria lobata* **(Luo et al., 2011**). Puerarin has many beneficial properties related to cardiovascular conditions, such as hypertension, hyperlipidemia, hemicrania, coronary heart disease, myocardial infarction, and angina pectoris **(Barthe et al., 1998)**. Puerarin can also improve microcirculation, expand the coronary artery, and increase the blood flow in the brain as well as coronary artery. Puerarin also has anti-thromboxane, anti-spasm and anti-platelet aggregation properties as well **(Zhao and Xiang, 2000).**Puerarin is currently available in the market as oral preparations, such as pellets, granules, and capsules. However, these preparations have low bioavailabiliy, which results in the high daily doses and poor compliance.

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2.Materials and Methods

2.1 Formulation of Nanosponge via Emulsion solvent evaporation technique

Emulsion solvent evaporation technique was used to developed nanosponge containing drug, Puerarin 20 mg of drug was dispersed in dichloromethane containing a specified amount of ethyl cellulose. The prepared dispersed phase was dropped slowly into aqueous solution containing a specified amount of polyvinyl alcohol (PVA), with stirring rpm a constant rate for 2 h. The nanosponges were separated and collected by filtration and further dried in oven at 40°C for 24 h. The dried nanosponges were stored in vacuum desiccators to facilitate removal of residual solvent**(Sana et al., 2023).**

Table 1: Trail formulation

S. N	Formulation Code	Drug	Ethyl Cellulose	DcmDichloro Methane (Ml)	Poly Vinyl Alcohol (Ml)	Distilled Water (Ml)	Stirring Time (Rpm For 2h)
1	NP1	20 mg	1	30	1	150	1000
2	NP2	20 mg	1	30	1.5	150	1500
3	NP3	20 mg	1	30	2	150	2000
4	NP4	20 mg	1.5	30	1	150	2500
5	NP5	20 mg	1.5	30	1.5	150	3000
6	NP6	20 mg	1.5	30	2	150	1000
7	NP7	20 mg	2	30	1	150	1500
8	NP8	20 mg	2	30	1.5	150	2000
9	NP9	20 mg	2	30	2	150	2500
10	NP10	20 mg	2.5	30	1	150	3000
11	NP11	20 mg	2.5	30	1.5	150	1000
12	NP12	20 mg	2.5	30	2	150	1500
13	NP13	20 mg	3	30	1	150	2000
14	NP14	20 mg	3	30	1.5	150	2500
15	NP15	20 mg	3	30	2	150	3000
16	NP16	20 mg	3.5	30	1	150	1000
17	NP17	20 mg	3.5	30	1.5	150	1500

2.2Characterization of the Nanosponges

2.2.1Determination of particle Size (PS)

The vesicle size was measured at 25 °C using the dynamic light scattering technique with a Zetasizer Nano ZS. Samples were diluted 100 times in Milli-Q water before analysis. For effective and stable formulations, parameters like the PDI are employed to characterize the homogenous size distribution of vesicles. The data's autocorrelation fit yielded the PDI and hydrodynamic diameter as Z-average**(Shirsand et al., 2012).**

2.2.2Entrapment Efficiency

Entrapment efficiency was found out by dialysis method. The Cellophane membrane was used as a semi permeable membrane. Here the cellophane membrane was soaked in Glycerol: water (1:3) mixture for 15 min. It was tied in an open ended tube and 1 gm of nanosponges was transferred into it. The was placed into a 250ml beaker containing 100 ml Distilled water and it was stirred by magnetic stirrer. The samples were taken every 15 min for 6 hours. The absorbance was measured at 370 nm by UV-spectrophotometer shimadzu using distilled water as blank and the entrapment efficiency was calculated by the following formula**(Patil et al., 2017).**

2.3. Optimisation

A three-factor, three-level BBD (box behnken design) was used to explore and optimize the main effects, interaction effects, and quadratic effects of the formulation ingredients on the performance of the nanosponges. This design was suitable for exploring quadratic response surfaces and constructing second-order polynomial models.Based on the analysis of the nanosponges, independent or formulation variables (ethyl cellulose; X1, PVA; X2, and Stirring time; X3) were identified . The significant response factors used to assess the quality of the formulation including mean particle size (PS; Y1), and entrapment efficiency (EE; Y2)

2.4Optimized formula

For the preparation of nanosponge solvent diffusion method was used. This method uses different proportion of ethyl cellulose and polyvinyl alcohol. The dispersed phase containing ethyl cellulose and drug was dissolved in 20 ml dichloromethane and slowly added to a definite amount of polyvinyl alcohol in 100 ml of aqueous continuous phase.

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The reaction mixture was stirred at 1500 rpm for 2hrs.Thennanosponge formed were collected by filtration and dried in the oven at 40° C for 24 hrs. The dried nanosponge was stored in vacuum desiccators to ensure residual solvent is evolved**(Srinivas et al., 2013).**

1.4.1 Percentage Yield
The nanosponges obtained after drying was weighed. Percentage yield value was calculated using the equation below.
Percentage yield= (Weight of nanosponges obtained ×100)/ (Total weight of drug and polymer)

1.4.2 Particle Size and Polydispersity Index
Particle size (z-average diameter) and Poly Dispersity Index (as a measure of particle size distribution) of drug loadedNanosponge dispersion was performed by dynamic light scattering also known as photon correlation spectroscopy (PCS) using a Malvern Zetasizer (Malvern Instruments, UK) at 25°C7 **(Patel et al., 2014).**

1.4.3 Zeta Potential
For Zeta Potential determination, 1ml of sample of nanosponges suspension was filled in clear disposable zeta cell, without air bubble within the sample, the system was set at 25ºC temperature, an electric field of about 15 V/cm and results wasrecorded . The more negative zeta potential indicates more stable the Nanospongeformulation**(Patil et al., 2014).**

1.4.4 Determination of Drug Entrapment Efficiency
Entrapment efficiency was found out by dialysis method. The Cellophane membrane was used as a semi permeable membrane. Here the cellophane membrane was soaked in Glycerol: water (1:3) mixture for 15 min. It was tied in an open ended tube and 1 gm of nanosponges was transferred into it. The samples were taken every 15 min for 6 hours. For the determination of drug entrapment, the nanosponge dispersion with known amount of drug was centrifuged at 4000 rpm for 15 minutes. The supernatant solution was separated. 5ml of supernatant was distributed with 100 ml of phosphate buffer solution pH 6.8 and the absorbance was measured .The absorbance was measured at 370 nm by UV-spectrophotometer shimadzu using distilled water as blank and the entrapment efficiency was calculated by the following formula**(Jadhao et al., 2021).**

Entrapment efficiency = % Drug content - % of maximum drug release of Unentrapped drug
1.5 Morphology of Nanosponge by Scanning Electron Microscopy
SEM analysis is significant for determination of surface characteristics and size of the particle. Scanning Electron Microscopy was operated at 15kV as an acceleration voltage. A aqueous suspension was spread in an equipment cell receiver and dried under vacuum. The sample was shown on a 20 mm thickened gold layer cathodic evaporator attached with computers which represents the images of the sample. The represented images were recorded and individual Nanosponge diameter was measured**(Subhash et al., 2016).**

2.6Nanospoges gel of optimised formulation
Precisely weighed amount of Carbopol-934 was soaked in water (around 100 mL) for 2 h and neutralized with triethanolamine and stirred continuously. Drug loaded NS (equivalent to topical doses of drugs) were dissolved in polyethylene glycol. This mixture was then transferred to the carbopol mixture and mixing was done for further 20 min. The dispersion was kept aside for 60 min, for complete hydration and swelling of gel components **(Shameem et al., 2021).**

2.7 Evaluation of nanosponges gel
2.7.1 Physical appearance
The physical appearance of the gel was analyzed by the visually and normal human senses. The organoleptic properties included color, odor, and texture.

2.7.2 Determination of pH
Digital pH meter was used to determine pH of the prepared gels. The samples were analyzed in triplicate. If slight deviations in pH were noted, it was adjusted to skin pH using dropwise addition of triethanolamine solution**(Pandey et al., 2018).**

2.7.3 Homogeneity
After placing the gels in the container, all formulations were tested for homogeneity (aggregates presence and appearance) by inspecting visually.

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2.7.4 Homogeneity
After placing the gels in the container, all formulations were tested for homogeneity (aggregates presence and appearance) by inspecting visually.

2.7.5 Spreadability studies
Spreadability is a mean of measuring the extent at which the semisolid formulations gets readily spread onto the administration site post application of little shear. Spreadability of the NS gel formulation was determined using wooden block-glass slide apparatus. Herein, approximately 20 g of weight was applied to upper sliding glass slide and estimation of time needed for complete detachment of upper movable slide from lower fixed slide was done. Higher spreadability is expressed by least time needed for separation of two slides **(Shameem et al., 2021).**

2.7.6 Viscosity studies
All measurements were carried out by viscometer (DV-II+, Brookfield engineering laboratories, Inc., MA, and USA) with spindle No. 6 at 10 rpm and at temperature of 37 ± 0.5 °C. The rheological properties of the formulated NS gels were studied at different rpm and the viscosity was recorded in cP**(Jadhao et al., 2021).**

2.8 In vitro drug release studies
NS based gels were permeated through a commercial semipermeable cellophane membrane (Hi-media) using Franz diffusion apparatus with a donor chamber and water jacketed receptor chamber maintained at 37 ± 0.5 °C. 1 g of gel was placed carefully on the cellophane membrane, which was placed between the donor and receptor compartments. The receptor compartment contained PBS pH 7.4, while the donor compartment was empty and open to atmosphere. The contents of the receptor section were kept at 37 ± 0.5 °C with continuous stirring at rate of 25 rpm, using a magnetic stirrer. Aliquots (2 mL) were withdrawn at regular intervals from the receptor compartment and an equal volume of fresh preheated receptor medium was replaced so as to maintain the constant volume of media and the sink condition. Collected samples were spectrophotometrically analyzed using UV visible spectrophotometer (1700, Shimadzu, Japan) at 370 nm and the amount of drug released from gel was calculated**(Manjula et al., 2014).**

Drug release kinetic study:
To study the kinetics of in vitro drug release, data was applied to kinetic models such as first order, Higuchi.

First order:
$\text{Log } C = \text{Log } C_o - K_t / 2.303$
Where C_o is the initial concentration of drug, K is the first order constant, and t is the time in hrs.
Plot a graph of Log cumulative percent drug remaining Vs time.

Higuchi:
$Q_t = k_t^{½}$
Where Q_t is the amount of the release drug in time t, K is the kinetic constant and t is time in hrs.
Plot a graph of cumulative percent drug release Vs square root of time.
The release exponent was studied **by Korsmeyer–Peppas Equation**
Qt/Qo = KPtn (4)
Where Qt is the amount of Tz released in time t; Qo is the initial amount

2.9 Stabilityof the optimized formulation
As per ICH Guidelines, stability studies were conducted for optimized NS based gel formulation for a period of 6 months and storage conditions were 25 °C/60% RH, 30 °C/60% RH and 40 °C/75% RH. The optimized formulation was analyzed for changes in physical appearance and viscosity at regular time intervals during the study period. Results that obtainedindicated that there was no significant change in appearance and drug content of NS formulation after subjection to stress testing for the 6 months period**(Manjula et al., 2014).**

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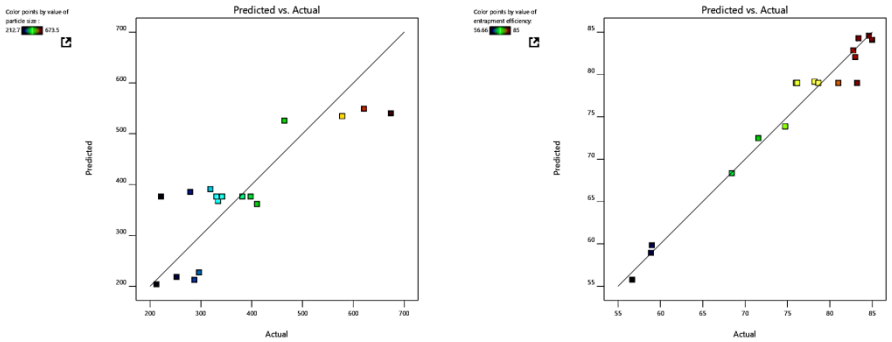
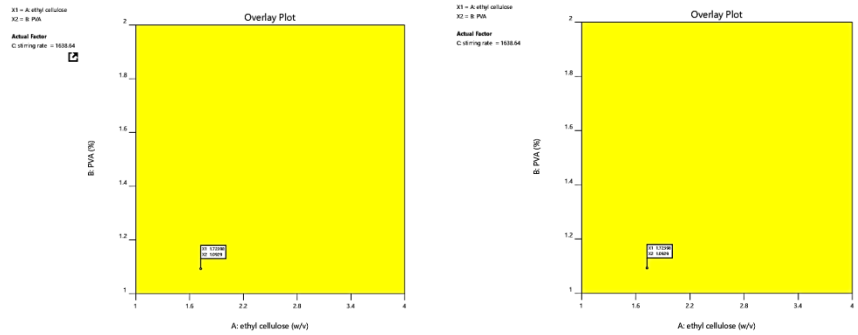
3. Results and discussion

3.1 Optimization



Graph 1: Predicted vs actual value of particle size and entrapment efficiency dependent variable



Graph 2: Overlay plot of particle size and entrapment efficiency dependent variable

3.2 Percentage Yield

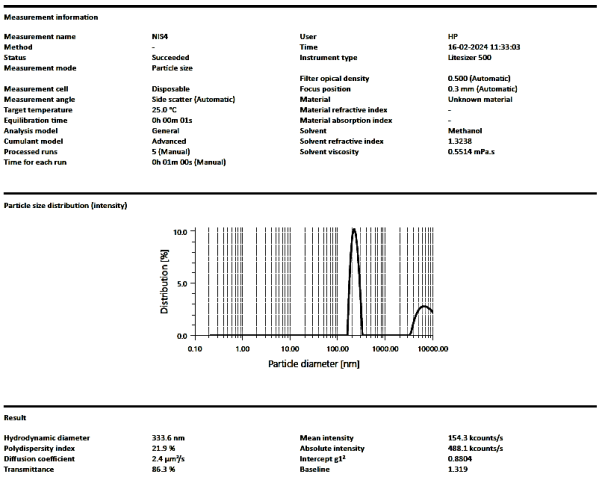
Table2:Percentage yield

S.N	Formulation code	Percentage yield
1	NP9	87%

3.3Particle Size and Polydispersity Index

Table 3: Particle Size and Polydispersity Index of the optimized formulation

S.N	Formulation code	Particle Size (nm)	Polydispersity Index
1	NP9	333.6	21.9%



Graph 3: Particle Size and Polydispersity Index of the optimized formulation

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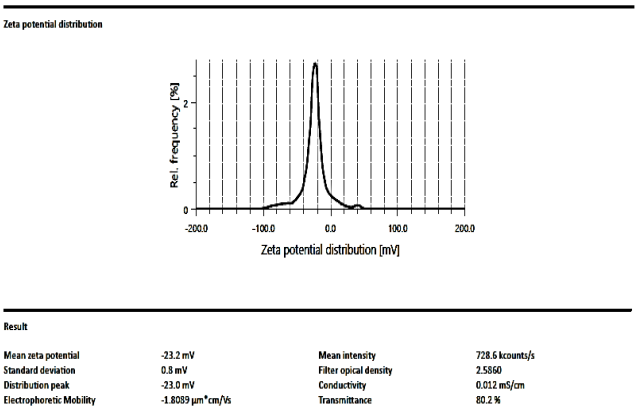
3.4 Zeta Potential

Table 4:Zeta Potential of the optimized formulation

S.N	Formulation code	Zeta Potential (mv)
1	NP9	-23.2



Graph 4 :Zeta Potential of the optimized formulation

3.5 Entrapment Efficiency

Table 5:Entrapment Efficiency of the optimized formulation

S.N	Formulation code	Entrapment Efficiency %
1	NP9	85 %

3.6 Morphology of Nanosponge by Scanning Electron Microscopy

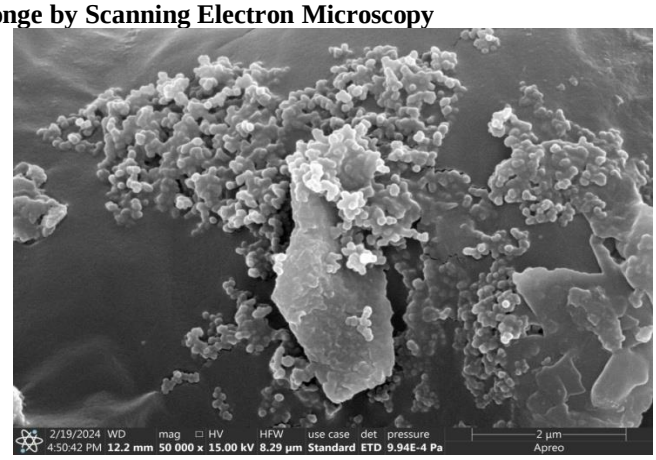


Fig 1: SEM of the optimized formulation

3.7 Evaluation of nanosponges gel

Table 6: Represent the physical properties of the optimized gel loaded with drug

S.N.	Formulation code	Parameters	Result
1	NP9G	Color	Whitish in color
		Texture	Smooth
		Appearance	Pleasant
		Odour	Odourless
		Gritty	Non gritty
2.	NP9G	pH.	6.98 ±0.054
3.	NP9G	Homogeneity	Homogenous mixture
4.	NP9G	Spreadability studies	26.22 ±0.024
5.	NP9G	Viscosity	5421 cp ±0.016

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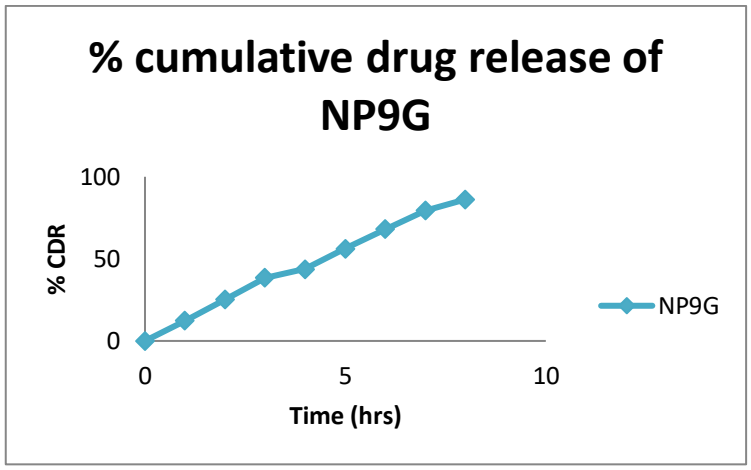
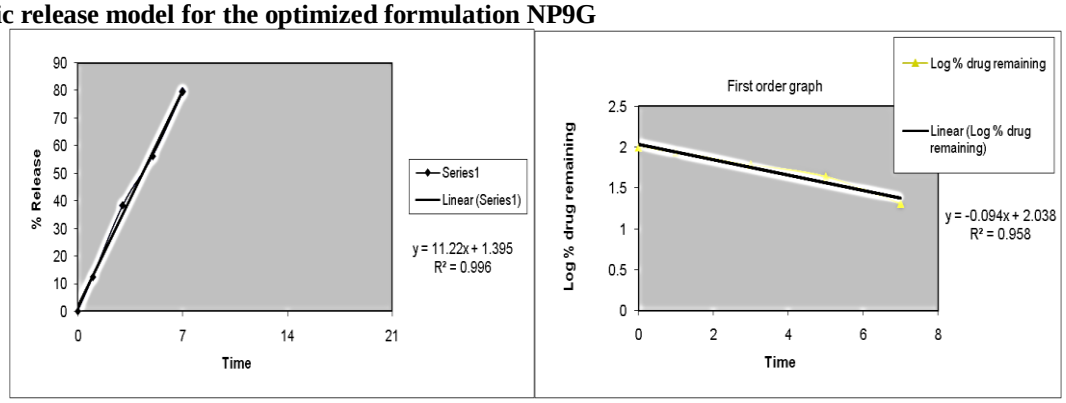
Table 7: Represent the % cumulative drug release of theNP9G gel loaded with drug

S.N.	Time (hrs)	NP9G
1	**0**	0
2	1	12 .40±0.62
3	2	25.26±0.66
4	3	38.50±0.42
5	4	43.74±0.38
6	5	56.19±0.73
7	6	68.23±0.66
8	7	79.47±0.23
9	8	86.24±0.73



Graph 5: % cumulative drug release of NP9G

3.8 Kinetic release model for the optimized formulation NP9G



Graph 6: zero order kinetic release model for NP9G and first order kinetic release model for NP9G

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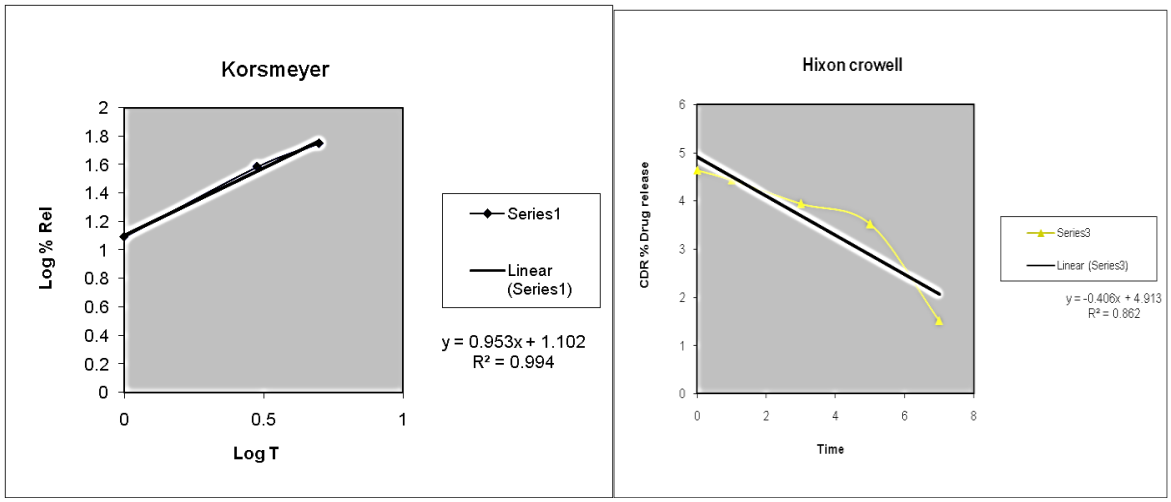
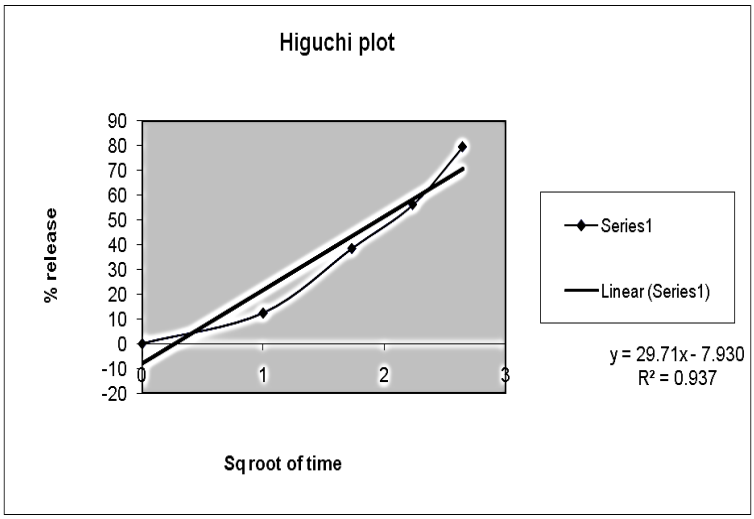


Graph 7: Korsmeyer kinetic release model for NP9G and Hixon crowell kinetic release model for NP9G



Graph 8: Higuchi kinetic release model for NP9G

3.9 Stability of the optimized formulation

Table 12:Stability study data of NP9G based gel formulation

Stability testing conditions	Sampling interval (months)	Physical appearance	Viscosity *
25 °C /60 ± 5% RH	0	No change	5421 ±0.016
	3	No change	5456± 0.21
	6	No change	5477± 0.35
30 °C /60 ± 5% RH	0	No change	5421 ±0.016
	3	No change	5469± 0.52
	6	No change	5487± 0.28
40 °C /75 ± 5% RH	0	No change	5421 ±0.016
	3	No change	5478± 0.64
	6	No change	5496± 0.52

4. Conclusion

In conclusion, the study successfully employed the Emulsion Solvent Evaporation technique to develop nanosponge formulations containing Puerarin. The optimization of key formulation parameters such as ethyl cellulose concentration, polyvinyl alcohol content, and stirring time was crucial for achieving desirable characteristics. Among the formulations tested, NP9 emerged as the optimal formulation, demonstrating a particle size of 252.1 nm, an entrapment efficiency of 84.6%, and a high percentage yield of 87%. The Zeta potential of NP9 was found to be -23.2 mV, indicating good stability.

The drug-loaded nanosponges were further incorporated into gel formulations, which were evaluated for physical appearance, homogeneity, spreadability, viscosity, and drug release. The NP9-based gel showed favorable characteristics with smooth texture, homogeneous composition, and a pH close to skin's natural pH (6.98). In vitro drug release studies revealed sustained drug release over time, with approximately 86.24% of the drug released by the eighth hour. The release kinetics followed the Higuchi model, indicating diffusion-controlled release.
Overall, the findings suggest that the nanosponge-loaded gels are promising candidates for controlled drug delivery systems, with potential applications in topical formulations due to their excellent stability, entrapment efficiency, and sustained release properties.

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