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Formulation and Evaluation Puerarin Loaded Nanosponges

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ABSTRACT

Objective: The objective of the present study was to develop and characterize optimal stable nanosponges of puerarin by using the emulsion solvent evaporation technique.

Methods: Pre-formulation studies of Peurarin were carried out. After that calibration curve was assessed for the determination of drug concentration present. The peurarin nanosponges was prepared by emulsion solvent evaporation technique (ESE-Tech) using the drug (Puerarin) 100 mg and polyvinyl alcohol (PVA) 0.3%, organic phase was prepared by dissolving ethyl cellulose (EC) (100–350 mg) and Puerarin in 20 mL dichloromethane (DCM). Fourier transform infrared spectroscopy (FTIR) estimated the compatibility of puerarin with polymer. All formulations evaluated for production yield, entrapment efficiency, in vitro drug release, scanning electron microscopy (SEM) and stability studies.

Results: The FTIR Studies revealed that no interaction between drug and polymer. The average particle size of optimized formula (F4) was 140.8nm. High drug entrapment efficiency F4 formulation, measured at 98.73%, was possessed by the puerarin loaded nanosponges. With the help of dialysis bag diffusion method in vitro drug release studies was performed for the optimized formulations and all the dilutions measured at the wave length 244nm. At the end of 24th hour F4 formulation shown highest drug release was found to be 97.92±1.12%. The release kinetics of the optimized formulation follows zero-order drug release. The stability study indicates no significant change in the particles size, zeta potential and entrapment efficiency of optimized formulation.

Conclusion: The results of various evaluation parameters revealed that peurarin nanosponges would be possible alternative delivery systems to conventional formulation to improve the therapeutic value of drug.

Keywords: Nanosponge, Peurarin, SEM, Ethyl cellulose, FTIR, Polymer.

1. INTRODUCTION

Drug delivery technology has rekindled interest in pharmaceuticals by focusing on therapeutic goals. Researchers are currently dealing with a fundamental problem: targeting medicine delivery. Therapeutics will focus on target-oriented drug delivery to improve efficacy,

minimize side effects, and optimize dose regimens. Targeted drug delivery involves selectively delivering pharmacologically active molecules to specific targets at therapeutic concentrations, while limiting access to non-target normal cellular linings. This minimizes toxic effects and maximizes the therapeutic index of the drug **(Vyas and Khar, 2008)**. Nanosponges are an interesting subject of study due to their potential for regulated drug delivery. Nanosponge delivery systems can accurately manage drug release rates and target specific body sites, significantly impacting the healthcare system. This nano sized delivery system offers advantages for drug delivery due to its high stability, carrier capacity, and ability to incorporate hydrophilic and hydrophobic compounds. Research in this area focuses on using nanosponges for targeted and localized therapeutic agent delivery **(Jilsha and Viswanand, 2013)**. Nanosponges are colloidal structures that have the ability to encapsulate a broad range of materials, including volatile oil, DNA, proteins and peptides, and antitumor medicines. The diameter of nanosponges is less than 1 μm, while that of microsponges is roughly 10–25 μm with a void size of 5–300 μm. That is why nanosponges are superior to microsponges. At 300 ℃, nanosponges exhibit strength and stability, whereas microsponges demonstrate stability and fragility only up to 130 ℃ **(Krabicova et al., 2020)**. Their lipophilic nature allows them to disperse in water, acting as a moving medium to assist cover up bad tastes and change the compound's liquid form to solid state. Measuring the medication's beneficial charge are biodegradable polyesters such polyglycolic acid (PGA) and cyclodextrin-dependent products **(Pawar et al., 2019).** The nanosponge is naturally degradable polyester that serves as a substance's backbone. It is around the size of a virus. The long polyester threads are mixed in solution with small molecules known as cross-linkers, which are better suited for specific polyester components **(Amin et al., 2020).** The polyester bits are cross-connected to form a spherical shape with several cavities or voids inside of it. These nanosponges are a collection of unique nanoparticles that are usually taken from their natural sources. Compared to the other nanoparticles, they are porous, non-toxic, and insoluble in organic solvents and water **(Cavalli et al., 2010).** In order to preserve degradable molecules, enhance lipophilic water solubility, and create channels for the delivery of medications for routes other than oral usage, nanosponges can be employed as a therapeutic tool **(Singireddy et al., 2019).** Simple manipulation, a rudimentary understanding of polymer chemistry, and the proper use of cross-linkers make preparation easy. Therefore, it is simple to scale up commercial manufacturing using this strategy. While soluble in water, nanosponges are not biodegradable in the presence of sunshine. It turns oils into solids and can be used to mask offensive smells. The nanosponges are able to adhere well to the target spot because of their chemical bonds **(Sherje et al., 2017).** Puerarin is the primary bioactive substance extracted from the root of Pueraria lobata (Willd.) Ohwi, also known as Gegen (Chinese) in traditional Chinese medicine**.** It can be found in regular meals and is employed in complementary therapies. Numerous conditions have been treated with it, including cancer, endometriosis, Parkinson's disease, Alzheimer's disease, diabetes and its complications, osteonecrosis, and cardiovascular and cerebrovascular illnesses. Puerarin's wide range of pharmacological qualities, which include vasodilatation, cardio protection, neuroprotection, antioxidant, anticancer, and anti-inflammatory effects, as well as pain relief, bone formation promotion, alcohol intake inhibition, and insulin resistance attenuation, may be the cause of its beneficial effects on a variety of medicinal purposes **(Zhou**

et al., 2014). The main objective of this study was to formulate and evaluate the puerarin loaded nanosponges.

2. MATERIAL AND METHOD

2.1 Chemicals

Peurarin was gifted by FDC, Mumbai, India and all other ingredients were of Analytical grade.

2.2 Pre-formulation studies

2.2.1 Organoleptic Properties

Organoleptic properties of puerarin were observed by visual observation. The organoleptic studies of puerarin like general appearance like color, odor, state, etc. were observed\ performed.

2.2.2 Solubility study

Qualitative solubility of puerarin in different solvents was determined according to USP NF, 2007. Approximately 1 mg of puerarin was weighed and transferred into a 10 ml test tube and dissolved in the respective solvents (1 ml each of methanol, ethanol, acetonitrile, and water)**(Jain and Verma 2020).**

2.2.3 Melting Point

Melting point was analyzed by open Capillary method using Thiele's tube. Few quantity of the puerarin was placed in a thin walled capillary tube 10-15 mm long, about 1mm inside diameter, and closed at one end. Liquid paraffin oil was filled in the thieles tube and placed in the contact of flame. The capillary was suspended into the thiele's tube and heat the sample slowly; thermometer was attached to check the temperature. The temperature at which the sample starts to melt was taken as the melting point of the sample **(Chowk, M. I. 2020).**

2.2.4 Partition coefficient of Puerarin

Partition coefficient (Log P) value is defined as ratio of unionized drug distributed between aqueous and organic phase. Oil-water partition coefficient gives the idea about drug's ability to cross the lipidic membrane. Lipophilic/hydrophilic balance is one of the most important contributing factors for optimum drug absorption and delivery. Due to lipidic nature of biological membrane, the amount of drug absorbed depends heavily on its lipophilicity. It is the unionized form of molecule that has better lipophilicity and hence it has received so much importance. 5 mg of drug was taken in separating funnel. The separating funnel was shaken for 2 hrs in a wrist action shaker for equilibration. Two phases were separated and the amount of the drug in aqueous phase was analyzed spectrophotometrically. The partition coefficient of the drug in phase's was calculated by using formula:

$$\text{Partition Coefficient} = \frac{\textbf{Concentrationofdruginoilphase}}{\textbf{Concentrationofdruginaqueousphase}}$$

2.2.5 Determination of Lambda max and calibration curve

2.2.5.1 Lambda (λ) max

A stock standard solution containing 1 mg/mL of Puerarin was prepared in 80% methanol. Working standard solution equivalent to 100 µg/mL of Puerarin was prepared by appropriate

dilution of stock solution with the same solvent. The solution was scanned in the range of 200 – 400 nm UV spectrum using Systronix 2202 double beam spectrophotometer (**Kumbhar and Salunkhe 2013)**.

2.2.5.2 Standard calibration curve of puerarin

Determination of absorption maximum (λmax)

100 mg of Puerarin was accurately weighted into 100 ml volumetric flask, dissolved in 80% Methanol and volume was made up with same solvent. Pipette 1ml of this solution into another 10 ml volumetric flask and the volume was made with Methanol and marked as Stock. The resultant solution is scanned in the range of (200-400 nm) by UV Spectrophotometer to get absorption maximum (λ max).

2.2.6 Preparation of calibration curve

The prepared stock solution was further diluted with solvent to get working standard solution of 2, 4, 6, 8, 10, 12, 14, and 16 µg/ml of Puerarin to construct Beer's law plot for the pure drug, the absorbance was measured at λ max at 244 nm, against solvent as blank. The standard graph was plotted by taking concentration of drug on X-axis and absorbance on Y-axis in the concentration range of 2-16µg/ml. (**Behera et al., 2012).**

2.2.7 Fourier transmission Infra-Red Spectroscopy

FT-IR spectrum of Drug and drug + excipients was recorded over the range of 4000 to 400 cm−1 by KBr pellet method using a FT-IR spectrophotometer. The KBr disc was prepared using 1 mg of each Drug and drug + excipients in 100 mg of spectroscopic grade KBr which has been dried using IR lamp. Both KBr and drug was mixed and subjected to hydraulic pressure to form disc. This disc was placed in FT-IR chamber. Infrared spectrum was recorded in the 4000 - 400 cm-1 region (**Chowk, M. I. 2020)**

2.3 FORMULATION OF NANOSPONGES

Puerarin loaded NS (PNS) were prepared by the emulsion solvent evaporation technique (ESE-Tech) using the drug (Puerarin) 100 mg and polyvinyl alcohol (PVA) 0.3%, w/v, compositions of formulations were tabulated in Table 1. Briefly, organic phase was prepared by dissolving ethyl cellulose (EC) (100–350 mg) and Puerarin in 20 mL dichloromethane (DCM). Separately, an aqueous phase was prepared composed of (0.3%, w/v) PVA in 100 mL of deionised water. Thereafter, the organic phase was emulsified drop wise into the aqueous phase by ultrasonication for 3 to 5 min (**Ahmed et al., 2020**). The formed NS was stabilized by PVA, which avoid particle agglomerations. Thereafter, the dispersion was kept on thermostatically controlled magnetic stirrer ‘‘(Remi)” with continuous stirring at under atmospheric pressure and room temperature for 3 to 4 h. After complete evaporation of the organic solvent, the puerarin nanosponges were washed three times with ultra-purified water to remove the adsorbed PVA, NSs were then collected by ultra-centrifugation and 4^0C for 30 min and freeze dried **(Ahmed et al., 2021).**

Table 1: Composition of Nanosponges formulation

Ingredients	F1	F2	F3	F4	F5
Puerarin (mg)	100	100	100	100	100
Ethyl cellulose (EC) (mg)	100	150	200	250	300

Poly vinyl alcohol (PVA) (%)	0.3	0.3	0.3	0.3	0.3
Dichlomethane (DCM) (ml)	20	20	20	20	20
Distilled water (ml)	100	100	100	100	100

2.4 EVALUATION PARAMETER OF NANOSPONGES

2.4.1Particle size

The particle size analysis of puerarin loaded NS was performed by using ‘‘Malvern Zetasizer Nano ZS (Malvern Instruments. The sample under investigation was diluted with distilled water (1: 200) and filled in disposable polystyrene cuvette. Measurement of particle size was done based on the dynamic light scattering (DLS) theory. **(Ahmed et al., 2021).**

2.4.2 Zeta potential

The zeta potential was measured for the determination of the movement velocity of the particles in an electric field and the particle charge. In the present work, the nanosponges was diluted 10 times with distilled water and analyzed by Zetasizer Malvern instruments. All samples were sonicated for 5-10 minutes before zeta potential measurements (**Kumar et al., 2018, Penjuriet al., 2016).**

2.4.3 Entrapment efficiency

To calculate the entrapment efficiency accurately weighed the quantity of nanosponges (10 mg) with 5 ml of methanol in a volumetric flask was shaken for 1 min using vortex mixer. The volume was made up to 10 ml. Then the solution was filtered and diluted and the concentration of entrapped puerarin was determined spectrophotometrically at 244 nm. **(Solunke et al., 2019).**

%EE = Initial amount of drug added - Drug amount in supernatant / Initial amount of drug added * 100

2.4.4 Scanning Electron Microscopic (SEM)

The electron beam from a scanning electron microscope was used to attain the morphological features of the puerarin loaded nanosponges were coated with a thin layer (2–20 nm) of metal(s) such as gold, palladium, or platinum using a sputter coater under vacuum. The pre-treated specimen was then bombarded with an electron beam and the interaction resulted in the formation of secondary electrons called auger electrons. From this interaction between the electron beam and the specimen’s atoms, only the electrons scattered at 90° were selected and further processed based on Rutherford and Kramer’s Law for acquiring the images of surface topography. **(Anwer et al., 2019)**

2.4.5 *In-vitro* drug release study

The *in-vitro* drug release study of puerarin loaded nanosponges formulation was studied by dialysis bag diffusion method. Puerarin loaded nanosponges were dispersed into dialysis bag and the dialysis bag was then kept in a beaker containing 100 ml of pH 7.4 phosphate buffer. The beaker was placed over a magnetic stirrer and the temperature of the assembly was maintained at 37 ± 2 ℃ throughout the experiment. During the experiment rpm was maintained at 100 rpm. Samples (2 ml) were withdrawn at a definite time intervals and replaced with equal amounts of fresh pH 7.4 phosphate buffers. After suitable dilutions the samples

were analyzed using UV–Visible spectrophotometer at 244 nm. To analyze the *in vitro* drug release data various kinetic models were used to describe the release kinetics.

To analyze the *in vitro* release data various kinetic models were use to describe the release kinetics. The zero order rate Eq. (2) describes the systems where the drug release rate is independent of its concentration. The first order Eq. (3) describes the release from system where release rate is concentration dependent. Higuchi described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion. The results of *in vitro* release profile obtained for all the formulation were plotted in modes of data treatment.

Zero - order kinetic model – Cumulative % drug released versus time.

First – order kinetic model – Log cumulative percent drug remaining versus time.

Higuchi's model – Cumulative percent drug released versus square root of time.

Korsmeyer-Peppas model - log cumulative % drug release vs log time (Kors-meyer–Peppas model)

2.4.5.1 Zero order kinetics

$$A_t = A_0 - K_0 t \quad \text{-------- Eq. (2)}$$

Zero order release would be predicted by the following equation:

Where,

At=Drug release at time't'

A0=Initial drug concentration.

K0=Zero-order rate constant (hr^{-1})

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys Zero– order kinetics and its slope is equal to Zero order release constant K0.

2.4.5.2 First order kinetics

$$\mathbf{LogC = logC_0 - Kt/2.303} \text{-------- Eq. (3)}$$

First-order release could be predicted by the following equation:

Where,

C=Amount of drug remained at time't'

C0=Initial amount of drug.

K=First-order rate constant (hr^{-1}).

When the data plotted as log cumulative percent drug remaining versus time, yields astraight line, indicating that the release follow first order kinetics. The constant 'K1'can be obtained When the data plotted as log cumulative percent drug remaining versus time, yields a straight line, indicating that the release follow first order kinetics. The constant 'K1' can be obtained by multiplying 2.303 with the slope value.

2.4.5.3 Higuchi's Model

Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation:

$$Q = [D\epsilon / \tau (2A - \epsilon Cs) Cst]^{1/2} \quad \text{.....eq (4)}$$

Where,

Q= Amount of drug release at time‘t’
D= Diffusion coefficient of the drug in the matrix.
A= Total amount of drug in unit volume of matrix.
Cs= Solubility of drug in the matrix
Є= Porosity of the matrix.
τ= Tortuosity.
t= Time (hrs at which amount of drug is released.

Above equation can be simplified as if we assume, that ‘D’, ‘Cs’ and ‘A’ are constant. Then equation: becomes**.**

2.4.5.4 Korsmeyer-Peppas model

Korsmeyer et al. (1983) derived a simple relationship which described drug release from a polymeric system equation (5). To find out the mechanism of drug release.

$$Mt / M\infty = Kt^n \quad \text{..... (5)}$$

Where Mt / M∞ are a fraction of drug released at time t, k is the release rate constant and n is the release exponent. The n value is used to characterize different release for cylindrical shaped matrices.

2.4.6 Stability study

Final puerarin loaded nanosponges were subjected to a stability testing for three months as per ICH guidelines at a temperature of 25° ± 2°C and 60% RH and 40^0C±2 ^{0}C and 70 ±5% RH . F4 Nanosponges formulation was analyzed for the change in, Particle size, *zeta potential and* entrapment efficacy.

3. RESULTS AND DISCUSSION

3.1 Pre-formulation studies

Pre-formulation studies of the drug were performed and the results are given below.

3.1.1Solubility Studies of Puerarin

Table 2: Solubility of puerarin

Drug	Solvents	Category
Puerarin	Methanol	Freely soluble
	Ethanol	Soluble
	Acetonitrile	Freely soluble
	Water	Soluble

Discussion

The solubility of Puerarin was determined in various non-volatile or volatile liquid vehicles such as Methanol, ethanol, acetonitrile, and water shown in **Table 2**. From the results, it was observed that the drug is freely soluble in Methanol, water and soluble in ethanol, water.

3.1.2 Melting Point determination.

Table 3: Melting point of Puerarin

S. No.	Drug	Specification	Inference
1	Puerarin	187-189ºC	188ºC

Discussion

The capillary method is used to determine the melting point of a substance. The melting point of the Puerarin was found to be 188ºC, which is well within the limits of the drug specification.

3.1.3 Partition coefficient of Puerarin

Table 4: Partition coefficient of puerarin

S. No.	Drug	Solvent	Inference
1	Puerarin	n-octanol : water	1.87

Discussion

Partition coefficient is calculated by the ratio of equilibrium concentration of a dissolved substance in a two Phase system they are n-octanol and water. The partition coefficient of puerarin was found out to be 1.87.

3.1.4 Determination of λmax

Solution was scanned under UV-Vis Spectrophotometer and λ max was determined. It was found to be as per the monograph.

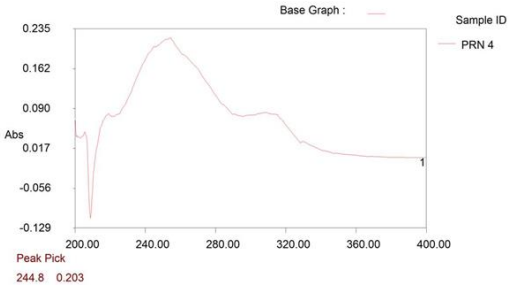

Figure 1: λmax of Puerarin

Double beam UV visible spectrophotometer (2202) was used to determine the lambda max (absorption maxima) of a substance. The lambda max of the puerarin was found to be 244 nm. This is well within the limits of the drug specification.

3.1.5 Standard calibration curve of Puerarin

All dilutions and measurements were made in methanol and the absorbance was taken at λ max 244 nm against a methanol blank. The standard curve was plotted between absorbance and concentration**.**

Table 5: Calibration curve of puerarin

S. No.	Concentration (µg/ml)	Absorbance (244nm)
1	2	0.059
2	4	0.101
3	6	0.192
4	8	0.278
5	10	0.359

6	12	0.496
7	14	0.539
8	16	0.618

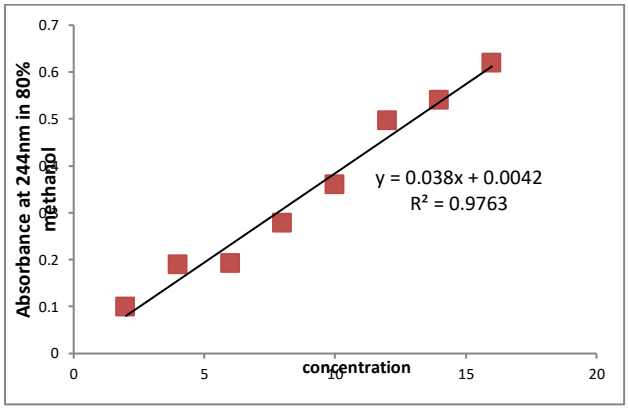

Figure 2: Calibration curve of Puerarin in 80% Methanol at 244 nm

3.1.6 Drug excipient compatibility by FTIR

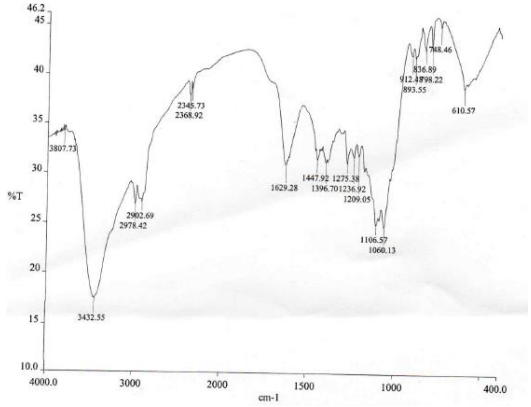

Figure 3: FTIR graph of puerarin

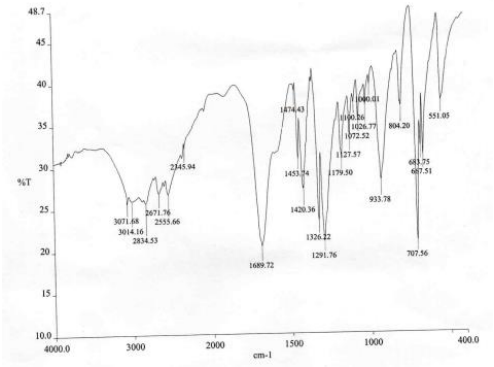

Figure 4: FTIR graph of Drug + Excipients

3.2 Evaluation parameter of nanosponges formulation

3.2.1 Particle size

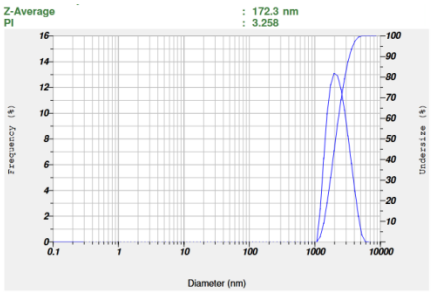

Figure 1: Particle size (Formulation 1)

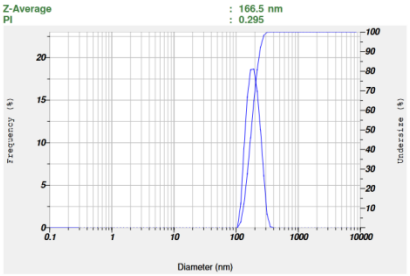

Figure 2: Particle size (Formulation 2)

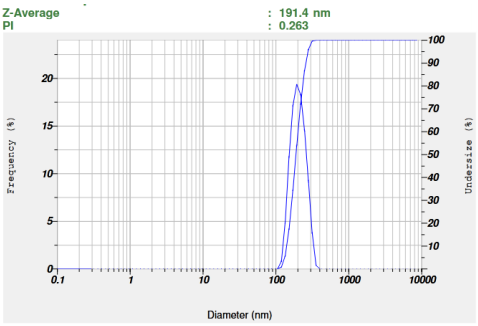

Figure 3: Particle size (Formulation 3)

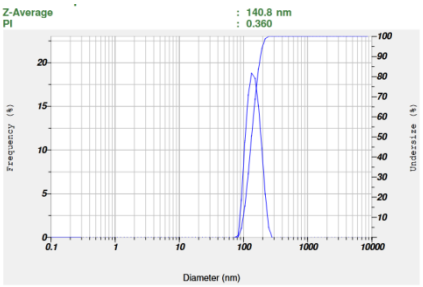

Figure 4: Particle size (Formulation 4)

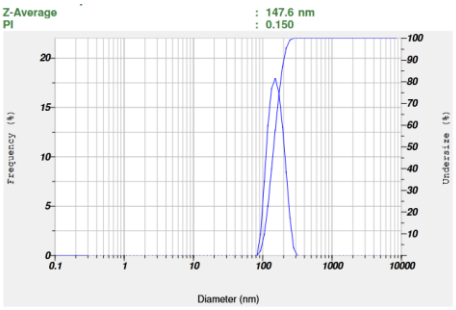

Figure 5: Particle size (Formulation 5)

Table 6: Particle size of Drug loaded nanosponges

S.No	Formulation	Particle size	PI value
1	F1	172.3nm	3.258
2	F2	166.5 nm	0.295
3	F3	191.4 nm	0.263
4	F4	140.8 nm	0.360
5	F5	147.6 nm	0.150

Discussion

The particle size is one of the most important parameter for the characterization of nanosponges. The average particle sizes of the prepared drug loaded nanosponges formulation were measured using Malvern zeta sizer. Particle size analysis showed that the average particle size of nanosponges was found to be range between 140.8 to 191.4 nm. These particle size values indicate that the all formulated nanosponges is under the range (Below 1000 nm) of nanosponges and F4 is the lowest particle size of all formulation shown in above **table 6**.

3.2.2 Zeta potential

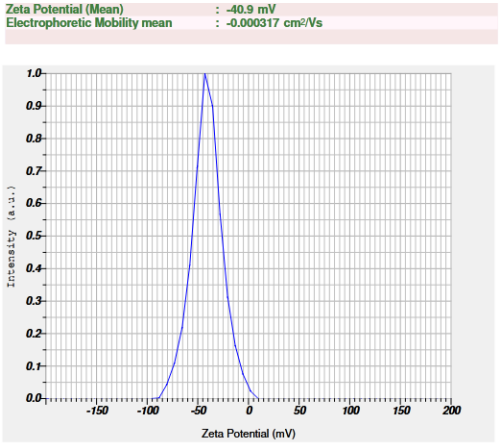

Figure 6: Zeta potential (Formulation 1)

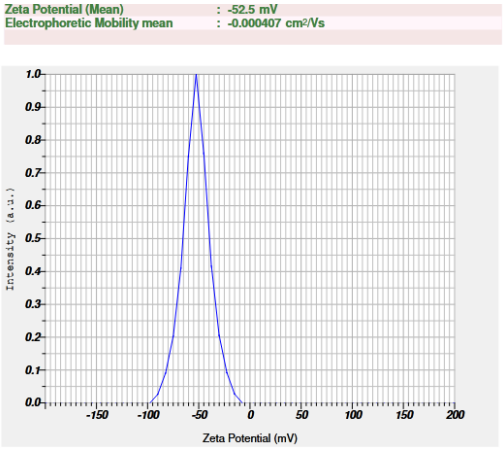

Figure 7: Zeta potential (Formulation 2)

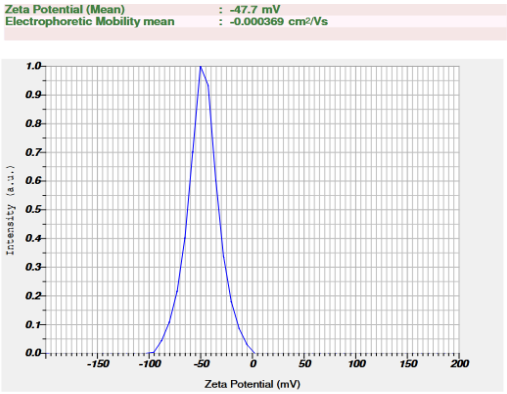

Figure 8: Zeta potential (Formulation 3)

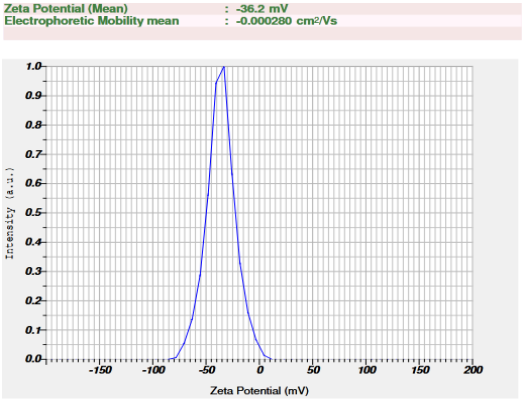

Figure 9: Zeta potential (Formulation 4)

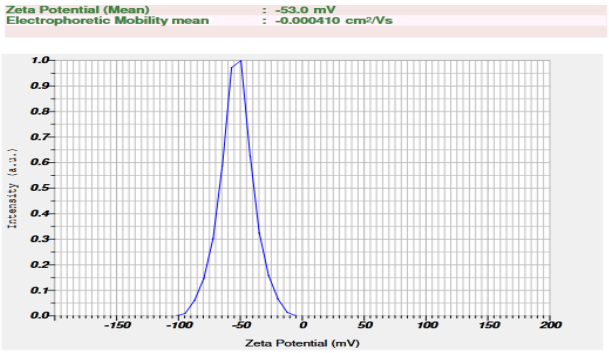

Figure 10: Zeta potential (Formulation 5)

Table 7: Zeta potential

S.No	Formulation	Zeta potential
1	F1	-40.9 mV
2	F2	-52.5mV
3	F3	-47.7mV
4	F4	-36.2mV
5	F5	-53.0mV

Discussion

Zeta potential analysis is carried out to find the surface charge of the particles. The magnitude of zeta potential is predictive of the colloidal stability. Zeta potential was found to be all formulation range -36.2 to -53.0 mV with peak area of 100% intensity. These values indicate that the all formulated nanosponges are stable.

3.2.3 Scanning Electron Microscopic (SEM) of F4 formulation

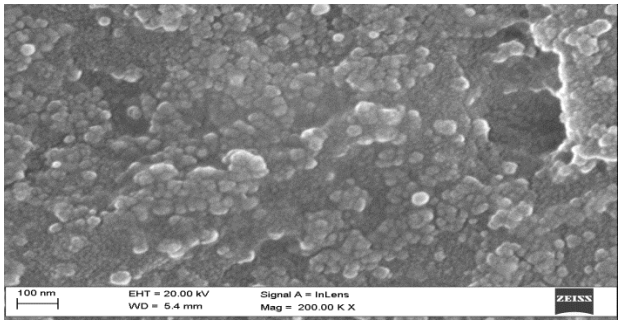

Figure 11: SEM (F3)

Discussion

SEM analysis was performed to determine their silver nanosponges characters (shape & morphology) of prepared drug loaded nanosponges. Puerarin loaded nanosponges were prepared and dried well to remove the moisture content and images were taken using scanning electron microscopy. Scanning electron micrograph of the prepared nanosponges at 200.00 kx magnification showed that the nanosponges were smooth surface morphology and spherical shape. The smooth surface morphology and spherical shape of silver nanosponges was clearly observed in the SEM images.

3.2.4 Entrapment efficiency

Table 8: Entrapment efficacy

S.No.	Formulations	Entrapment efficacy (%)
1.	F1	78.49
2.	F2	89.53
3.	F3	91.25
4.	F4	98.73
5.	F5	97.14

Discussion

This might be due to the fact that the variation in entrapment efficiency was due to the changes in the polymer concentration. The prepared puerarin loaded nanosponges possesses high drug entrapment efficiency F4 formulation and found to be 98.73%

3.2.5 *In-vitro* drug release study of F4 Formulation

Table 9: Release kinetics study of final formulation

Time (Hr)	cumulative % drug released	% drug remaining	Square root time	log Cumu % drug remaining	log time
0	0	100	0.000	2.000	0.000
2	14.12	85.88	1.414	1.934	0.301
4	25.01	74.99	2.000	1.875	0.602
6	39.56	60.44	2.449	1.781	0.778
8	56.19	43.81	2.828	1.642	0.903

10	67.32	32.68	3.162	1.514	1.000
12	72.08	27.92	3.464	1.446	1.079
16	86.89	13.11	4.000	1.118	1.204
24	97.92	2.08	4.899	0.318	1.380

Table 10: Correlation value (R^2 value)

Formulation	Model	Kinetic parameter values
Nanosponges	Zero Order	$R^2 = 0.904$
	First Order	$R^2 = 0.795$
	Higuchi	$R^2 = 0.955$
	Korsmeyerpeppas	$R^2 = 0.860$

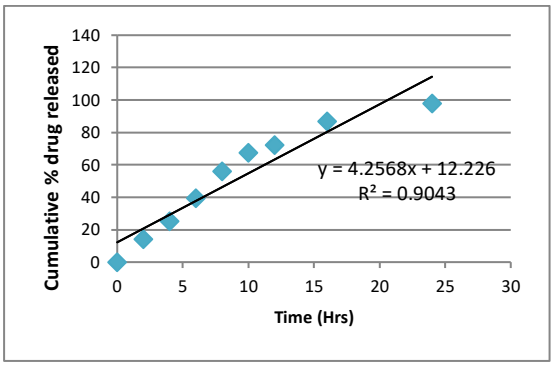

Figure 12: Zero order kinetic model

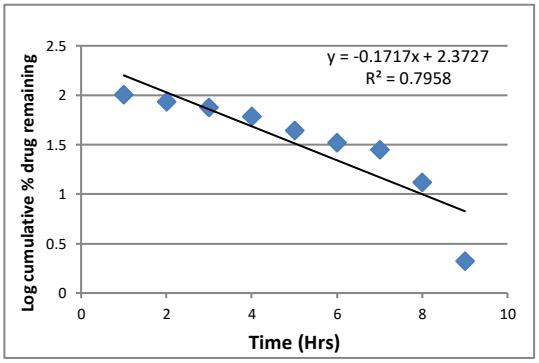

Figure 13: First Order kinetic model

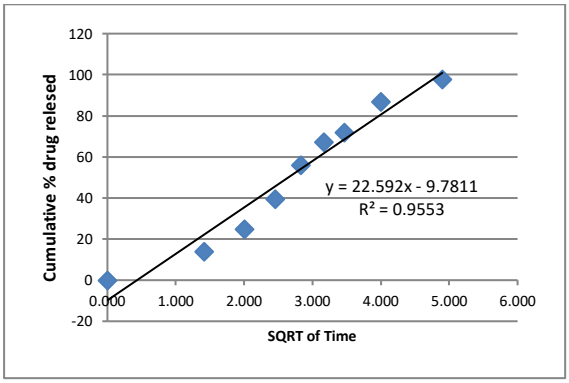

Figure 14: Higuchi model

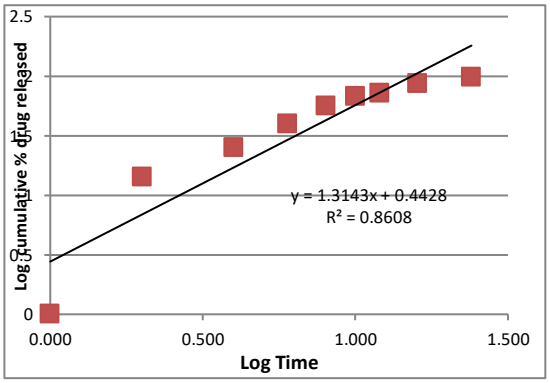

Figure 15: Korsmeyerpeppas

Discussion

The data of percentage drug release formulation were shown in Fig. 16 to 19. For kinetic study following plots were made: cumulative % drug release vs. time (zero order kinetic models); log cumulative % drug remaining vs time (first order kinetic model); cumulative % drug release vs square root of time (Higuchi model); log cumulative % drug release vs log time (Korsmeyer–Peppas model). All Plots are shown in Fig. 16 to19 and results are summarized in Table 10. Zero order kinetic models refer to the process of constant drug release from a drug delivery device independent of the concentration. The zero order graph of optimized formulation showed the constant drug release from the nanosponges, the results of the zero order model was found to be y = 4.256x + 12.22 R^2 = 0.904. The first order kinetic model describes the release from system where release rate is concentration dependent. The results of first order kinetic model was found to be y = -0.171x + 2.372 R^2 = 0.795. The Higuchi model is used to describe the limits for transport and drug release. The Higuchi model of formulation

was found to be, y= 22.59x - 9.781 R^2 = 0.955. And the results of Korsmeyer peppas kinetic model was found to be y = 1.314x + 0.860 R^2 = 0.860. *In-vitro* drug diffusion studies were carried out using dialysis bag method. In the above table R2 is correlation value. On the basis of best fit with the highest correlation (R2) value it is concluded that in the final formulation of nanosponges follow the Higuchi kinetic model.

5.5 Stability Study

Table 11: Stability Study of Nanospongess

S.No	Time (Days)	25^0C±2 ^{0}C and 60 ± 5% RH			40^0C±2 ^{0}C and 70 ±5% RH		
		Particle size	EE (%)	Zeta potential	Particle size	EE (%)	Zeta potential
1.	0	140.8	98.73	-36.2mv	140.8	98.73	-36.2mv
2.	30	140.9	98.55	-36.1mv	140.9	98.02	-36.2mv
3.	60	140.7	98.08	-35.9mv	141.0	97.99	-36.9mv
4.	90	140.8	98.60	-36.8mv	141.1	98.75	-35.8mv

Discussion

The selected formulation F4 was evaluated for stability studies which were stored at temperature of 25° ± 2°C and 60% RH and 40^0C±2 ^{0}C and 70 ±5% RH for 90 days (3 month) and were analyzed for their assay, zeta potential, particle size and entrapment efficiency etc. There was no significant change in the physicochemical properties of nanosponges formulation during the stability period. There was a slight increase in particle size for the stored formulation, but it was well within the acceptable limit.

CONCLUSION

Nanosponges are tiny mesh-like structures with a size less than 1µm. Due to their porous structure and small size; they can easily bind to drugs which are poorly-soluble leading to better bioavailability and solubility of such drugs. In the present research work the Puerarin loaded nanosponges was prepared, this drug is poorly soluble in water (BCS IV) and has less bioavailability, it's a basic problem faced by a formulation scientist. Therefore, in this study puerarin loaded in nanosponges to improve solubility and bioavailability. So this can be overcome by developing nanotechnology based formulation like nanosponges which may increase the bioavailability. Through the different evaluation parameters the formation of nanosponges confirmed and via drug release study as well as solubility study proven the enhancement of the bioavailability of the drug. The discovery of nanosponges have become a significant step in overcoming certain problems such as drug toxicity, poor bioavailability and release of drug in a predictable fashion as they can accommodate both hydrophilic and hydrophobic drug. Nanosponges exhibit a porous structure in nature which has the unique ability to entrap the drug moieties and offers a merit of desire release.

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