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Plumbagin Loaded Nonionic Surfactant Vesicle is An Effective Phytochemical Nanocarrier: Formulation, Optimization and Characterization

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Abstract

The aim of the present study was to develop and evaluation of Plumbagin loaded niosomes as an effective nanocarrier for delivery of phytochemical. Plumbagin loaded into niosomes to increase its solubility and hence efficacy. Plumbagin loaded niosomes was prepared by reverse phase evaporation method followed by sonication. The particle size, PDI, zeta potential, entrapment effectiveness, invitro drug release, in vitro kinetic release were used to characterize the Plumbagin niosomes. Based on particle size, zeta potential, PDI and entrapment efficiency FC5 batch formulation was chosen as optimized formulation, resulted in the particle size (123.5± 4.82 nm), PDI (0.143±0.067), zeta potential (-26.8±2.13 mV). The entrapment efficiency was (91.42±2.19) %. Invitro drug release, niosomes shown sustain release behavior as compared as compared pure Plumbagin. The initial drug release from formulation was found to be 24.62 % in 1 hour followed by sustained release of drug was observed till 24 hours. The drug release in 24 hours was 95.28.

Keywords: Niosomes, Plumbagin, Phytoconstituents, Solubility, Entrapment Efficiency.

Introduction

In the recent year, the various plant derivatives have been suggested as natural remedies for the treatment of various kind of disease, due to its high bioavailability, low side effects, low cost [1]. The use of natural materials for medicinal purposes and alternative therapies have garnered increased attention in recent years [2]. Over the past few decades, a class of phytoconstituents known as 1, 4-naphthoquinones has been extracted from natural resources. These compounds have a wide range of biological attributes and regulate several pharmacological functions, which makes them promising new targets for a variety of illnesses [3, 4]. Plumbagin (5-hydroxy-2-methyl-1, 4-napthoquinone, PLB), a naturally occurring naphthoquinones, has been found in the roots of the Plumbago zeylanica L. plant used in traditional medicine. Numerous biological benefits of Plumbagin have been demonstrated, including analgesic, anti-inflammatory, antioxidant, anti-cancer, antibacterial, antifungal, and anti-atherosclerosis properties [5]. Plumbagin's bioavailability is only 39% when taken orally due to its restricted biopharmaceutical properties, which include high lipophilicity (log P = 3.04), insolubility in water (79.3 ± 1.7 µg/ml) a short biological half-life (35.89 ± 7.95 min), and a low melting point. Plumbagin has the potential to address problems pertaining to its biopharmaceutical characteristics when administered in a different and appropriate configuration [6, 7, 8]. By moving from traditional form to natural substances put into nanocarrier, problems with solubility, penetration, toxicity, bioavailability, etc. can be resolved.

Formulation scientists are interested in using nanotechnology to deliver medications with low side effects and increased efficacy. When medications build up in particular target locations regardless of the mode and route of administration, this is known as targeted drug delivery. Active or passive targeting strategies can direct drugs to the intended biological targets at higher concentrations. In comparable fashion, it reduces drug localization in normal, non-targeted, and sensitive tissues. Additionally, it prevents drug degradation and early clearance and minimizes drug leakage during transit to the targets. Targeted drug delivery promotes cellular uptake and intracellular trafficking while keeping the medication at the intended locations of action for the optimal amount of time [9].

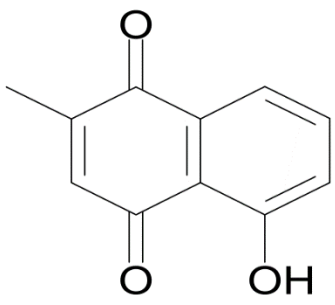


Fig: 01 Plumbagin Structure

Niosomes is nonionic surfactant with cholesterol liposomes like vesicles. The nonionic surfactant and cholesterol are the main ingredient of niosomes formulation, nonionic surfactant provide the stability to niosomes formulation by avoiding aggregation between vesicles and cholesterol provide rigidity to vesicles [10]. The peculiar structure of niosomes, which are vesicular systems made of nonionic surfactants, allows for the dispersion and integration of hydrophilic and hydrophobic therapeutic compounds, respectively. In addition, niosomes are biocompatible, biodegradable, nontoxic, immunosuppressive, and osmotically sensitive [11].

Objectives

The aim of the research work was to develop Plumbagin loaded niosomes for targeted drug delivery system to improve efficacy of a phytochemicals. The plant derived compounds have less toxicity as compared to synthetic drugs, but their free consumption can decrease their efficacy because of their drawback. Although many studies proved that pharmacological applications of phytochemicals have been limited due to its poor water solubility, poor permeability, low bioavailability and low stability in biological systems. Formulating these phytochemicals in niosomes as Nano carrier to address these limitations effectively.

Material & Method

Material

Plumbagin powder was obtained from Yucca Enterprises Mumbai, Cholesterol, Sorbitan Monostearate (Span 60) and Diactyl Phosphate was obtained from Loba chemise, India. All other materials used in this study were of analytical grade

Method

The Plumbagin niosomes was prepared by reverse phase evaporation method. The specific molar ratio of span 60, cholesterol and Plumbagin (5:5:1) were taken and then span 60 and cholesterol dissolved in to 250 ml round bottom flask which containing 10 ml chloroform, DCP was added in above solution. The Plumbagin was dissolved separately in 10 ml PBS pH 7.4. The amount of Plumbagin was same in all formulations. Both solutions were emulsified by using a sonicator for 5 min. Transfer the emulsified mixture in rotary evaporator to dry the contents until a thick gel was formed. The organic solvent was removed by rotary evaporator (Labline PBV 7D) at 100 rpm

at 60 ^{0}C for 30 min in thermostatically controlled water bath at 60 ^{0}c under vaccum 35^{o}c. The organic solvent was removed by this process; the thin lipid film was formed on the inner surface of the flask. The resulted thin lipid film was hydrated by using 10 ml PBS pH 7.4 at 60 ^{o}c for 1hrs to obtain niosomes formulation. The formulation was sonicated for 5min to achieve niosomes with uniform size distribution [12, 13].

Characterization of Niosomes

Preformulation Study

Melting Point Determination

The Plumbagin melting point was determined by using the capillary method and a thieles tube melting apparatus. A glass capillary tube was filled with a small amount of Plumbagin (2-4 mg) and one end was sealed. This capillary was tied with a thermometer and placed in a thieles tube containing liquid paraffin so that the drug-containing end is submerged in the paraffin. Heat was applied to the thieles tube using a burner, and the temperature at which the drug sample was start to melt up to complete melting of sample [14].

Saturation Solubility

Plumbagin saturation solubility was ascertained by the solubility test. The excess amount of Plumbagin was added to separate vials containing distilled water, ethanol, and phosphate buffer pH 7.4. These vials containing solutions were then allowed to stand at 37°C for 24 hours while being periodically shaken to achieve equilibrium. This process produced the saturated solution of Plumbagin. Using the supernatant as a reference, the saturation solubility was ascertained, and the concentration of Plumbagin was measured using a Shimadzu UV 2501 double beam spectrophotometer against the appropriate solvent at λmax of 263 nm [14].

Determination of Wavelength of Maximum Absorbance

10 mg of accurately weighed Plumbagin was dissolved in 10 mL methanol to produce stock solution (1000 µg/ml). Aliquots were taken and diluted with methanol to prepare analytical standards. All the standards were analyzed by UV-Visible spectrometry in the range of 400-200 nm. The concentration verses absorbance responses were plotted to find to slope and regression. [14].

FTIR Analysis

Kbr were previously activated. The sample (1mg) was triturated with Kbr and pellet was formed. This pellet was then filled into die and analyzed by FTIR spectrometry in the range of 400- 4000 nm [15].

Differential Scanning Calorimetry

The Plumbagin (3–5 mg) was analyzed under dry nitrogen purge (50 mL/ min) in a sealed and pinhole Aluminum pan. The sample was heated from room temperature to five degrees (K) above the melting points of Plumbagin and held for 5 min, then the sample is cooled to 223 K (- 50 C) and held for 15 min, then the sample is again heated to five degrees (K) above the melting point of Plumbagin. A constant heating and cooling rate of 10 ^{0}C/min was used [16].

Analysis of Particle Size, PDI and Zeta Potential

The Horiba SZ 100, which operated on the principle of dynamic light scattering technology, was used to determine the particle size, PDI and zeta potential. The formulations diluted, then put into a cuvette for analysis at 25 ^{0}C ± 5^{0}C. The zeta potential of formulations was carried out using a zeta potential cuvette with an applied potential of 3.3 mV. With an increase in cholesterol levels, niosomes particle size increased [17, 18, 19].

Determination of % Entrapment Efficiency

Entrapment efficiency is the percentage fraction of the entire drug entrapped in the niosomes. Entrapment efficiency of niosomal formulation was determined by centrifugation method.

After the separation of the produced niosomes following the ultracentrifugation method at 18000 rpm for 25 min, the entrapment efficiency (%) was calculated according to the difference between the total entrapped drug content in the niosomes and the free drug content in the supernatant. The free drug concentration in the supernatant was assayed by UV spectrophotometry at 263 nm. The entrapment efficiency (EE) percentage was calculated as follows [20, 21].

$$\text{Entrapment Efficiency} = \frac{\text{Initial amount of drug} - \text{Free drug in supernent}}{\text{Initial amount of drug}} \text{ X } 100$$

In Vitro Drug Release

The dialysis bag technique was used to determine the in-vitro dissolution study of pure Plumbagin and Plumbagin niosomes formulation. Lyophilized optimized formulation equivalent to 10 mg Plumbagin was taken into dialysis bag which was previously soaked overnight in phosphate buffer pH 7.4 and both ends of bags was closed. The sample containing dialysis bag was tied to paddles of dissolution test apparatus (Electrolab Dissolution Tester TDL-08L, India) which containing 900 ml phosphate buffer pH 7.4 as dissolution medium. The whole system was kept at 37 ± 0.5 ^{o}c with speed of rotation 100 rpm. The sample was withdraw at predetermined time period and replaced with an equal volume of fresh medium in order to maintain sink condition. The drug release from formulation was estimated UV spectrophotometer at 263 nm [22].

Kinetic Release Study

The in vitro drug release data was subjected to kinetic treatment. The release kinetics of Plumbagin from niosomes was assayed by means of different kinetics models such as zero order, first order, Higuchi and Korsmeyer –Peppas (KP) models.

$Q_t = Q_0 + k_0 t$

$Q_t = Q_0 e^{-k1t}$ $k_1/2.303$

$Q_t = k_H . t^{1/2}$

$M_t/M = k_p t^n$

Where Q_t is the cumulative amount of released drug at time t, M_t/M is the fraction of drug released, and k_0, k_1, k_H and k_p are the constant for zero, first, Higuchi and Peppas model respectively. Also Q_0 is the total concentration of loaded drug and n is diffusional exponent. The determination coefficient (r2) for all models was calculated to find the best fitting model [1].

Result and Discussion

Preformulation Study

The preformulation study of Plumbagin and excipients were performed in order to check purity and quality of drug and excipients. In preformulation study various parameters such as melting point, saturation solubility, identification by FTIR and DSC were studied. The result of these all parameters were found within standard limits and thus it indicates procured Phytoconstituents and

excipients were found in pure quality. The melting point was found to 76.5 **°C.** The Plumbagin was found to be very less soluble in water whereas in phosphate buffer pH 7.4 the solubility was found to be 2.25 mg/ml. The concentration verses absorbance responses were plotted to find to slope and regression. The maximum absorbance was found to be at 263 nm and shown in fig. 2.

The compatibility study between Phytoconstituents and excipients were done by FTIR and DSC. There was no appearance of new absorption band in FTIR spectra as well as in endotherm of DSC as it indicate no new peaks were observed. This shown that Plumbagin was compatible with vesicle components. Thus result of FTIR spectrum and DSC endotherm indicated that there was no chemical reaction happens between Phytoconstituents and excipients.

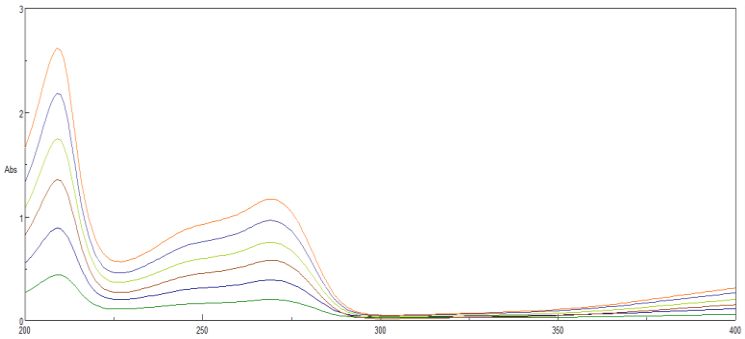


Fig: 2 UV Spectra of Plumbagin

FTIR Analysis

FT-IR spectra of Plumbagin exhibit intense band at 3733.50 cm^{-1} represents –OH group, strong intense band at 1644.98 cm^{-1} represents ketone C=O group and strong intense band at 2995.02 cm^{-1} represents alkyl -CH group. FT-IR of physical mixture (Plumbagin with cholesterol and span-60) exhibit strong intense band at 3442.31 cm^{-1} represents –OH group, strong intense band at 1645.95 cm^{-1} represents ketone C=O group, medium intense band at 1744.3 cm^{-1} represents ester C=O group, strong intense band at 2963.09 cm^{-1} represents alkene =CH group and strong intense band at 2845.45 cm^{-1} represents alkyl -CH group.

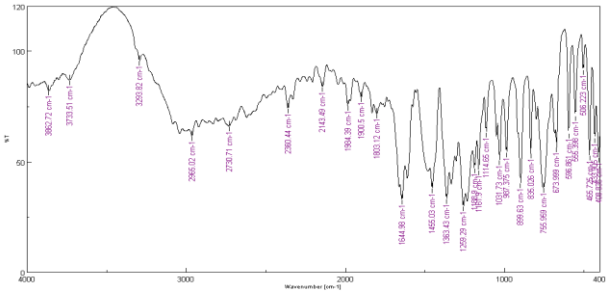


Fig: 3a FTIR Spectra of Plumbagin

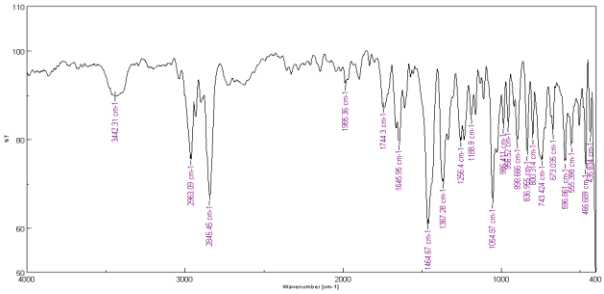


Fig: 3b FTIR Spectra of Physical Mixture

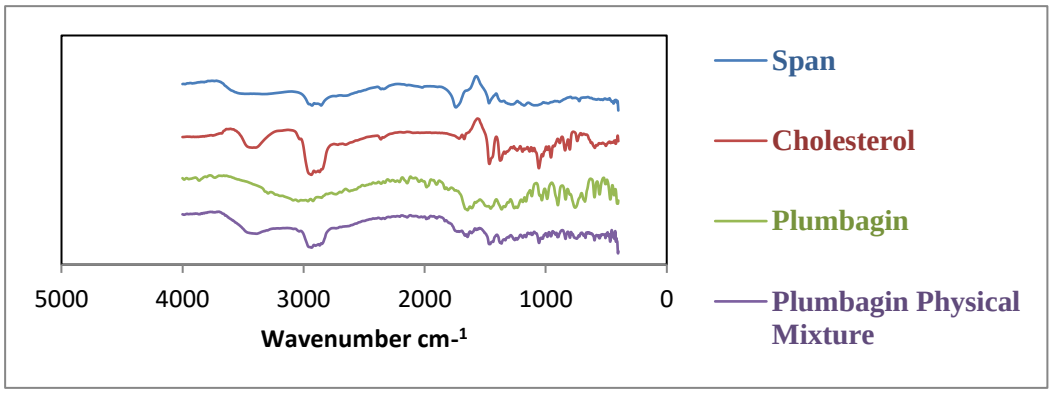


Fig: 4b Overlay FTIR Spectra

Differential Scanning Calorimetry

The differential Scanning calorimetry curve of Plumbagin is shown below. The graph shows melting. The melting point of Plumbagin was found to be 79. 61°C.

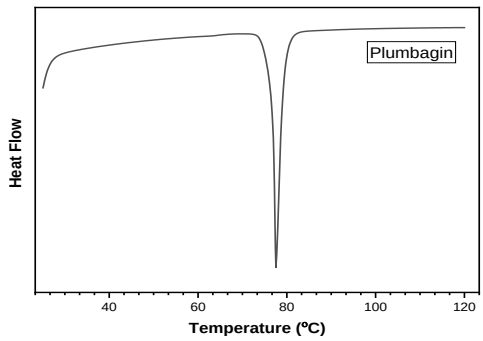


Fig: 5a DSC Thermogram of Plumbagin

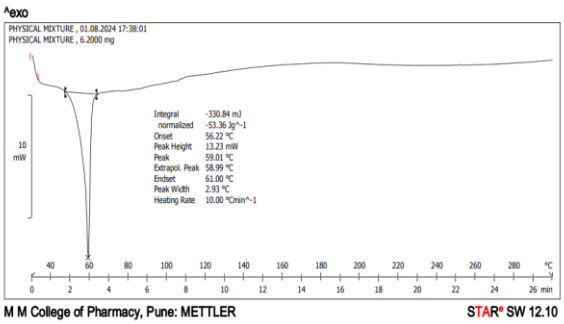


Fig: 5b DSC Thermogram of Physical Mixture

Analysis of Particle size, PDI and Zeta potential

The particle size data for the various formulation codes (FC1 to FC5) were analyzed. The diameter of niosomes was mainly in the range of 91.45± 6.81 nm to 123.5± 4.82 nm. After the loading of niosomes by Plumbagin, the spherical integrity of formulation did not significantly change. It is evident that FC5 stands out as the optimized batch in terms of particle size. With a particle size of 123.5± 4.82 nm, this finer particle size can be advantageous in many applications where a specific particle size range is desired, such as in pharmaceuticals, cosmetics, or materials science. The optimization of FC5 suggests that it meets the desired criteria for particle size, making it a favorable choice for further development or use in applications where particle size is a critical parameter. The homogeneity of the formulation was known as the polydispersity index (PDI). Small value of PDI < 0.1 indicate a homogeneous population, while a PDI > 0.3 indicates high heterogeneity. The PDI value of optimized niosomes (FC5) was found to be 0.143 ± 0.067 had satisfactory homogeneity and stability. The stability of niosomes formulation is dependent on the zeta potential. A zeta potential near ± 30 mv can guarantee a long –term stable formulation. Zeta potential for optimized batch was found to be -26.8 ± 2.13 mV. High and negative zeta potential values are an indication of stable preparation and prevent aggregation phenomena. The loading of Plumbagin into niosomes resulted in more negative zeta values and hence more stable formulations.

Determination of % Entrapment Efficiency

The entrapment efficiency data for the various formulation codes (FC1 to FC5) reveal that FC5 is the optimized batch with the highest entrapment efficiency. The EE of optimized formulation (FC5) was found to be 91.42 ± 2.19. Many studies mentioned that hydrophobic drugs are entrapped into bilayers of niosomes through electrostatics interactions. High encapsulating efficiently of Plumbagin in niosomes is because of hydrophobic nature of Plumbagin that results in more affinity and thus more incorporation in niosomes bilayers. Such high drug loading will be beneficial for the systemic administrations and result in the release of a significant amount of Plumbagin at the site of action. Barani et al. (2018) prepared lawsone –loaded niosomes with a particle size of 300 nm and loading efficiency of 69% [2]. The Plumbagin niosomes prepared in this study had a higher loading efficiency and smaller particle size in comparison to the one mentioned above.

Table No: 1 Formulation optimization and development of Plumbagin Niosomes

Sr. NO.	Formulation Code	Span 60: Cholesterol; Plumbagin (% w/w/w)	Average Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)	Entrapment Efficiency (%)
1.	FC1	5:1:1	91.45± 6.81	0.256 ± 0.057	-29.87± 1.83	57.63 ± 2.16
2.	FC2	5:2:1	101.6± 4.52	0.097 ± 0.08	-31.4 ± 2.31	64.51 ± 3.12
3.	FC3	5:3:1	110.9± 4.98	0.125 ± 0.11	-35.47 ± 2.54	74.62 ± 2.83
4.	FC4	5:4:1	119.58± 7.24	0.208 ± 0.09	-33.13 ± 2.18	84.65 ± 2.87
5.	**FC5**	**5:5:1**	**123.5± 4.82**	**0.143 ± 0.067**	**-26.8 ± 2.13**	**91.42 ± 2.19**

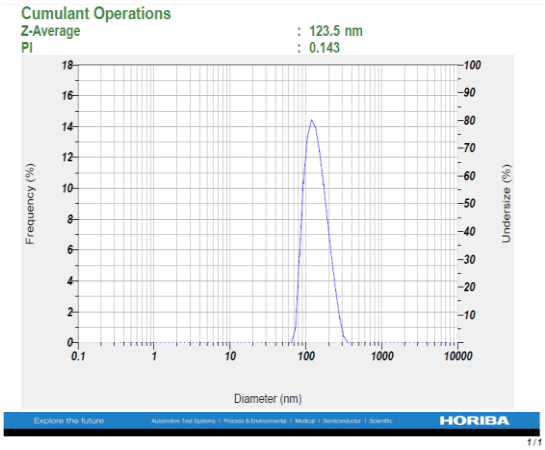


Fig: 6 Particle Size of FC5 Formulation

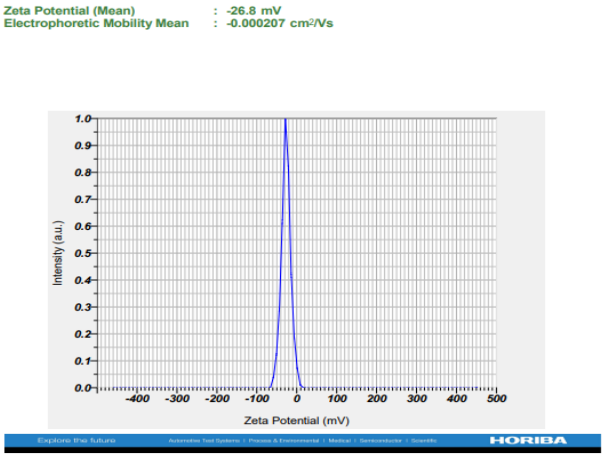


Fig: 7 Zeta Potential of FC 5 Formulation

In vitro Plumbagin Release Study

Figure 8 shown that Plumbagin Niosomes slow down the rate of drug release compared with the free Plumbagin solution. The efflux of Plumbagin from niosomes was biphasic, with an initial faster release which lasts for about 1–6 h, followed by a period of slow but sustained release (9-24 h). This biphasic release pattern of the drugs seems to be characteristic of the bilayer vesicles.

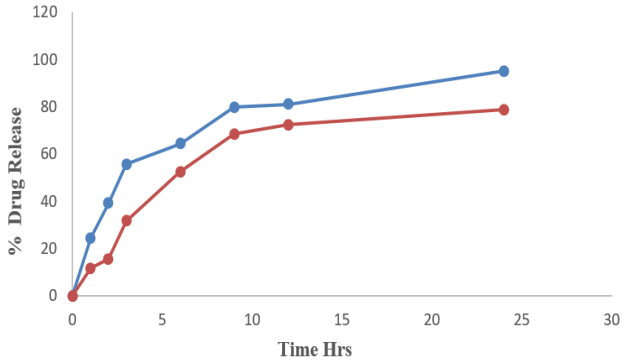


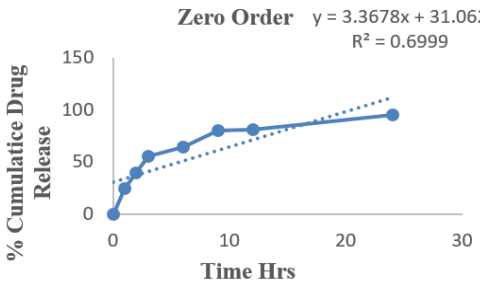
Fig: 7 In vitro Release Profiles of free Plumbagin (Red Line) and Plumbagin Niosomes (Blue Line) in PBS 7.4 at 37 °C

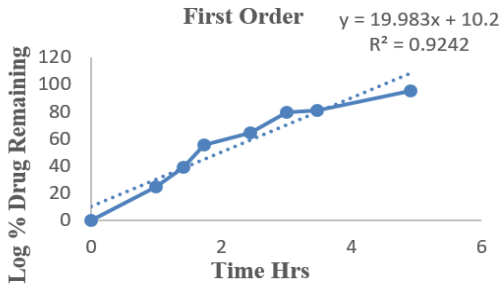
Kinetic Release Study

The drug release kinetics was evaluated using zero order, first order, Higuchi and KP kinetic models. Table No.2 Shows that the Higuchi Model best fitted the experimental data on the basis of the correlation coefficient ($R^2 > 0.97$).

Table No: 2 Model, Equation, and Regressions of Plumbagin Release from Niosomes

Model	Equation	R^2
Zero-Order	y = 3.3678x + 31.062	R^2 = 0.6999
First Order	y = 19.983x + 10.2	R^2 = 0.9242
Higuchi	y = -0.0526x + 1.8883	R^2 = 0.971
Kors-peppas	y = 0.0457x + 1.2158	R^2 = 0.3128





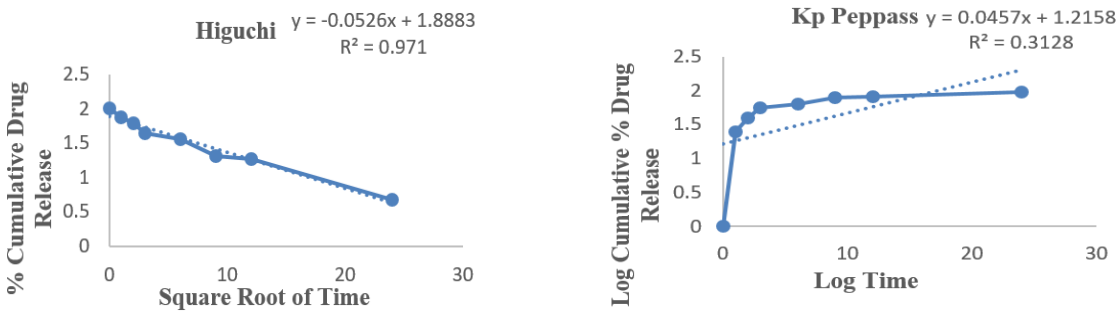


Fig: 8 Release Kinetic of Optimized Plumbagin niosomes formulation

Conclusion

Plumbagin was loaded into niosomes by reverse phase evaporation method and its characteristics were evaluated. The prepared niosomes were shown an average particle size from 91.45± 6.81to 123.5± 4.82. The Zeta potential value of optimized batch was found to be -26.8 ± 2.13 Mv and % and drug entrapment efficiency was found between 57.63 ± 2.16 to 91.42 ± 2.19. SEM shown a spherical structure of Plumbagin loaded niosomes. Invitro drug release, niosomes shown sustain release behavior as compared as compared pure Plumbagin. The initial drug release from formulation was found to be 24.62 % in 1 hour followed by sustained release of drug was observed till 24 hours. The drug release in 24 hours was 95.28.

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