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APPLICATION OF ETHANOL-INJECTION TECHNIQUE FOR FORMULATION OF VITEXIN LOADED NOISOMES AND CHARACTERIZATION OF THE VESICLES

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Abstract
In the present study niosomes of vitexin were developed by ethanol injection method and were characterized for particle size, entrapment and drug release and release kinetics. The niosomes prepared using either span 60, tween 80 or tween 20 as the non-ionic surfactants and cholesterol as the lipid were evaluated for particle size, entrapment efficiency and *in vitro* release. Increasing the HLB value of the surfactant was found to increase the particle size of the vesicles. On the other hand, surfactant with lower HLB also exhibited better entrapment efficiency. The entrapment efficiency of the formulations was found to be in the range of 55 – 71%. The release of vitexin from the prepared niosomes ranged from 60.78 % to 76.37%. Formulation **F3** presented the lowest particles size (229.6 nm), higher entrapment efficiency (71%) and sustained release of vitexin over 24 hours (65.53) and was considered the best formulation. The release of vitexin from the **F3** depicted Higuchi and Korsemeyer-Peppas type of mechanism at these two models presented higher linearity compared to the first-order and zero-order models

Keywords: Vitexin, inflammation noisome, ethanol-injection, entrapment

Introduction

The primary objective of the novel drug delivery system is to attain a steady state blood or tissue concentration of the drug that is therapeutically effective and non-toxic for an extended period of time.[1] Newer perspectives on controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition, and efficacy of drugs have therefore been explored and studied. Drug delivery via vesicular system (prepared by the self-assembly of the lipids/surfactants to form the bilayers where an aqueous space is present in the core) offer many advantages like increased solubility, high permeability, acts as a carrier for various drugs which exhibits different solubility.[2] Niosomes are lamellar vesicles composed of non-ionic surfactants and cholesterol. In comparison with liposomes, they offer some advantages, such as lower cost, greater chemical stability and longer storage time.

Vitexin is an apigenin flavone glycoside, which is found in the passion flower, bamboo leaves and pearl millet. The flavone is associated with platelet aggregation inhibition, antineoplastic and antidiabetic actions.[3] The phytomolecule is poorly absorbed and has a half-life of 0.5-2.5 h.[3]

Poor water solubility, high metabolic clearance, short half-life and other pharmacokinetic problems hinder the pharmacodynamic efficacy of vitexin. In order to increase the bioavailability, increase the half-life and prolong the duration of action several formulations like solid dispersions, nanoparticles,

solid-lipid nanoparticles, nanoemulsions etc have been formulated.[4-8] The non-ionic surfactant play important role in solubilizing the drug as well as the permeation enhancers due to their ability to increase membrane fluidity and their capacity to solubilize and extract membrane components. On the basis of these evidences, it was envisioned that non-ionic surfactant based vesicles (niosomes) can help in achieving the goals of increasing bioavailability and half-life of vitexin.

Material and Methods

Vitexin was obtained from National Analytical Corporation, Mumbai; cholesterol, diacetylphosphate and Triton X- 100 were purchased from Himedia. All other chemicals were obtained from oxford fine chemicals.

Calibration Curve of Vitexin by HPLC

The estimation of vitexin in samples was done by HPLC method previously reported[53]. The stock solution was prepared by dissolving 1mg of pure vitexin in 10 mL of warm methanol. Aliquots of 0.2, 0.4, 0.6, 0.8 and 1.0 mL were transferred to 10 mL volumetric flasks and the volume of each was made up to 10 mL with warm methanol to obtain working standards of 2-10 µg/mL vitexin. The chromatographic conditions included 0.1% orthophosphoric acid: acetonitrile (80:20) as the mobile phase, flow rate of 1.0 mL/min, C-18 column and a UV detection wavelength of 335 nm. The working standard were injected into the column and the peak area of the chromatogram was measured for each. A calibration curve of peak area vs. concentration was constructed and the regression equation was obtained. This equation was used to evaluate the concentration of vitexin in samples.

Formulation of Vitexin Niosomes[9]

Vitexin loaded niosomes were prepared by ethanol injection technique. Accurately weighed quantity of vitexin, surfactant and cholesterol (Table 1) were dissolved in ethanol and the solution was warmed to 60°C on water bath (organic phase). In a separate flask, phosphate buffer saline (PBS) was prepared and heated to 60°C on water bath (aqueous phase). While maintaining the temperature at 60°C, the organic phase (6mL) was injected in to the aqueous phase (50 mL) at constant rate using a hypodermic syringe, stirring the solution at 400 rpm on a magnetic stirrer. On completion of addition, the organic phase was eliminated by using rotary vacuum evaporator, at 50°C. The resulting solution was sonicated under cooling using a probe sonicator at 65% power for 20 min.

Table 1 Composition of Vitexin Niosomes

Formulation Code	Vitexin (mg)	Tween 80 (mg)	Span 60 (mg)	Tween 20 (mg)	Cholesterol (mg)
F1	1	50	-	-	50
F2	1	100	-	-	50
F3	1	-	50	-	50
F4	1	-	100	-	50
F5	1	-	150	-	50
F6	1	-	-	50	50
F7	1	-	-	100	50

Evaluation of Vitexin loaded Niosomes[9]

FTIR spectroscopic analysis

The Fourier transformed infrared spectroscopic analysis of the procured drug sample was performed and the major absorption bands were compared with that of the spectral database of the drug to ascertain its identity. FTIR of physical mixture of the drug and the used polymers was also performed to observe to any possible interaction between the drugs and excipients (Cholesterol, span 60, tween 20, tween 80 and vitexin).

Particle Size and zeta potential

The particle size and zeta potential of the prepared niosomes was determined using zeta sizer.

Removal of un-entrapped drug from niosomes

The unentrapped drug from niosomal formulation was separated by centrifugation method. The niosomal suspension was taken in centrifuge tube. The formulation was centrifuged at 15,000 rpm for 30 min using cooling centrifuge and temperature was maintained at 5°C. The supernatant was separated. Supernatant contained unentrapped drug and pellet contained drug encapsulated vesicles. The pellet was resuspended in methanol to obtain a niosomal suspension free from un-entrapped drug.

Encapsulation efficiency[10]

Drug entrapped vesicles were separated from un-entrapped drug by centrifugation method. 0.5 ml of Vitexin loaded niosome preparation was added with 0.5 ml of 10% triton X 100 and mixed well then incubated for 1 hour. The triton X 100 was added to lyse the vesicles in order to release the encapsulated vitexin. The solution was diluted with phosphate buffer saline pH 7.4 and filtered through Whatman filter paper. The filtrate was determined by HPLC method.

$$\text{Percent entrapment} = \frac{\text{Amount of drug entrapped}}{\text{Total amount of drug added}} \times 100$$

In vitro release study for niosomal preparation[9]

The niosomal formulation was taken in a dialysis membrane of 5 cm length and suitably suspended in a beaker containing 100 ml diffusion medium of phosphate buffer saline pH 7.4. The temperature of medium was maintained at 37±0.5°C. The medium was stirred by means of magnetic stirrer at a constant speed. 1 ml of sample was withdrawn at every 1 hour and replaced with 1 ml of fresh buffer, so that the volume of diffusion medium was maintained constant at 100 ml. The withdrawn samples were analyzed by HPLC for determining the amount of vitexin.

Kinetics of drug release

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing zero order (Q v/s t), first order [Log(Q0-Q) v/s t], Higuchi's square root of time (Q v/s √T) and Korsemeyer Peppas double log plot (log Q v/s log t) respectively, where Q is the cumulative percentage of drug released at time t and (Q0-Q) is the cumulative percentage of drug remaining after time t.

Results and Discussion

The calibration curve of vitexin was prepared according to the reported procedure by HPLC method. The retention time of vitexin was found to be 11.00 min. The peak area obtained for the working standards was plotted against concentration and the calibration curve along with the equation for straight line was obtained (Figure 1).

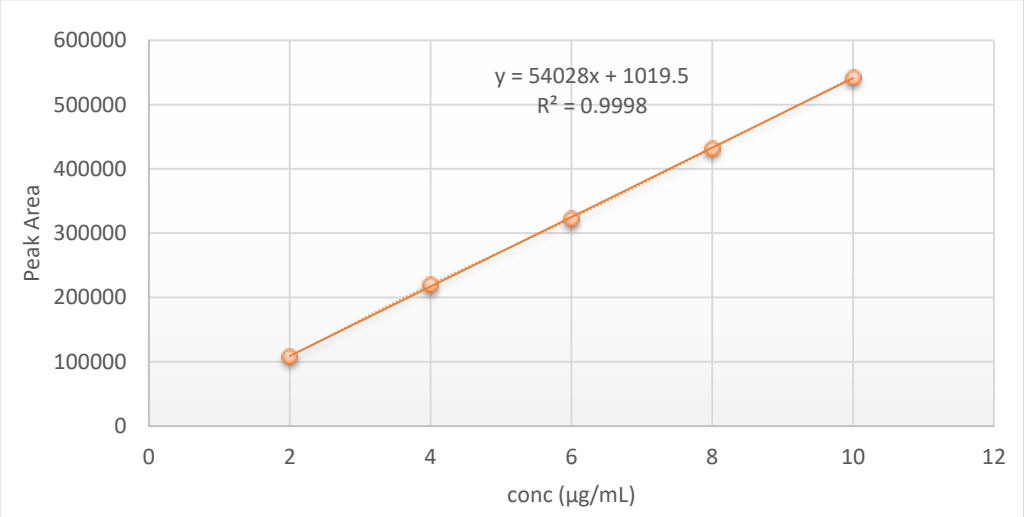

Figure 1 Calibration curve of Vitexin by HPLC

Development of Vitexin niosomes

In this study, vitexin loaded niosomes were prepared by ethanol injection technique using cholesterol and non-ionic surfactants such as span 60, tween 80 and tween 20. Ethanol was used as solvent for dissolving the components of niosome.

The organic phase was removed from the aqueous phase (phosphate buffer saline) by rotary evaporation by azeotropic distillation and the size of the vesicles in formulation was reduced by sonicating the formulation in Probe sonicator. The solution was kept cooled throughout the sonication step to prevent loss of solvent resulting from the heat generated during sonication.

The effect of the surfactant used for formulation on particle size and entrapment efficiency of niosomes was studied at two levels.

Evaluation of vitexin Niosomes

FTIR and compatibility study

The FT-IR spectra (Figure 2) exhibits the major peaks of the functional groups present in vitexin. All these peaks were observed in the FT-IR spectra of the physical mixture of drug and excipients (Figure 3) also providing evidence for the absence of any chemical incompatibility between pure drugs with the excipients.

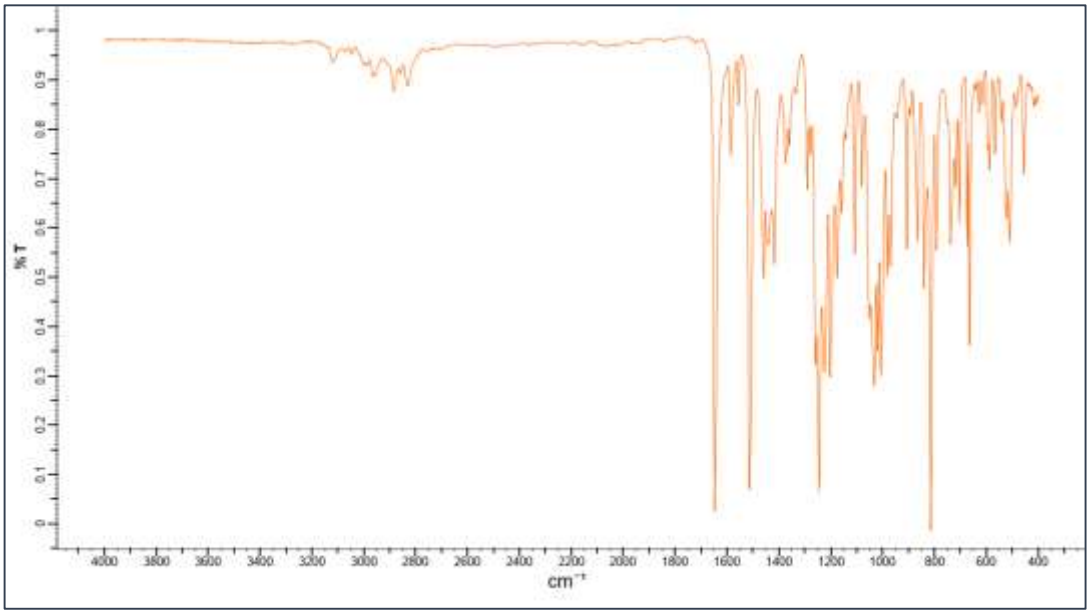

Figure 2 FT-IR spectra of vitexin

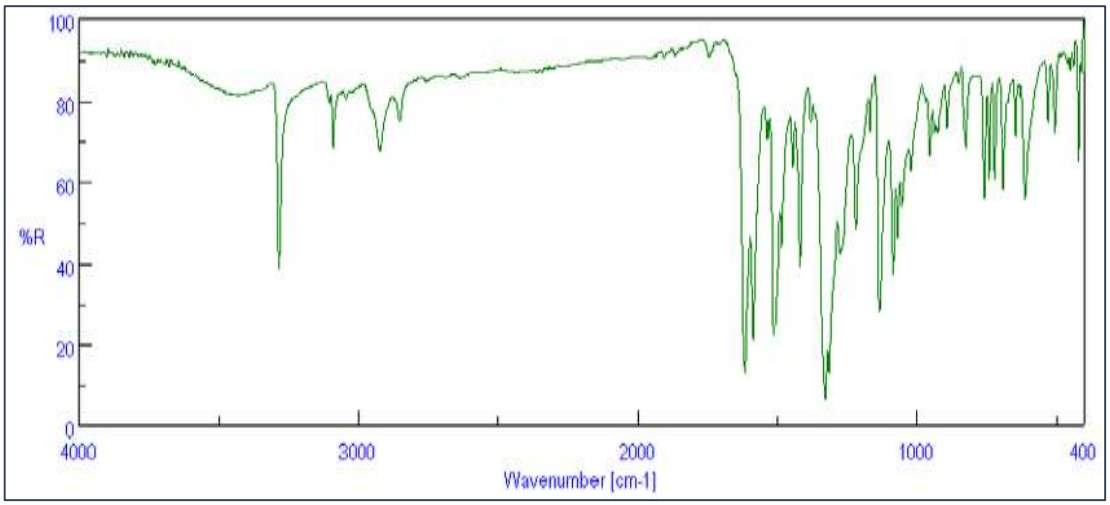

Figure 3 FT-IR spectra of physical mixture of vitexin, cholesterol. Tween 20, Tween 80 and Span 60

Particle Size and zeta potential of niosomes

The effect to the concentration and type of surfactant was evident on the particles size of the niosomes. Increasing the HLB value of the surfactant was found to increase the particle size of the vesicles. As it can be seen from Table 5.5, the niosomes produced by Span 60 (HLB 4.7) were of lower size than those produced by Tween 20 (HLB 16.7) and Tween 80 (HLB 15.0). On the other hand the polydispersity of the niosomes prepared using Span 60 was higher as compared to the others. Also it was evident that increasing the ratio of surfactant to cholesterol decreased the particle size. As it has been found in several of the previous studies on niosomes, an equimolar ratio of span60 and cholesterol produced the vesicles with the smallest diameter.[11,12]

Table 2 Particle size, polydispersity and zeta potential

Formulation Code	Particle size (nm)	PDI	Zeta potential
F1	231.7	0.183	-8.1
F2	248.5	0.197	-8.9
F3	229.6	0.214	-16.9
F4	221.3	0.269	-18.3
F5	221.3	0.297	-19.8
F6	242.7	0.151	-5.4
F7	267.1	0.162	-5.9

The zeta potential of the niosomes was found to be in range of -5.4 to -18.3. It was found that a higher electrostatic repulsion existed between cholesterol and span 60 resulting in a larger negative value of zeta potential. On the contrary, the lower zeta potential values of the niosomes could be explained due to the non-ionic character of the surfactants. Similar zeta potential values have been reported in previous studies for niosomes prepared with span and tween.[13]

Percentage drug entrapment efficiency

The un-entrapped drug from niosomes was removed by centrifugation technique. The results are presented in Table 3. The entrapment efficiency of the niosomes is governed by the ability of formulation to retain drug molecule in aqueous core or in bilayer membrane of vesicles. After removal of un-entrapped drug, the entrapment of all formulation was studied.

Table 3 Entrapment efficiency

Formulation Code	Percentage Entrapment Efficiency (%)
F1	63
F2	61
F3	71
F4	74
F5	76
F6	59
F7	55

The entrapment efficiency also followed a pattern similar to the particle size. It was visible from the results that the lower the HLB value of the surfactant, the higher was the entrapment of vitexin in the vesicles. Also it was observed that increasing the ratio of surfactant led to increased entrapment when span 60 was used whereas a decrease in entrapment of vitexin was observed when the ratio of tween 20 or tween 80 to cholesterol was increased.

In vitro release study

The release of vitexin from niosomes was determined using the membrane diffusion technique. Release study was carried for 24 hours and results are noted in following Table 4.

Table 4 *In vitro* release of vitexin from niosomes

Time (h)	Cumulative percentage of drug released (%)						
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
0	0	0	0	0	0	0	0
1	9.11	9.61	4.06	3.97	4.36	12.85	10.16
2	14.06	14.19	8.09	6.05	15.13	19.12	16.33
4	16.25	19.14	19.21	15.22	20.28	23.21	20.19
6	24.32	29.42	34.12	26.23	29.83	29.24	26.43
8	33.82	35.62	39.53	33.92	38.44	39.31	35.27
10	41.53	41.63	45.14	48.49	47.69	44.41	40.14
12	47.37	51.84	50.29	52.54	55.16	50.48	45.32
24	67.61	76.37	65.53	68.65	71.68	62.33	60.78

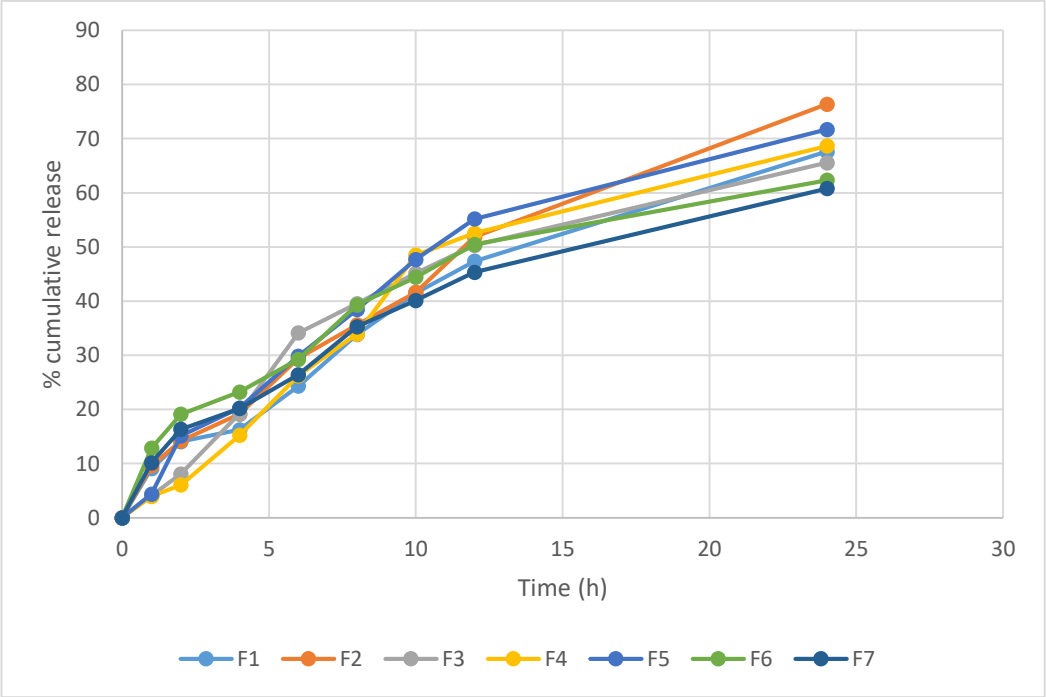

Figure 4 Comparative i*n vitro* release from niosome formulations

As it can be seen from the result, the release of vitexin from the prepared niosomes ranged from 60.78 % to 76.37%. The highest amount of drug was released from F2 whereas the lowest form F7. The niosomes prepared using span 60 were found to release 65.53 to 71.68 % vitexin. It was also observed that the niosomes prepared with span 60 did not produce burst release of the drug in the 1st hour. On the other hand the release from the remaining niosomes witnessed a burst release (9.11-12.85%) in the 1st hour. This could be attributed to the presence of lesser un-entrapped drug on the surface of the vesicles prepared by span 60.

Release kinetics

The mechanism of release of vitexin from the niosomal vesicles (**F3**) was studied by graphical exploration of the release data fitted with various mathematical models. F3 was considered as the best formulation as it exhibited the lowest particle size and the highest entrapment efficiency of all the formulations. Also the high negative zeta potential of the formulation ensued that it might exhibit good stability over longer period of time in comparison to the remaining formulations. The slope of the graph (Figure 5-8) and regression constant were used to explain the release behavior.

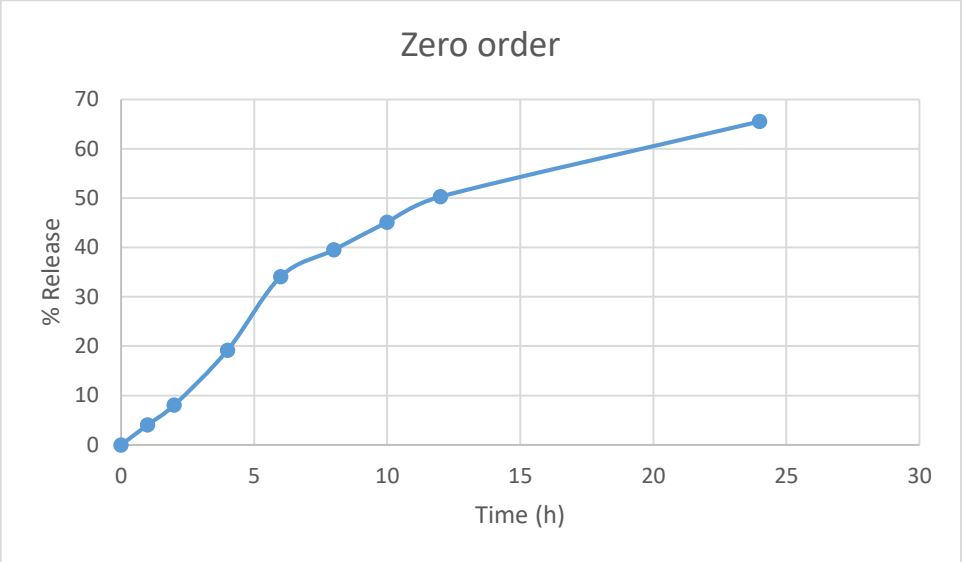

Figure 5. Zero order release plot for F3

Figure 6 First order release plot for F3

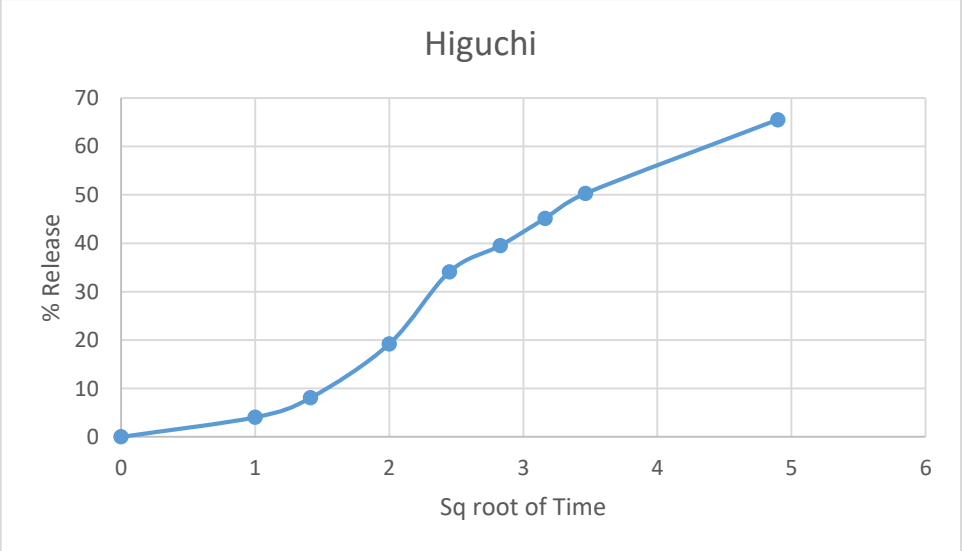

Figure 7 Higuchi release plot for F3

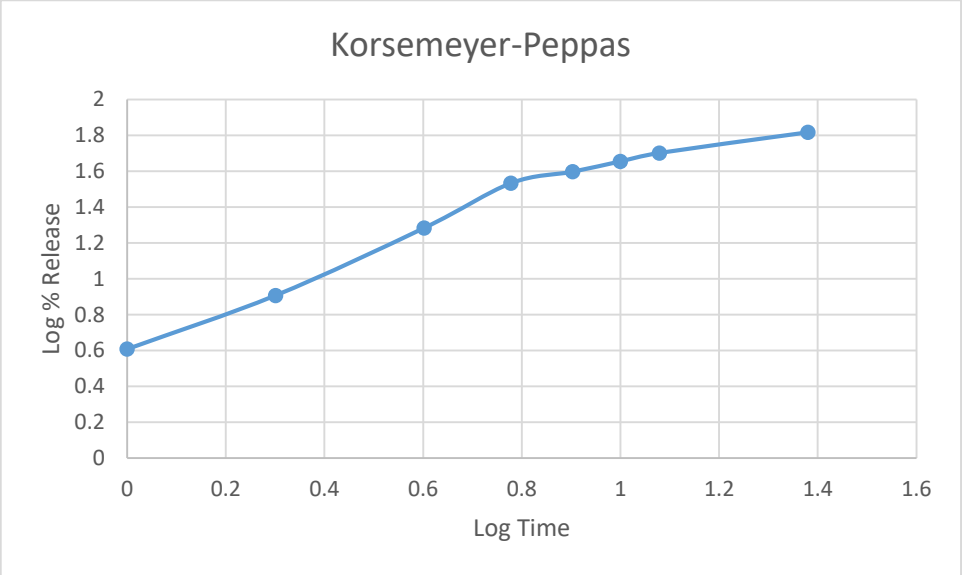

Figure 8 Korsemeyer-Peppas release plot for F3

The release of vitexin from the niosomes depicted Higuchi and Korsemeyer-Peppas type of mechanism at these two models presented higher linearity compared to the first-order and zero-order models (Table 5).

Table 5 Regression coefficient of various mathematical models for release of F3

	Zero-order	**First-order**	**Higuchi**	**Korsemeyer-Peppas**
Regression coefficient (R^2)	0.8669	0.6089	0.9547	0.9557
Slope	2.862	0.045	15.328	0.9372

The higher linearity of the Higuchi and Peppas models suggest that vitexin is released primarily by diffusion from the vesicles followed by an erosion of the carrier matrix.

Conclusion

The objective of the present investigation was to develop non-ionic surfactant based delivery system for topical application of vitexin for treatment of inflammatory condition. The idea was to increase the bioavailability of vitexin. Niosomes are known to present a solution to these side effects and the study proved that niosomes of vitexin could be easily prepared using ethanol injection method and can provide improved drug bioavailability via sustained release of the drug.

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