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Formulation and characterization of chrysin loaded niosomal vesicular delivery system

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ABSTRACT

In the present study niosomes of chrysin were developed by thin film hydration technique and characterized. The niosomes prepared using varying ratio of surfactant (span 20 and tween 20) and cholesterol were evaluated for entrapment efficiency and *in vitro* release. From the result of experimental investigations it was observed that using either of the surfactants a 1:2 ratio of surfactant and cholesterol exhibited the maximum entrapment of the drug in the core. From the results of the release studies it was found that the maximum amount of drug was released from formulations **F2** (79.4 %) and **F5** (71.8 %) over a period of 18 h at an almost steady rate. The higher amount of drug release along with the higher entrapment efficiency make the formulations F2 and F5 containing surfactant to cholesterol ratio of 1:2 the most promising formulations. The release kinetic was studied using the graphical method and it was found that chrysin released from the niosomes following a mixed order kinetics wherein both concentration of the drug and erosion of the noisomal matrix played vital role in the release of chrysin.

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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]. The method by which a drug is delivered can have a significant effect on its efficacy[2]. To minimize drug degradation and loss, to prevent harmful side-effects and to increase drug bioavailability and the fraction of the drug accumulated in the required zone, various drug delivery and drug targeting systems are currently under development. Vesicular systems are prepared by the self-assembly of the lipids/surfactants to form the bilayers where an aqueous space is present in the core. Drug delivery via vesicular system offer many advantages like increased solubility, high permeability, acts as a carrier for various drugs which exhibits different solubility. 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. They also have high compatibility with biological systems and low toxicity due to their non-ionic nature[3-8].

Chrysin (Figure 1) is a dihydroxyflavone in which the two hydroxy groups are located at positions 5 and 7. It has a role as an anti-inflammatory agent, an antineoplastic agent, an antioxidant, a hepatoprotective agent, an EC 2.7.11.18 (myosin-light-chain kinase) inhibitor and a plant metabolite. The flavone is poorly water soluble and its half-life is reported to be 13 min[9].

Poor water solubility, high metabolic clearance, short half-life and other pharmacokinetic problems hinder the pharmacodynamic efficacy of chrysin. 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.

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 chrysin.

Material and Methods

Standard curve for Chrysin

Chrysin 25 mg was weighed and dissolved in methanol in a 25 ml volumetric flask. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 1000 µg/ml. From the primary stock solution, pipette out 2 ml and placed into 100 ml volumetric flask. The volume was made up to mark with methanol to give a stock solution containing 20 µg/mL. Appropriate volume of aliquots (1 to 10 ml) from chrysin secondary stock solution were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with methanol to obtain concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg/mL. Absorbance of each solution against methanol as blank were measured at 212 nm.

Formulation of chrysin Niosomes[10]

Chrysin loaded niosomes were prepared by thin film hydration technique. Accurately weighed quantity of cholesterol and surfactant were dissolved in chloroform methanol mixture ratio (2:1v/v) in a 100 mL volumetric flask. The weighed quantity of drug and dicetyl phosphate was added to the solvent mixture. The solvent mixture was removed from liquid phase using rotary evaporator at 60°C to obtain a thin film on the wall of the flask at a rotation speed of 150 rpm. The complete removal of solvent can be ensured by applying vacuum. The dry lipid film was hydrated with 5 ml phosphate buffer saline of pH 7.4 at a temperature of 60±2°C for a period of 2 hour until the formation of niosomes. All the batches were subjected to sonication process for 2 min using probe sonicator. The ratios of cholesterol and surfactant used in the formulation are presented in Table 1.

Evaluation of Chrysin loaded Niosomes

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 20, tween 20).

Removal of un-entrapped drug from niosomes

The unentrapped drug from niosomal formu-

lation 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[11]

Drug entrapped vesicles were separated from un-entrapped drug by centrifugation method. 0.5 ml of chrysin 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 chrysin. The solution was diluted with phosphate buffer saline pH 7.4 and filtered through Whatman filter paper. The filtrate was measured spectrophotometrically at 212 nm using methanol and triton X 100 mixture as blank.

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

***In vitro* release study for niosomal preparation[12]**

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 made upto 10 mL using phosphate buffer saline pH 7.4. The samples were measured spectrophotometrically at 212 nm.

Drug release kinetics

The data obtained from the *in vitro* release studies was fitted into various graphical models (Zero order, first order, Higuchi and Korsemeyre-Peppas) in order to infer the release kinetics of chrysin from the niosomes.

Results and Discussion

The calibration curve of chyrsin was prepared according to the reported procedure using methanol as the solvent (Figure 2).

Development of chrysin niosomes

In this study, chrysin loaded niosomes were prepared by thin film hydration technique using cholesterol and non-ionic surfactants such as span 20 and tween 20. Chloroform-methanol mixture (2:1v/v) was used as solvent. After evaporation of solvent from the formulation, thin film was formed. The thin film was hydrated and removed by phosphate buffer saline pH 7.4. Size of the vesicles in formulation was reduced by sonicating the formulation in probe sonicator. Formulations with different ratios of surfactant and cholesterol were prepared. Several physicochemical characteristics of niosomes such as morphology, vesicle size determination, drug release profile was investigated. Dicetyl phosphate (DCP) was included in the formulation as charge inducing agent. The inclusion of charge inducing agent (DCP) prevented the aggregation and fusion of vesicles. Integrity and uniformity also maintained by dicetyl phosphate.

Evaluation of chrysin niosomes

Percentage drug entrapment efficiency

The unentrapped drug from niosomes was removed by centrifugation technique. The results are presented in Table 2. 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 unentrapped drug, the entrapment of all formulation was studied. Entrapment efficiency was varied with varying the surfactant and cholesterol ratio. Various factors like lipid concentration, drug to lipid ratio, and cholesterol content are liable to affect the entrapment efficiency.

The entrapment efficiency using both the surfactants was determined and it was found that a 1:2 ratio of surfactant and cholesterol exhibited the maximum entrapment of the drug in the core.

***In vitro* release study**

The release of chrysin from niosomes was determined using the membrane diffusion technique. Release study was carried for 24 hours (Table 3, Figure 3).

From the results of the release studies it was found that the maximum amount of drug was released from formulations **F2** (79.4 %) and **F5** (71.8 %) over a period of 18 h at an almost steady rate. The higher amount of drug release along with the higher entrapment efficiency make the formulations **F2** and **F5** containing surfactant to cholesterol ratio of 1:2 the most promising formulations.

Release kinetics

The graphical models for the release of F2 and F5 were studied (Figure 3-7). The slope and regression coefficients were calculated from the graph and the release kinetics was predicted (Table 4).

The regression coefficients obtained from the graphical models of kinetic reveal that the release of chrysin from the niosomes releasing drug by virtue of concentration as well as erosion of the matrix.

Conclusion

The objective of the present investigation was to develop non-ionic surfactant based delivery system for topical application of chrysin for treatment of inflammation. The idea was to increase the bioavailability of chrysin by sustaining the release. Niosomes are known to present a solution to the problems of bioavailability and the study proved that niosomes of chrysin could be easily prepared with improved drug bioavailability.

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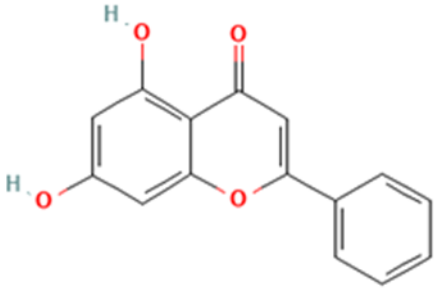

Figure 1 Structure of Chrysin

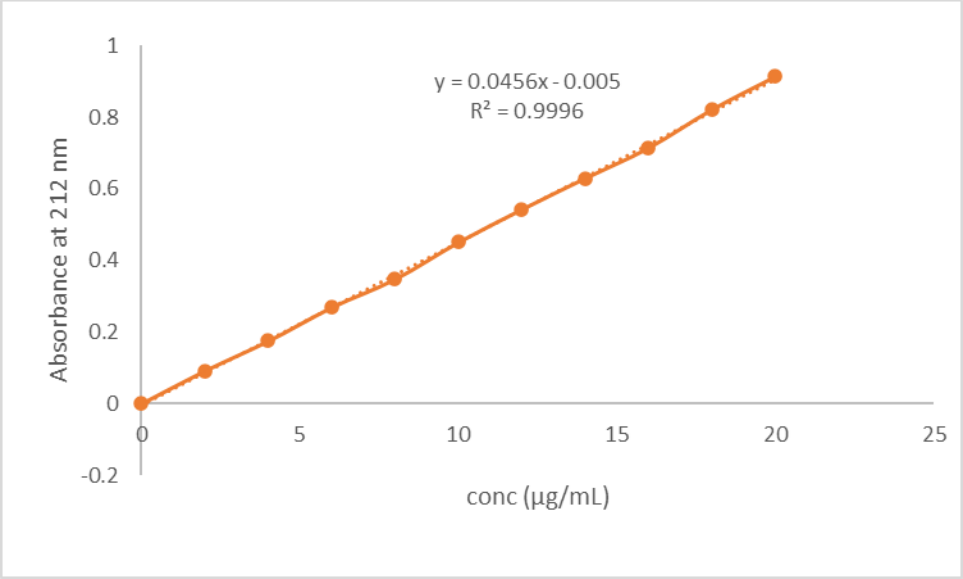

Figure 2 Calibration curve of chrysin

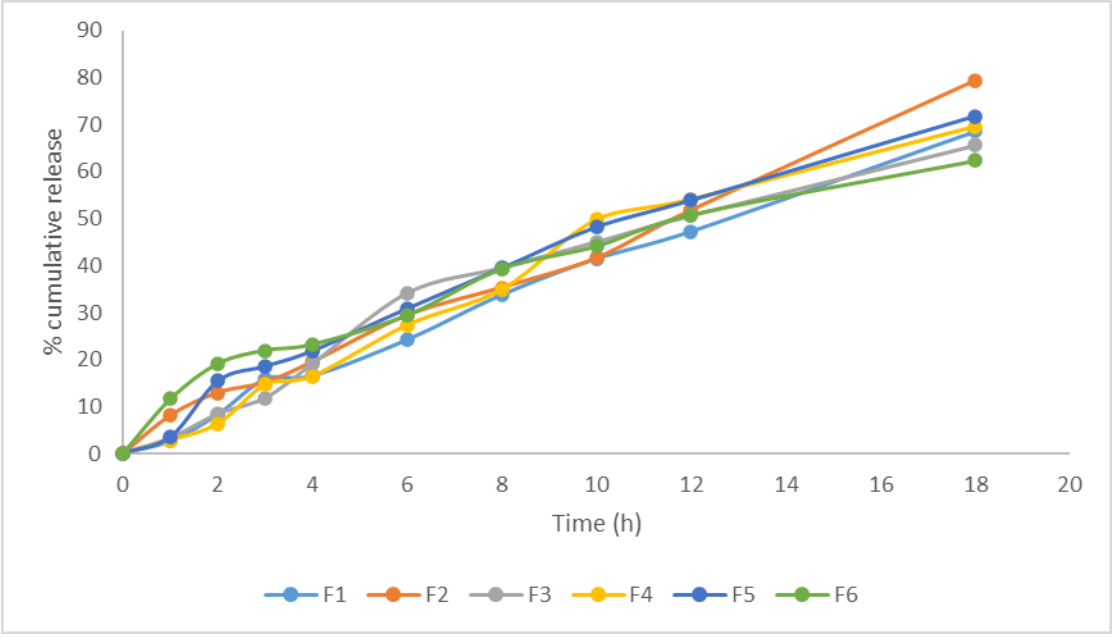

Figure 3 Comparative i*n vitro* release from niosome formulations

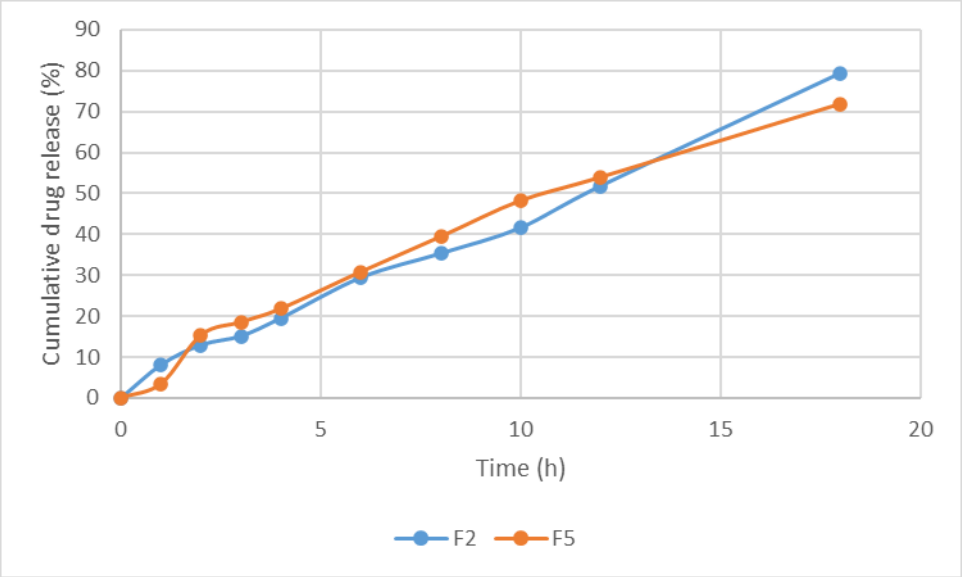

Figure 4 Zero order release of F2 and F5

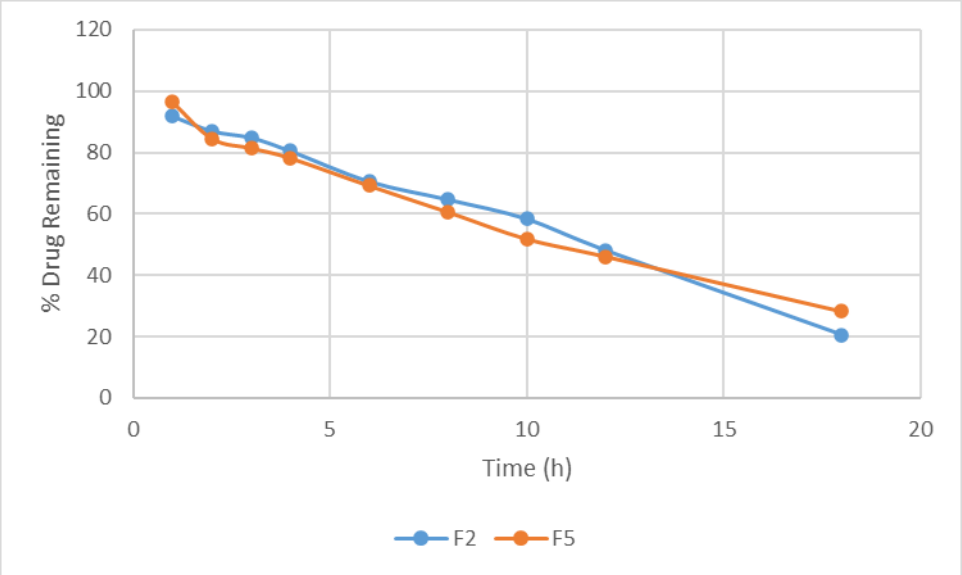

Figure 5 First order release of F2 and F5

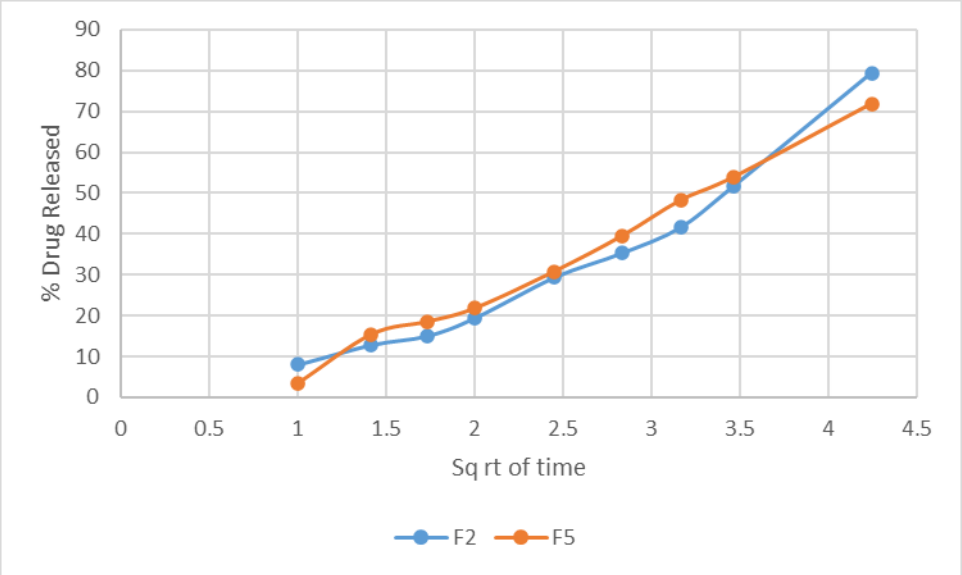

Figure 6 Higuchi plot of F2 and F5

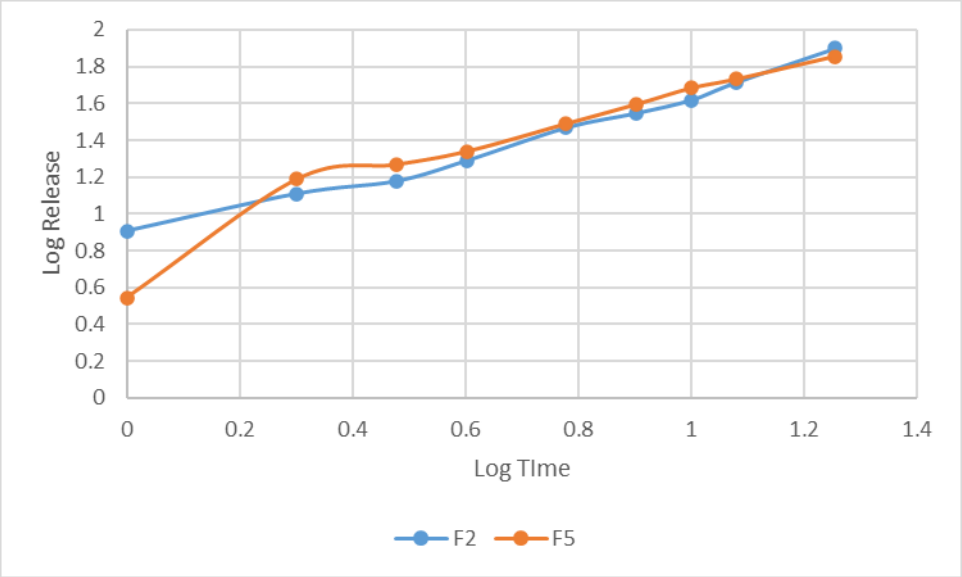

Figure 7 Korsemeyer-Peppas plot of F2 and F5

Table 1 Composition of chrysin Niosomes

Formulation Code	Chrysin (mg)	Surfactant	Surfactant: Cholesterol (µM)
F1	100	Span 20	100:100
F2	100	Span 20	100:200
F3	100	Span 20	100:300
F4	100	Tween 20	100:100
F5	100	Tween 20	100:200
F6	100	Tween 20	100:300

Table 2 Entrapment efficiency

Formulation Code	Surfactant: Cholesterol Ratio	Surfactant Used	Percentage Entrapment Efficiency (%)
F1	100:100	Span 20	66
F2	100:200	Span 20	76
F3	100:300	Span 20	64
F4	100:100	Tween 20	70
F5	100:200	Tween 20	73
F6	100:300	Tween 20	63

Table 3 *In vitro* release of chrysin from niosomes

Time (h)	Cumulative percentage of drug released (%)					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	2.6	8.1	3.3	2.8	3.5	11.6
2	8.3	12.9	8.4	6.3	15.4	19.1
3	15.8	15.1	11.8	14.6	18.5	21.9
4	16.5	19.5	19.1	16.4	21.8	23.2
6	24.2	29.4	34.2	27.3	30.8	29.4
8	33.8	35.3	39.6	34.9	39.4	39.3
10	41.5	41.6	45.1	49.8	48.2	44.1
12	47.3	51.8	50.7	54.1	53.9	50.8
18	68.6	79.4	65.7	69.6	71.8	62.3

Table 4 Slope and regression coefficient of various graphical models

Formulation	Zero Order		First Order		Higuchi		Korsemeyer-Peppas	
	Slope	R^2	Slope	R^2	Slope	R^2	Slope	R^2
F2	4.185	0.9947	-4.1111	0.9955	21.095	0.9474	0.7833	0.8586
F5	4.0121	0.9768	-3.8758	0.9781	20.535	0.9924	0.9478	0.9376