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FORMULATION OF NIOSOMAL SUSPENSION WITH ENHANCED ORAL BIOAVAILABILITY OF DICLOFENAC SODIUM

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ABSTRACT

In order to establish and maintain drugs from any concentrations at target sites for a longer period of time, niosomes was formulated. The main aim of this study was to formulate niosomal suspension containing diclofenac sodium multilamellar vesicle (MLVs). Diclofenac sodium was encapsulated into niosomes through the thin film hydration method. Different ratios of cholesterol and surfactant (span 60) were used. The optimized ratio was 1:2:1 with highest entrapment efficiency. The *in-vitro* release of the drug was consistent. The time course of drugs and their effects in the body are assessed through pharmacokinetics which was provided by mathematical basis. The data collected from the release were incorporated into release kinetic analyses which are Higuchi equation, Korsmeyer-Peppas equations and Hixson-Crowell model along with zero order kinetics and first-order kinetics. The best fit with higher correlation ($R^2 >$ 0.98) was found with the Korsmeyer-Peppas equation with the R^2 value of 0.993. A stability study was done in order to discover the ability of drug to be leached out from the niosomal formulation upon storage at room temperature 25 ± 2^0C and refrigerated condition (2^0C to 8^0C). The drug release and the stability studies have been shown up to 28 days, but considerable stability was found for 2 weeks only. In conclusion, the encapsulation of diclofenac sodium in the niosomal formulation was assumed to be more suitable compared to the conventional drug

delivery system. The diclofenac sodium was released slowly as the niosomal vesicles act as depot and offer a controlled release.

Key words: niosomes, span 60, diclofenac sodium, release kinetics

INTRODUCTION

From this, new thoughts on scheming the pharmacokinetics, pharmacodynamics, toxicity, immunogenicity, and efficiency of drugs were generated, often called as novel drug delivery systems.[1] The primary niosome formulations were developed and patented by L'Oreal in 1975.[2] They are liposome-like vesicles formed from the hydrated mixtures of cholesterol, charge inducing substance, and nonionic surfactants such as monoalkyl or dialkyl polyoxyethylene ether.[3] Basically, these vesicles do not form impulsively. Thermodynamically stable vesicles form only in the presence of proper mixtures of surfactants and charge inducing agents.[4] Niosomes are now widely studied as an alternative to liposomes because they alleviate the disadvantages associated with liposomes.[5] Drug delivery systems using colloidal particulate carriers such as niosomes have distinct advantages over conventional dosage forms. The low cost, stability and resultant ease of storage of nonionic surfactants has led to the exploitation of these compounds as alternatives to phospholipids.[6] Niosomes consist of two main component which is non-ionic surfactant and additives The vesicular layer is formed by the non-ionic surfactants. Cholesterol is the additive used in niosomes preparation. The presence of the steroidal system in cholesterol improves the rigidity of the bilayer and their presence in membrane affects bilayer fluidity and permeability.[7] NSAIDs are a class of drugs with analgesic, anti-inflammatory, and antipyretic effects. The most common pain reliever used in whole worldwide are NSAIDs or nonsteroidal anti-inflammatory drugs.[8] Diclofenac sodium, is a phenylacetic acid derivative, structurally similar to both the phenylalkanoic acid and the anthranilic acid series of compounds. [9] Diclofenac is more than 99% bound to human serum proteins, primarily to albumin.[10] Diclofenac metabolites undergo further glucuronidation and sulfation followed by biliary excretion. Diclofenac sodium was formulated in the form of niosomes to establish the enhanced therapeutic effect upon its sustaining drug release pattern.

MATERIALS AND METHODS

Diclofenac sodium was a benevolent gift from Ranbaxy (Malaysia) Sdn. Bhd. Cholesterol (CHOL), ethyl alcohol and sorbitan monostearate (span 60) were purchased from R & M Marketing Essex UK. Chloroform was purchased from HmbG Chemicals (Malaysia). All other chemicals used were of analytical grade.

Thin film hydration technique

The multilamellar vesicles containing diclofenac sodium was prepared by using thin film hydration technique with slight modification as reported by Pratap S. Jadon *et.al.* (2009). Initially, the cholesterol and surfactant (span 60) was dissolved in a little amount of chloroform (about 2-3 ml) and placed in a sonicator (Elma S 80 H Elmasonic) to form a homogenous solution. Meanwhile, diclofenac sodium was dissolved in ethyl alcohol (about 2-3 ml) separately and placed in a sonicator for complete solubility of the drug. Later on, the two mixtures were mixed together and again placed in a sonicator to form a homogenous clear solution. The organic solvents were slowly evaporated using rotary evaporator (Rotavapor R-210 Buchi Switzerland) at 60^{o}C. This will form a very thin film of dry lipids on the inner surface of the round bottom flask. This layer was rehydrated with phosphate buffer saline (PBS) pH 7.4. Upon continuous gentle shaking by hand, results in swelling of the surfactant layer. The dispersion was maintained for 2 hrs at room temperature to allow the niosomes to form vesicles which entrap the diclofenac sodium.[11]

Separation of free drug

The prepared MLV diclofenac sodium niosomes was separated from unentrapped diclofenac sodium by ultracentrifugation at 6,000 rpm for 1 hr using a centrifuge at 2^{o}C. The isolated particles were washed twice each with 10 ml phosphate buffered saline, and recentrifuged again for 1 hr.[12]

Determination of entrapment efficiency

The amount of entrapped diclofenac sodium was determined by lysis of the vesicles with absolute ethanol. A 0.1 ml sample of niosomes was mixed with 5 ml of absolute ethanol and covered well with parafilm to prevent evaporation.[12] The solution then sonicated for 10 min in a sonicator to obtain a clear solution. The concentration of diclofenac sodium in absolute ethanol was determined spectrophotometrically at 277 nm using UVspectrophotometer.

Drug entrapment efficiency

$$\text{Percentage drug loading} = \frac{\text{Amount of drug in niosome}}{\text{Amount of niosome taken}} \text{ X } 100$$

$$\text{Percentage entrapment efficiency} = \frac{\text{Practical drug loading}}{\text{Theoretical drug loading}} \text{ X } 100$$

Drug content

(i) Standard preparation

Standard solution was prepared with 50 mg of diclofenac sodium dissolved in 50 ml of phosphate buffer pH 6.8 in a 50 ml volumetric flask. Then, 1 ml from the solution was pipette out and transferred into 100 ml of volumetric flask, diluted and made up to 100 ml with phosphate buffer pH 6.8.

(ii) Sample preparation

5 ml of niosomal suspension was dissolved into a 100 ml of volumetric flask. 2 ml of ethanol was added into the volumetric flask and was shaken well for a few minutes until it was completely mixed. The solution in the volumetric flask was then made up to 100 ml with phosphate buffer pH 6.8.

The resulting solution was filtered by using 0.45 μ filter membrane. 1 ml of filtered solution was pipette out and was transferred into 10 ml volumetric flask and made up to the volume with phosphate buffer pH 6.8. The solution was analyzed for drug content by using UV spectrophotometer at 277 nm. The percentage of drug content was calculated by using the following formula:

$$\% \text{ Drug content} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \text{ x } \frac{\text{Standard dilution}}{\text{Sample dilution}} \text{ x } 100$$

$$\text{Amount of drug (mg)} = \frac{\% \text{ drug content}}{100} \text{ x } 50$$

Vesicle morphology analysis

The freshly formulated niosomal dispersion was scanned and imaged using an optical microscope (Olympus CX21, ME00001947) with a magnification power of 40x and attached to video camera (Sony Cybershot DSC-T900, 12.1 Megapixel). The shape of the niosomes was observed.

Size analysis

Optical microscope with a calibrated eyepiece micrometer was used to analyze particle size. The average was taken for their size distribution range and mean diameter were calculated by measuring roughly about 200 niosomes individually.[13] Further the niosomes were analyzed by Scanning Electron Microscopy (SEM) for more specific characterization of the size of the formed vesicles.

***In-vitro* drug release studies**

A visking tube containing 2 ml of prepared niosomes is tied at both ends and placed in 1 liter beaker of a dissolution apparatus (USP II TDT-08L Electrolab) containing 900 ml of phosphate buffer saline solution of pH 6.8 maintained at 37°C. At specified interval of time, sample solution of 5 ml is withdrawn from the beaker and replaced with fresh buffer solution. The sample was then analyzed after necessary dilutions at 277 nm under UV spectrophotometer.

Stability studies

The prepared niosomes with encapsulated diclofenac sodium was stored for 30 days at room temperature 25 ± 2^0C and refrigeration condition (2^0C to 8^0C). The stability of the niosomes was evaluated in terms of drug release, formation of precipitation and the observation of the shape of the particles under optical microscope.

Drug release kinetic studies

To investigate the possible mechanisms of diclofenac sodium released from the prepared niosomes, the release data were analyzed mathematically according to different kinetic models such as, zero order kinetics, first order kinetics, Higuchi's model, Korsmeyer-peppas model and Hixson Crowell model.[14]

RESULTS

The formulated niosomes were analyzed for percentage of drug entrapment efficiency. The findings were recorded in table 2. The formulation code, DF3 has showed the highest entrapment (74.09 ± 0.77). The lowest entrapment efficiency was seen in formulation code DF5 which was 64.46 ± 1.07. The result obtained was recorded in table 2. The graph plotted for entrapment efficiency is shown in figure 1.

Particle size distribution for diclofenac sodium

The mean particle sizes for the formulated niosomes were calculated and a graph was plotted (figure 2). Then, the average number of particle size for the niosomal formulation with the best entrapment efficiency (Formulation code: DF3) was plotted in a chart (figure 3). The obtained result is recorded in table 4.

Vesicle morphology study

Observation of niosomes under optical microscope

The formulated niosomes were observed under optical microscope and the particles observed were spherical in shape and surrounded by the formation of lipid layer. (Figure 4)

Observation of niosomes under SEM

The obtained results were shown in figure 5 and 6**.**

Drug release profile

The formulation code of DF3 was found to be significant because it has showed a cumulative release of 94.46% over a period of 8 hrs. The rate of drug release recorded in table 5. The drug release data obtained for diclofenac sodium was plotted according to different modes of formulated treatment were shown in figure 7.

Stability studies of final formulation (DF3)

The drug release and the stability studies have been shown up to 28 days, but considerable stability was found for 2 weeks only. The obtained result was recorded in table 6. The graph was plotted and shown in figure 8 for room temperature and figure 9 for refrigerated condition

Study of drug release kinetics

The results obtained for DF3 were recorded in table 5. The kinetics models all were represented in its respective graphs and shown in figure 10, 11, 12, 13 and 14. The best fit with higher correlation ($R^2 > 0.98$) was found with the Korsmeyer-Peppas equation with the R^2 value of 0.993.

Table 1: Compositions of drug, cholesterol and surfactant used to formulate niosomes

Formulation code	Drug : Cholesterol: Surfactant (ratio)	Drug (mg)	Span 60 (mg)	Cholesterol (mg)
DF1	1 : 1 : 1	50	50	50
DF2	1 : 1.5 : 1	50	75	50
DF3	1 : 2 : 1	50	100	50
DF4	1 : 2.5 : 1	50	125	50
DF5	1 : 2 : 2	50	100	100
DF6	1 : 2.5 : 2	50	125	100

Table 2: Entrapment efficiency of the formulated niosomes

Formulation Code	Entrapment efficacy (%)
DF1	72.88 ± 3.22
DF2	69.50 ± 0.63
DF3	74.09 ± 0.77
DF4	67.20 ± 1.01
DF5	64.46 ± 1.07
DF6	65.46 ± 0.91

Table 3: Percentage of drug content in formulated niosomes

Formulation code	% Drug content
DF1	75.56
DF2	82.88
DF3	95.05
DF4	84.26
DF5	76.65
DF6	88.80

Table 4: Mean particle size and the shape of niosomes from niosomal formulations

Formulation Code	Mean particle size (µm)	Morphology
DF1	4.12 ± 0.89	Spherical
DF2	4.58 ± 0.61	Spherical
DF3	4.09 ± 1.22	Spherical
DF4	6.17 ± 0.87	Spherical
DF5	5.76 ± 0.43	Spherical
DF6	5.12 ± 0.43	Spherical

Table 5: The percentage of drug release from of niosomal formulations

Time (hrs)	% Drug release					
	DF1	DF2	DF3	DF4	DF5	DF6
1	13.23	18.76	18.64	34.26	5.98	10.32
2	27.14	27.87	34.24	46.01	12.48	32.18
4	42.36	48.45	53.02	75.56	34.03	46.78
6	65.78	66.09	68.12	92.18	51.12	58.45
8	80.97	85.26	94.46	102.54	62.23	78.12

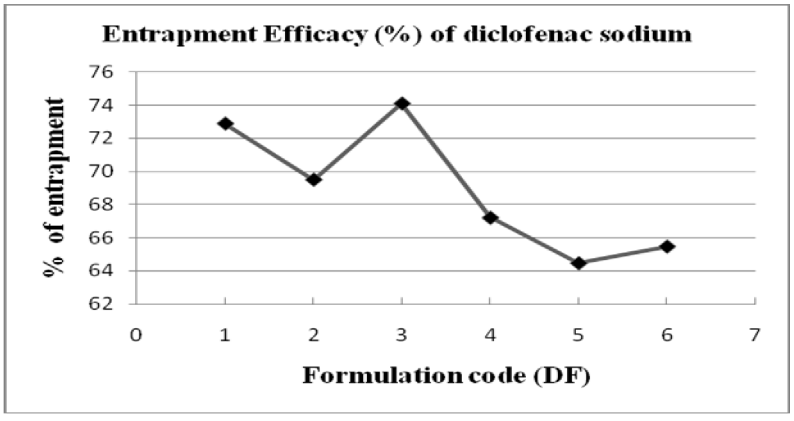


Fig. 1: The percentage of entrapment efficiency of diclofenac sodium niosomal formulations

Table 6: The percentage drug content and physical stability of diclofenac sodium niosomes

S. No	Time (Days)	Storage condition	Drug content (%)	Physical stability (Microscopically observed)
1	Initial	RC	94.46	Spherical
		RT	94.46	
2	7th day	RC	88.23	Spherical
		RT	82.40	Spherical
3	14th day	RC	79.12	Non spherical
		RT	63.81	Non spherical
4	21st day	RC	61.26	Non spherical & clumps
		RT	32.50	Non spherical & clumps
5	28th day	RC	33.33	Non spherical & clumps
		RT	12.01	Non spherical & clumps

RC *– Refrigerated condition (2 to 8^{0}C);* ***RT*** *–Room temperature (25 ^{0}C ± 2 ^{0}C)*

Table 7: The kinetic analysis of niosomal formulation loaded with diclofenac sodium

Time (hrs)	**√t**	**log t**	**Amount released (mg)**	**%Drug released**	**%Drug to be released**	**log% Drug released**	**log% Drug to be Released**	**(D)⅓**
1	1.0	0.0	9.32	18.64	81.36	1.270	1.910	4.333
2	1.414	0.301	17.13	34.24	65.76	1.535	1.818	4.036
4	2.0	0.602	26.51	53.02	46.93	1.724	1.671	3.607
6	2.449	0.778	34.06	68.12	31.88	1.833	1.501	3.171
8	2.828	0.903	47.23	94.46	5.54	1.975	0.744	1.769

Where,

D – Percentage drug to be released,

t – Time

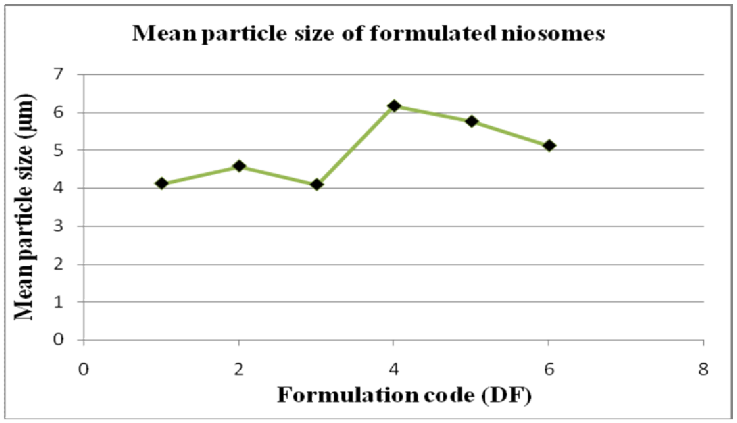


Fig. 2: Mean particle size of six different niosomal formulations containing diclofenac sodium

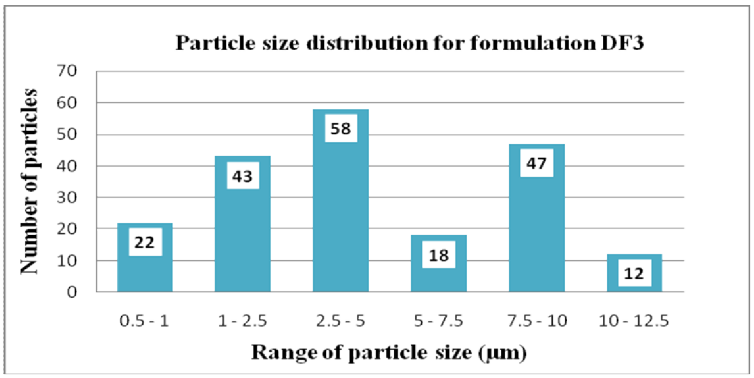
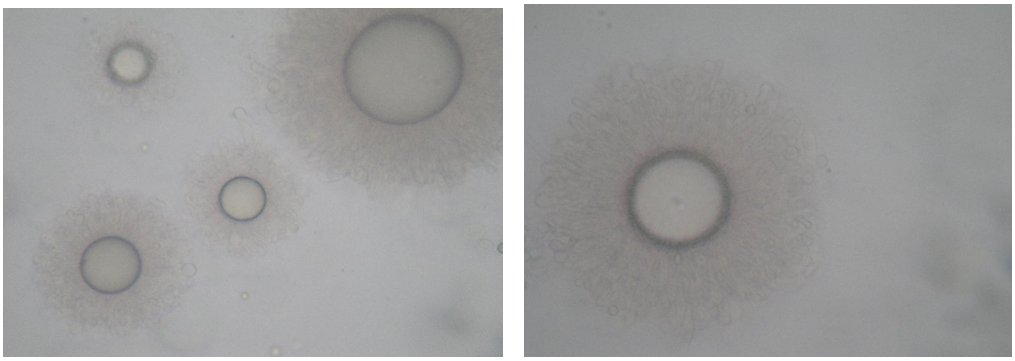
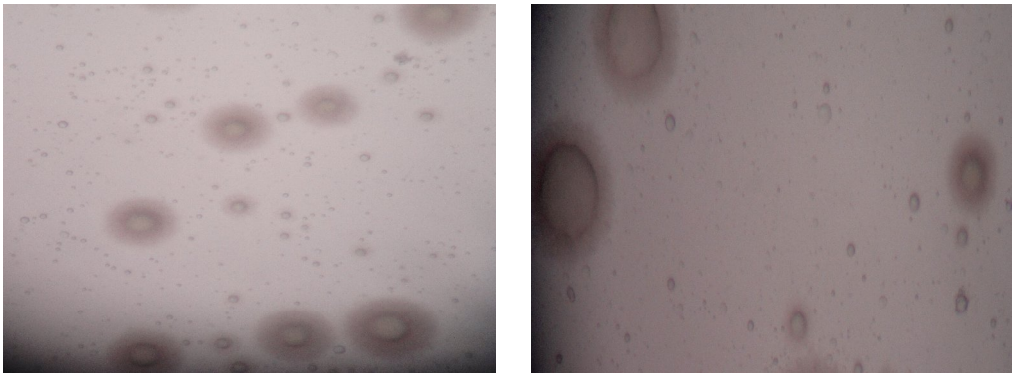


Fig. 3: Mean particle size of niosomal formulations containing diclofenac sodium for the formulation DF3

Figure 4 A, B, C, and D: Microphotographs of optimized niosomes observed under optical microscope (Olympus CX21, ME00001947) with a magnification power of 40x, attached to a video camera (Sony Cybershot DSC-T900, 12.1 Megapixel).



4A 4B



4 C 4D

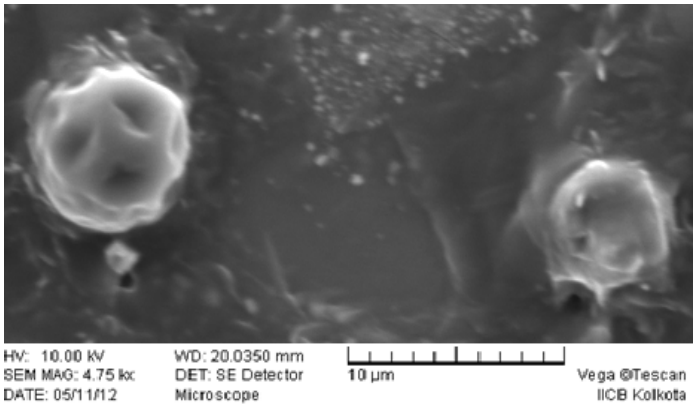


Fig. 5: Niosomes observed under SEM with a magnification power of 4.75kx

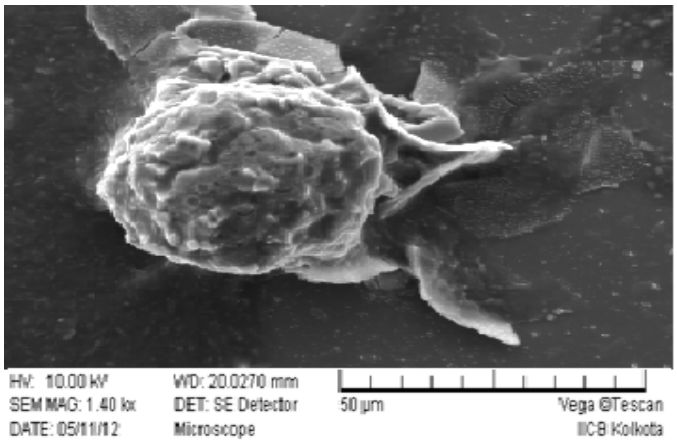


Fig. 6: Niosomes observed under SEM with a magnification power of 1.40kx

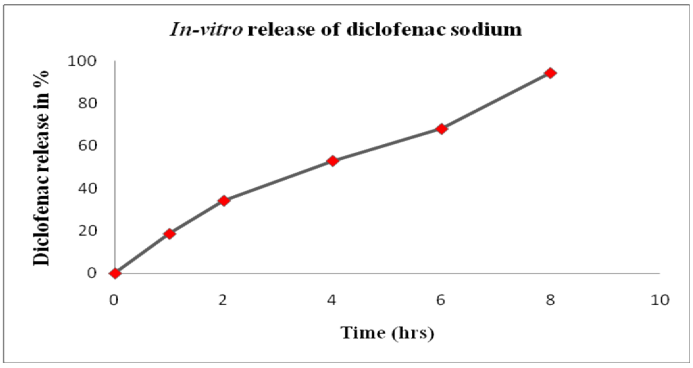


Fig. 7: The *in-vitro* release rate of diclofenac sodium in niosomal formulation

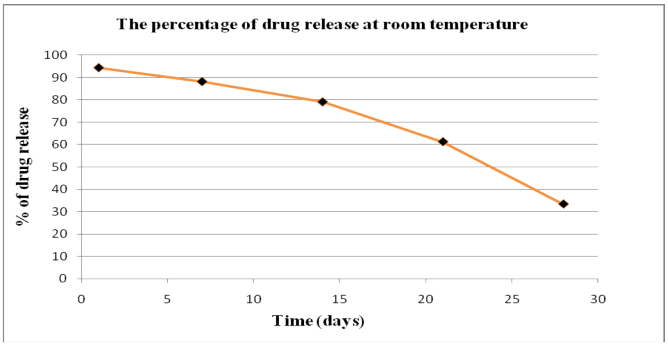


Fig. 8: The percentage of diclofenac sodium released at room temperature for 28 days

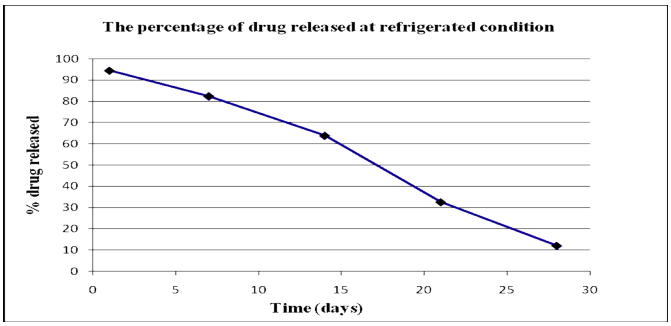


Fig. 9: Percentage of diclofenac sodium released at refrigerated condition for 28 days

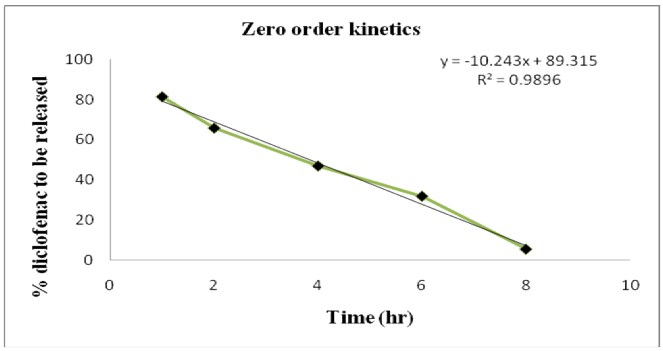


Fig. 10: The zero-order kinetics (cumulative amount of diclofenac sodium released *vs*. time)

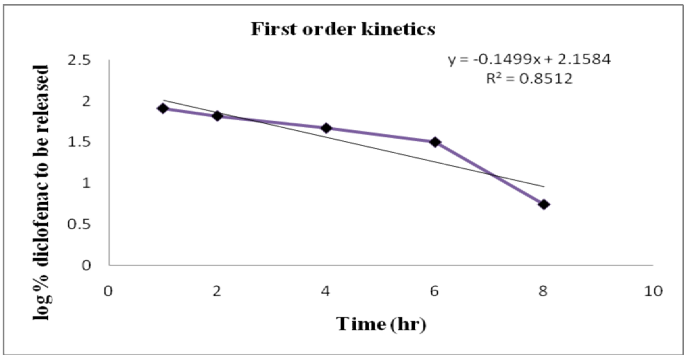


Fig. 11: First-order kinetics (log cumulative percentage of diclofenac sodium remaining *vs.* time)

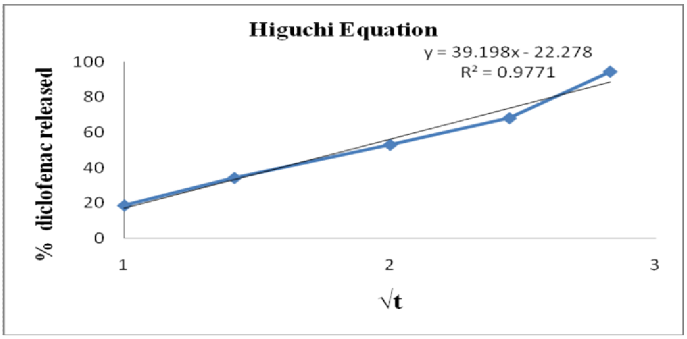


Fig. 12: Higuchi equation for diclofenac sodium niosomes

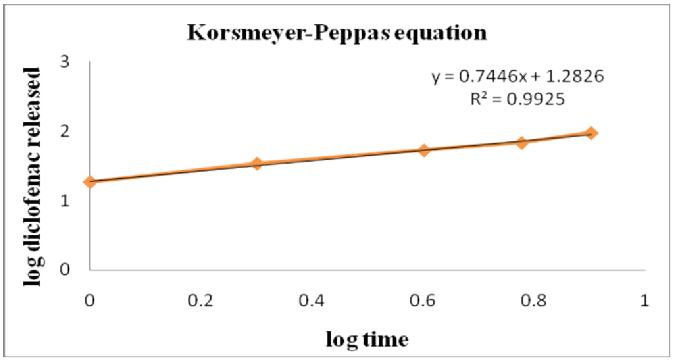


Fig. 13: The Korsmeyer-Peppas equation for diclofenac sodium niosomes

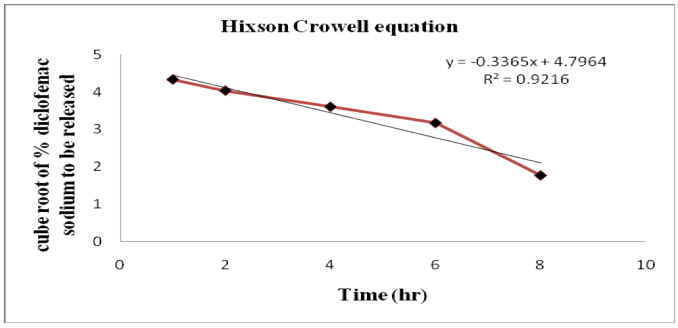


Fig. 14: The Hixson Crowell equation model for diclofenac sodium niosomes

DISCUSSION

Prepared niosomes were optimized on the basis of vesicle size and entrapment efficiency. The optimum of drug: surfactant: cholesterol was obtained at the ratio of 1:2:1 in the formulation of DF3. The formulation of DF3 showed the highest cumulative drug release of 94.46% over a period of 8 hrs. This is may be due to the solid nature, hydrophobicity, and high-phase transition temperature of the surfactant. The particles in the niosomal formulations were spherical in shape for the first week and non-spherical at the second week of storage at both room temperature and refrigerated condition. Then, following the 3^{rd} and 4^{th} week the particles were non-spherical in shape and there was formation of clumps. The formulation code DF3, obeys zero order kinetics, Higuchi and Korsmeyer-Peppas release pattern. All the equations for the formulation code DF3 shows high linearity of (R^2 = 0.971 to 0.998) except for first order kinetics (R^2 = 0.851) and Hixson equation model (R^2 = 0.922). The best fit with higher correlation (R^2 > 0.98) was found with the Korsmeyer-Peppas equation with the R^2 value of 0.993. The high linearity indicates coupling of diffusion and erosion mechanism. Release of a drug from a lipid bilayer generally involves both pore diffusion and matrix erosion.

CONCLUSION

The incorporation of diclofenac sodium in the niosomal drug targeted delivery system was assumed to be more appropriate compared to the conventional drug delivery system. The main aim of this study was to formulate niosomal suspension containing diclofenac sodium multilamellar vesicle (MLVs). The formulation code, DF3 (1:2:1) is the optimized ratio which showed the highest entrapment (74.09 ± 0.77) and the mean particle size was 4.09 ± 1.22. Diclofenac sodium was released slowly as the niosomal vesicles act as depot and offer a controlled release. Thus, the adverse effects due to the drug accumulation can be minimized or prevented. By incorporating the drug in the form of niosomes, it may enhance the bioavailability and improves the drug absorption. In future, *in-vivo* studies should be carried out for niosomal formulations containing diclofenac sodium to prove the enhancement of the drug delivery system composing niosomes.

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