AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Formulation and Evaluation of Proniosomal Gel of Flurbiprofen

Dinesh Chandra*[1], Ashish Kumar[1], Rakesh singh[1], Tirth Raj[1], Jayant Kumar Maurya[1], Shrikant Verma[2]

1.Kamla Nehru Institute of Management and Technology, Sultanpur, India. Babu Banarasi Das University, Lucknow, India

ABSTRACT

The aim of present study, To formulate and characterized proniosome contain flurbiprofen in a gel formulation for the treatment of rheumatoid arthritis and enhanced skin targeted effect, sustained & prolonged drug release, enhanced skin bioavailability by using different type of non ionic surfactant & cholesterol. The batches were designed using Box Behnken Design and prepared by coacervation phase separation method. Optimized formulation (PNGopt) showed drug entrapment efficiency of 74.46% and particle size 215nm. *In-vitro* drug release from PNGopt was found to be 84.15 in 24 hrs. The *In-vitro* drug release was best explained by zero order kinetics as the plot showed highest linearity and release was governed by Quasi Fickian diffusion.

Keywords: Proniosomes, Flurbiprofen,Brij93,in-vitro release, Stability studies

*Corresponding Author Email: ashishkumar8081405109@gmail.com

Received 02 November 2013, Accepted 11 November 2013

Please cite this article in press as: Chandra D. *et al.*, Formulation and Evaluation of Proniosomal Gel of Flurbiprofen. American Journal of PharmTech Research 2013.

INTRODUCTION

Drug delivery systems using vesicular carriers such as liposomes and niosomes have distinct advantages over conventional dosage forms. They may serve as a solubilization matrix, as local depot, as permeation enhancer or as a rate limiting membrane barrier for the modulation of systemic absorption of drugs via the skin.[1]Proniosomes are recent development in Novel drug delivery system. These are most advanced drug carrier in vesicular system which overcomes demerits of liposomes and niosomes. These, hydrated by agitation in hot water for a short period of time, offer a versatile vesicle delivery concept with the potential for drug delivery via the transdermal route[2] Liposomes or niosomes can carry hydrophilic drugs by encapsulation or hydrophobic drugs by partitioning of these drugs into hydrophobic domains. Liposomes are unilamellar or multilamellar spheroid structures composed of lipid molecules, often phospholipids, assembled into bilayers. Because of their ability to carry a variety of drugs, liposomes have been extensively investigated for their potential application in pharmaceutics; such as drug delivery , drug targeting; controlled release or increased solubility.[3]The proniosomal approach minimizes the above-mentioned problems, as it involves a dry product or a liquid crystalline gel that can be hydrated immediately before use. Ease of transfer, distribution, measuring and storage makes proniosomes a versatile delivery system. Proniosomes are water-soluble carrier particles that are coated with surfactant and can be hydrate to form a niosomal dispersion immediately before use on brief agitation in hot aqueous media.[4]

Flurbiprofen is a derivative of phenylalkanoic acid, a nonsteroidal anti inflammatory drug(NSAID) related to ibuprofen in structure. it is used for the relief of pain and inflammation associated with rheumatoid arthritis and osteoarthritis.1 It exhibits anti-inflammatory, analgesic and antipyretic activities. It is also used in mild to moderated pain including dysmenorrheal and migraine. Flurbiprofen is more potent than Ibuprofen but has more gastric side effect like peptic ulceration and severe gastrointestinal bleeding may occurs. The plasma half life (t1/2) of flurbiprofen is 4-6 hours. Hence repeated administration of high dose (100 mg: three time a day) is required for effective management of rheumatoid arthritis and osteoarthritis. It will be also affected through transdermal route because of its size, nature and chemistry, these systems give better drug permeability from biological bioavailability membranes and helps in solubilization of some practically insoluble drugs and hence solve problems of many drug. To overcome the problem like gastric side effect, short half life and low bioavailability etc of flurbiprofen can be solved by developing the formulation of flurbiprofen as proniosome gel.[5]

MATERIALS AND METHOD

Material

Flurbiprofen was a gift from F.D.C. Mumbai, cholesterol, and dialysis tubing was purchased from Hi-Media Laboratories (Mumbai, India). BRIJ 93 was purchased from Central Drug House (Delhi, India). All other chemicals and solvents were of analytical grade and obtained from Central Drug House Delhi, India.

Method

Proniosomal gel was prepared by a coacervation-phase separation method. Precisely weighed amounts of surfactant, cholesterol and drug were taken in a clean and dry wide mouthed glass vial of 5.0 ml capacity and alcohol (0.5 ml) was added to it. All the ingredients were mixed well with a glass rod; the open end of the glass bottle was covered with a lid to prevent the loss of solvent from it and warmed over water bath at 60-70°C for about 5 min until the surfactant mixture was dissolved completely. Then the aqueous phase (0.1% glycerol solution) was added and warmed on a water bath till a clear solution was formed which was converted into proniosomal gel on cooling. The gel so obtained was preserved in the same glass bottle in dark conditions for characterization.[1,2,3,4,5,11,20,22,23,37]

Experimental Design :

A three-factor, three-level Box-Behnken experimental design was used to optimize the formulation development. It was suitable for investigating the quadratic response surfaces using Design Expert 8.0.7.1® (Version) software. In this study, 3 independent variables drug-polymer ratio, Cholesterol, Brij 93 were taken which significantly influence the observed response for particle size, entrapment efficiency.[11,22,38,39,40,45,46]

Table .1 Values for Independent Variables

Independent variables	Low level(-1)	Medium level (0)	High level (+1)
Drug : polymer	1:1	1:1.5	1:2
Cholesterol	50	100	150
Surfactant	50	75	100

Table 2: Formulation of Proniosomal Gel of Flurbiprofen

Formulation. Code	Drug: Polymer (mg) Flurbiprofen: Lecithin	Surfactant-Brij93 (mg)	Cholesterol (mg)	Solvent ratio(ml)
PNG 1	50:50	100	100	0.5
PNG 2	50:100	100	100	0.5
PNG 3	50:75	75	100	0.5
PNG 4	50:75	100	50	0.5
PNG 5	50:75	50	50	0.5
PNG 6	50:75	75	100	0.5

PNG 7	50:100	75	50	0.5
PNG 8	50:75	75	100	0.5
PNG 9	50:50	50	100	0.5
PNG 10	50:75	75	100	0.5
PNG 11	50:50	75	150	0.5
PNG 12	50:50	75	50	0.5
PNG 13	50:100	50	100	0.5
PNG 14	50:75	75	100	0.5
PNG 15	50:100	75	150	0.5
PNG 16	50:75	100	150	0.5
PNG 17	50:75	50	150	0.5

CHARACTERIZATION OF PRONIOSOMAL GEL

Optical microscopic examination

Hydration of proniosomal gel was done by adding saline solution (0.9% solution) in a small glass vial with occasional shaking for 10 min. The dispersion was observed under optical microscope at 100 x magnification. The sizes of 200-300 vesicles were measured using a calibrated ocular and stage micrometer (Erma, Tokyo) fitted in the optical microscope[9,11,23,28,53]

Entrapment efficiency

To evaluate the loading capacity of proniosomal systems for flurbiprofen, proniosomal gel (100mg) was dispersed in distilled water and warmed a little for the formation of niosomes. Then the dispersion was centrifuged at 18000 rpm for 40min at 5ºC (Remi CPR-24 centrifuge). The clear fraction was used for the determination of free drug at 247.0 nm spectrophotometrically. The percentage encapsulation efficiency was calculated from Equation 1.

% Encapsulation Efficiency=[1-(Unencapsulated drug / Total drug)] x 100……1[11,13,20,22,26,29,31]

Vesicle physical analysis:

The morphology of two dimensional vesicles was further evaluated by TEM. The niosomal suspension was diluted to 1:100 or more so that it looks clear. From the clear suspension one drop was mounted on copper grid. Samples were negatively stained with a 1% aqueous solution of PTA. It was then completely air dried and visualized under Transmission electron microscope.[11,35,38]

In-Vitro Release

In vitro drug release study was carried out using Keshary-Chien (K-C) cell of 25 ml capacity using egg membrane, in phosphate buffer saline (PBS) pH 7.4. The receptor compartment was filled with phosphate buffer saline pH 7.4 while a 2ml volume of formulation was taken in the donor compartment. The temperature of the receptor compartment was maintained at 37 ± 0.5^0C with the help of a circulating water bath. Samples (1 ml) were withdrawn at regular interval and

replaced with equal volume of PBS pH 7.4 to maintain the sink conditions. Samples were diluted with buffer solution and analyzed spectrophotometrically at 246 nm against reagent blank. The percentage drug release was calculated from the calibration curve.[11,18,19,22,26,33,36,40,46]

Rate of spontaneity:

Approximately 10 or 20 mg of proniosomal gel was transferred to the bottom of a clean stoppered glass bottle and spread uniformly around the wall of the glass bottle with the help of a glass rod. At room temperature, 2 ml of phosphate saline was added carefully along the walls of the glass bottle and left in a test-tube after 20 minutes, a drop of this dispersion was withdrawn and placed on Neubaures chamber to count the number of vesicles. The number of niosomes eluted from proniosomes were counted.[11,20,22,26,33,41,44,46]

Vesicular Size Analysis

Zetasizer was used to determine the size of the optimized vesicular formulation. The optimized formulation was hydrated with 7.4 pH phosphate buffer saline and was converted to niosomes. The size of the vesicles is determined by the instrument.[11,22,26,38,45,46]

Stability Studies

The ability of vesicles to retain the drug (Drug Retention Behaviour) was assessed by keeping the proniosomal gel at three different temperature conditions, i.e., Refrigeration Temperature (4-8^0 C), Room Temperature (25 ± 2^0 C) and oven (45 ± 2^0 C). Throughout the study, proniosomal formulations were stored in aluminum foil-sealed glass vials. The samples were withdrawn at different time intervals over a period of 1-3 month and drug content was analysed spectrophotometrically. [11,20,22,26,40,44,45,49]

RESULTS AND DISCUSSION

Optical microscopy

The photomicroscopic examination of the prepared niosomal vesicles were taken at suitable magnification. One formulation were optimized here for further studies which is formulation no.PNG 12[11,23,38,53]

Entrapment Efficiency

Vesicular entrapment efficiency is an important parameter that conveys the stability of vesicles and this depends upon the amount of surfactant as well as amount of cholesterol used. The entrapment efficiency of these formulation varies from 59.32 to 74.46%. The entrapment efficiency of various formulation is tabulated in table 2. From the data in table 2, it is clear that entrapment efficiency depends upon both the amount of surfactant and cholesterol. Effect of

independent variables on the entrapment efficiency can be represented in the 3D surface plot using Design Expert 8.0.7.1® (Version) software.[11,20,22,26,29,40]

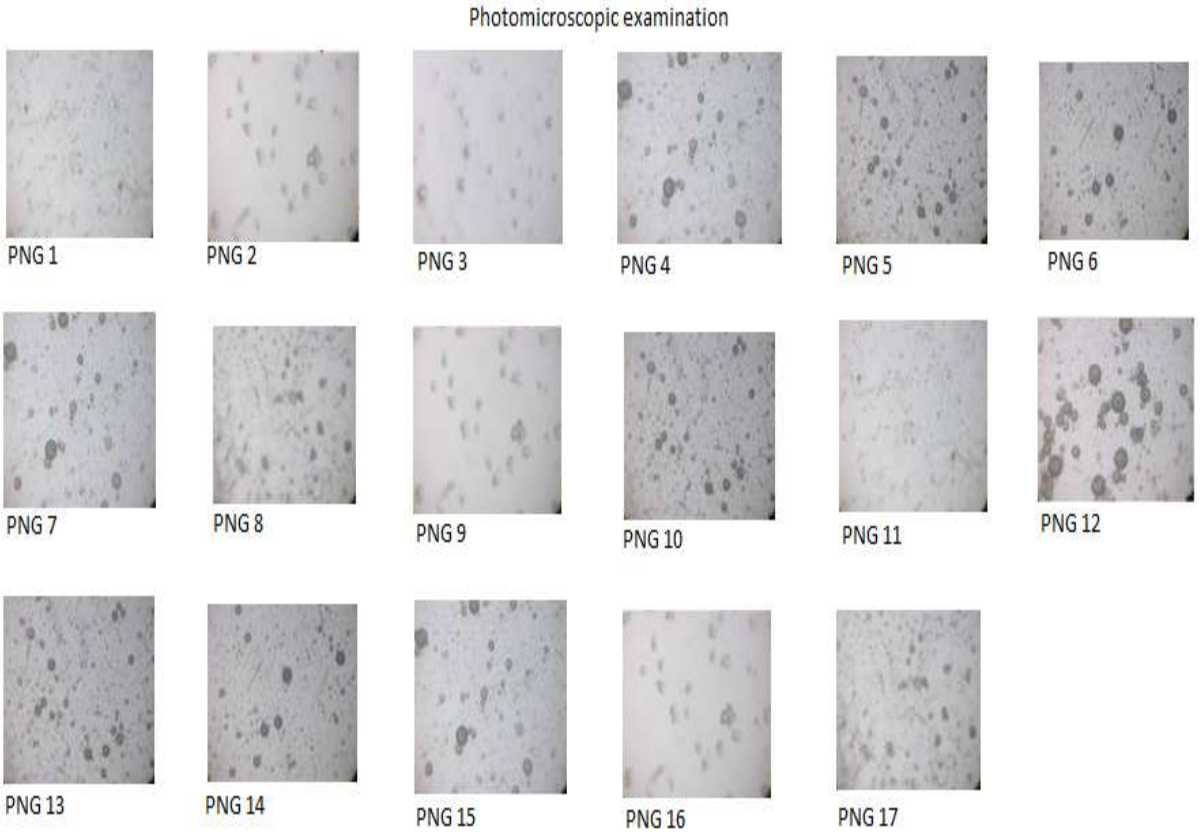

Figure .1 Photomicroscopic view of all prepared niosomal formulation from proniosomes (PNG 1-PNG 17) after their hydration:

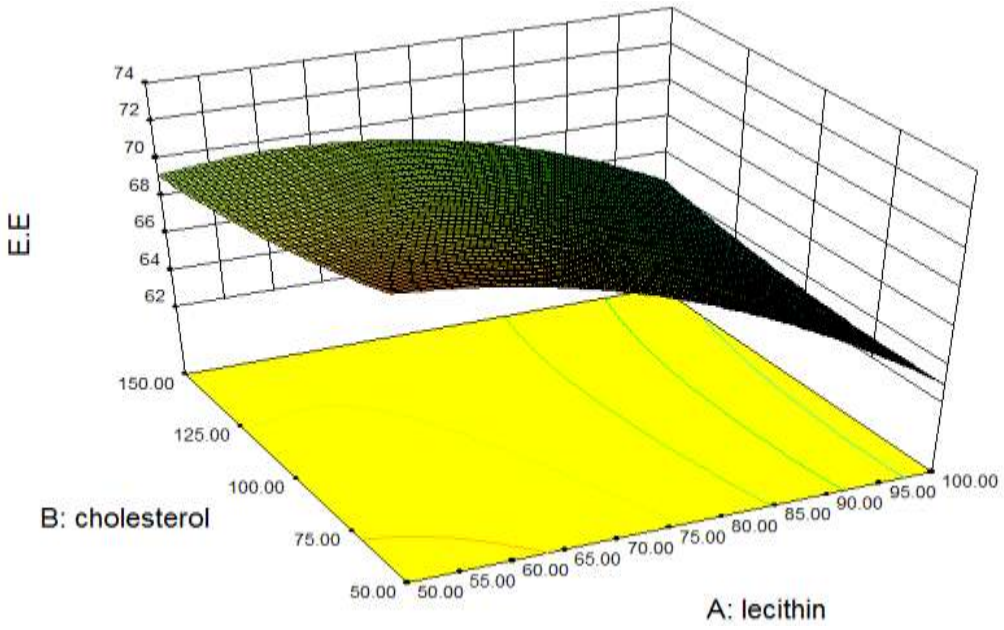

Figure 2. Response surface plot for Drug Entrapment efficiency

Table 3. Drug entrapment efficiency of proniosomal gel of various batches

Batches	Drug Entrapment Efficiency (%)
PNG1	68.88
PNG2	59.32
PNG3	70.92
PNG4	69.32
PNG5	68.76
PNG6	72.01
PNG7	67.44
PNG8	70.96
PNG9	71.09
PNG10	72.63
PNG11	70.12
PNG12	74.46
PNG13	64.52
PNG14	71.11
PNG15	68.32
PNG16	68.87
PNG17	67.02

The entrapment effiency of different batches was found to be 59.32 to 74.46%. The Drug entrapment efficiency of optimizes batch (PNG12) was found to be 74.46%.

Effect of amount of surfactant properties

Surfactant is an important component in the formation of niosomal vesicles and the variation in the amount may affect the entrapment efficiency. PNG12 gives better entrapment than other formulation.[11,20,22,23,34,45]

Effect of cholesterol

The concentration of cholesterol plays an important role in the entrapment of drug in the vesicles. The increase in cholesterol concentration maximizes the drug loading but to a maximum value above which this will disrupt the vesicular membrane structure. The best result was shown in PNG12 (50 mg) from table 2 & 3.[11,20,22,23,34]

Vesicular size analysis

Particle size of prepared proniosomal gel was determined by Malvern Zetasizer Ver. 6.01

Table 4 Particle Size of proniosomal gel

Batches	Drug: Polymer	Cholesterol	Surfactant (Brij 93)	proniosomal gel size(nm)
PNG1	50:50	100	100	250
PNG2	50:100	100	100	265
PNG3	50:75	100	75	225
PNG4	50:75	50	100	236
PNG5	50:75	50	50	260
PNG6	50:75	100	75	230

PNG7	50:100	50	75	248
PNG8	50:75	100	75	228
PNG9	50:50	100	50	245
PNG10	50:75	100	75	229
PNG11	50:50	150	75	257
PNG12	50:50	50	75	215
PNG13	50:100	100	50	246
PNG14	50:75	100	75	220
PNG15	50:100	150	75	290
PNG16	50:75	150	100	285
PNG17	50:75	150	50	270

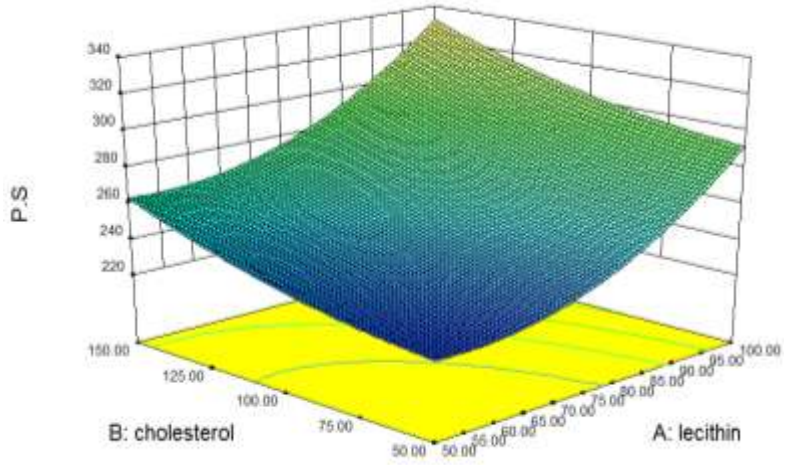

Figure 3. Response Surface Plot For Particle Size

The results of particle size is given in Table 4 Particle size of different batches was found to be 215 to 365 nm.. The particle size of optimized batch was (PNG12) found to be 215nm.[11,22,28,40,45,46]

TEM imaging of prepared niosomal vesicles after hydration of optimized proniosomal gel

TEM studies show that niosomes appeared as vesicles here. The outer membrane of the vesicles was properly visible by transmission electron microscopy (figure 4)[11,35,38]

Figure.4. TEM of optimized formulation (PNG12)

Cumulative Drug Release Study of the Optimized formulation

In vitro drug release study was carried out using Keshary-Chien (K-C) cell of 25 ml capacity using egg membrane, in phosphate buffer saline (PBS) pH 7.4. The receptor compartment was filled with phosphate buffer saline pH 7.4 while a 2ml volume of formulation was taken in the donor compartment. The temperature of the receptor compartment was maintained at 37 ± 0.5^{0}C with the help of a circulating water bath. Samples (1 ml) were withdrawn at regular interval and replaced with equal volume of PBS pH 7.4 to maintain the sink conditions. Samples were diluted with buffer solution and analyzed spectrophotometrically at 246 nm against reagent blank. The percentage drug release was calculated from the calibration curve.[1,5,11,20,22,23,28,30,34,36,37]

Table 5 *In- vitro* release of flurbiprofen in PBS pH 7.4 of optimized formulation

Time in hrs	% Cumulative drug released
1	2.92
2	9.30
3	17.27
4	26.02
6	36.63
8	47.77
12	59.45
16	71.68
24	84.15

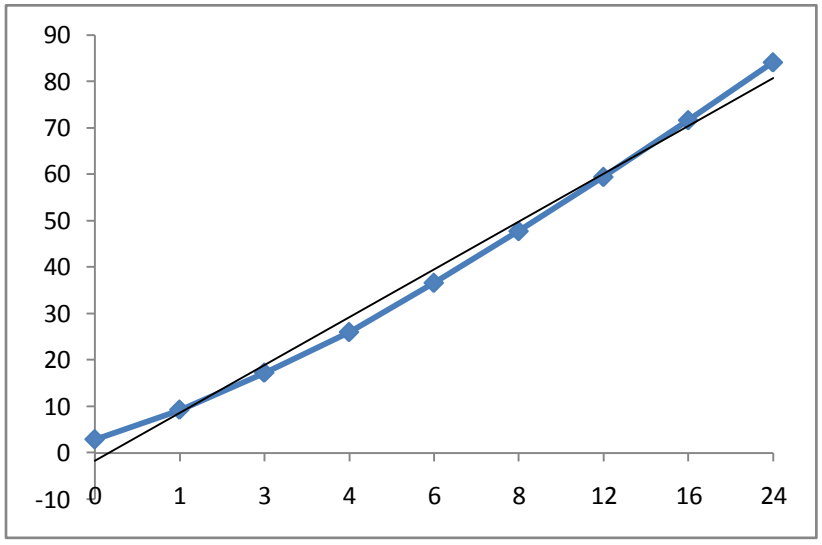

Figure 5. Cumulative Drug Release% of Optimized formulation

Analysis of Drug Release Data of the Optimized formulation

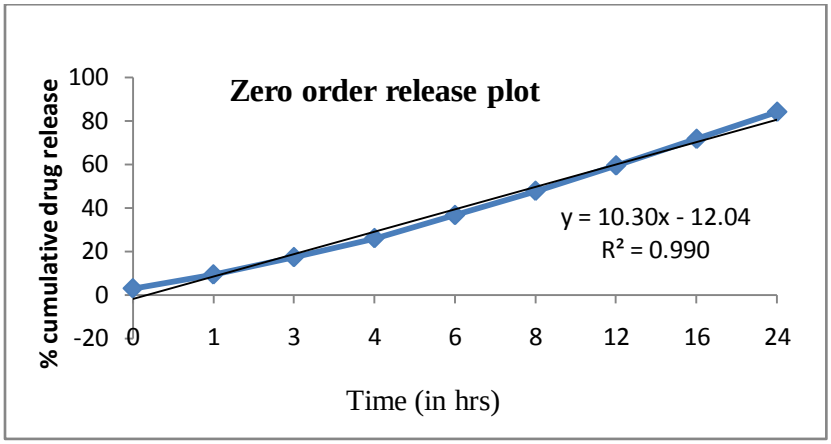

Figure 6: Plot of Zero order release kinetics of the optimized batch

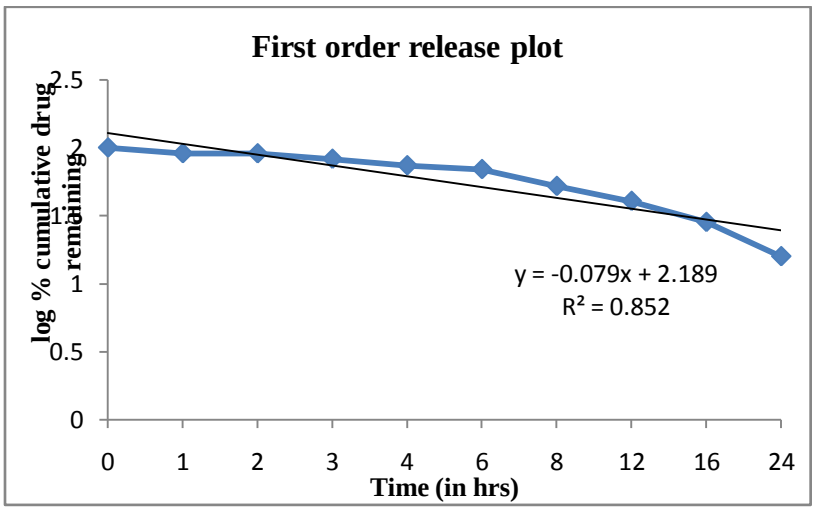

Figure.7. Plot of First order release kinetics of the optimized batch

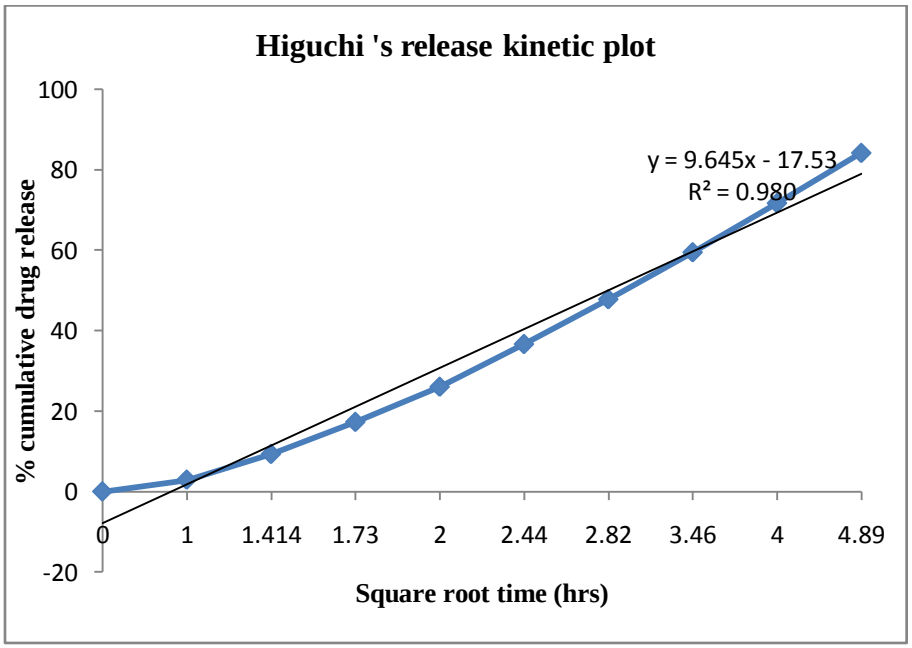

Figure.8. Plot of Higuchi release kinetics of the optimized batch

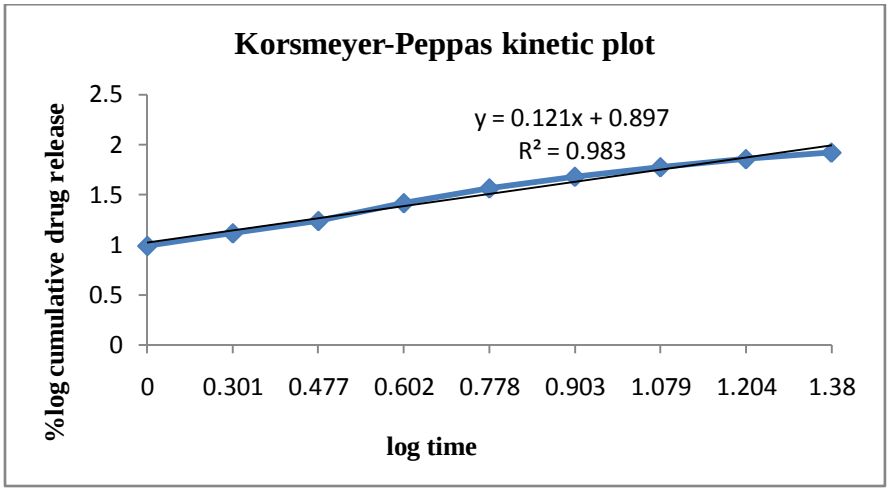

Figure.9. Plot of Korsmeyer- Peppas release kinetics of the optimized batch

Table.6. n value for different release kinetics

'n' values	Mechanism	dM_t/d_t dependence
n < 0.5	Quasi-Fickian Diffusion	$t^{0.5}$
0.5	Fickian Diffusion	$t^{0.5}$
0.5 < n < 1.0	Anomalous (non-fickian) Diffusion	t^{n-1}
1	Non-Fickian Case II	Zero Order
n > 1.0	Non-Fickian Super case II	t^{n-1}

Table.7. Release parameters of optimized proniosomal gel

Formulation	Zero order		First order		Higuchi		Korsmeyer-peppas	
	k	R^2	K	R^2	k	R^2	N	R^2
NPopt	10.30	0.990	-0.079	0.852	9.645	0.980	0.121	0.983

It was found that the *in vitro* drug release of PNG opt was best explained by zero order (Figure 6), as the plots showed the highest linearity (R^2 = 0.990), followed by higuchi's equation (Figure 8) (R^2 = 0.980) and first order (Figure 7) (R^2 = 0.852). The corresponding plot (log % cumulative drug release vs log time) for the Korsmeyer-Peppas equation indicated good linearity (Figure 9) (R^2 = 0.983). The release exponent 'n' was found to be 0.121, which appears to indicate the Quasi Fickian diffusion.[1,5,11,20,22,23,28,30,34,36,37]

CONCLUSION

A three-factor, three-level Box Behnken design was used for formulation optimization. A total 17 batches were prepared by Co-acervation phase separation method. and evaluated on the basis of particle size, and entrapment efficiency . The particle size of different batches was found to be between 215 to365 nm. Drug entrapment efficiency of various batches were found to be in the range of 59.32to74.46% respectively. The Optimized formulation (PNG12) was determined by using software Design Expert (Version 8.0.7.1). The optimized formulation was prepared with drug: polymer ratio 1:1 ,100mg cholesterol and using brij93(75mg). Optimized formulation

showed drug entrapment efficiency of 74.46 and particle size 215nm. In-vitro drug release of optimised formulation was found to be 84.15 in 24 hrs. *In vitro* drug release of PNG opt was best explained by zero order (Figure 6), as the plots showed the highest linearity (R^2 = 0.990), followed by higuchi's equation (Figure 8) (R^2 = 0.980) and first order (Figure 7) (R^2 = 0.852). The corresponding plot (log % cumulative drug release vs log time) for the Korsmeyer-Peppas equation indicated good linearity (Figure 9) (R^2 = 0.983). The release exponent 'n' was found to be 0.121, which appears to indicate the Quasi Fickian diffusion.

ACKNOWLEDGEMENT

The authors acknowledgement the financial support received from my parents. FDC Pvt Ltd Mumbai, Dr. Dinesh Chandra(Guide), Friend's and KNIMT Sultanpur for their support and encouragement to carry out this work.

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