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FORMULATION AND EVALUTION OF PROLIPOSOMAL GELS OF NAPROXEN USING VARIOUS GRADES OF HPMC POLYMERS

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ABSTRACT

The development of in situ gel systems has received considerable attention over the past few years. In situ gel forming drug delivery systems are principle, capable of releasing drug in a sustained manner maintaining relatively constant plasma profiles. Naproxen is classified as a nonsteroidal anti-inflammatory drug (NSAID) Given its overall tolerability and effectiveness, naproxen can be considered a first line treatment for a variety of clinical situations requiring analgesia. Preparation of gels using appropriate amounts of excipients to obtain stable gel formulation for anti-arthritic drugs. Variables such as amount of phospholipid, mannitol and cholesterol have a profound effect on vesicle size and entrapment efficiency. FTIR studies concluded that there is no interaction between drug and excipients. Rheological studies of all gels prepared with 1%, 2% and 3%w/w carbopol gave clear idea of concentration of carbopol (1%) is require for preparation of stable gel formulation. In-vitro studies of gels encapsulating anti-diabetic drugs were found to increase the skin permeation and deposition showing a sustain effect when compared to marketed gel (conventional gel). Stability studies performed for optimized gel formulation indicate that prepared gels have more stability at freezing temperature than that room temperature, suggesting the storage at low temperatures

KEYWORDS: Naproxen, proliposomal gel, HPMC K15M, PEG, Carbopol.

INTRODUCTION

The development of in situ gel systems has received considerable attention over the past few years. In situ gel forming drug delivery systems are principle, capable of releasing drug in a

sustained manner maintaining relatively constant plasma profiles. These hydrogels are liquid at room temperature but undergo gelation when in contact with body fluids or change in pH. These have a characteristic property of temperature dependent, pH dependent and cation induced gelation.[1-5] Compared to conventional controlled release formulations, in situ forming drug delivery systems possess potential advantages like simple manufacturing process, ease of administration, reduced frequency of administration, improved patient compliance and comfort. Oral dosage forms pose low bioavailability problems due to their rapid gastric transition from stomach, especially in case of drugs which are less soluble at alkaline pH of intestine. Similarly, drugs which produce their local action in stomach get rapidly emptied and do not get enough residence time in stomach. So, frequency of dose administration in such cases is increased. To avoid this problem floating drug delivery system has been developed.[6-8]

Naproxen is classified as a nonsteroidal anti-inflammatory dug (NSAID) Given its overall tolerability and effectiveness, naproxen can be considered a first line treatment for a variety of clinical situations requiring analgesia. Naproxen is available in both immediate and delayed release formulations, in combination with sumatriptan to treat migraines, and in combination with esomeprazole to lower the risk of developing gastric ulcers. As with other non-selective NSAIDs, naproxen exerts it's clinical effects by blocking COX-1 and COX-2 enzymes leading to decreased prostaglandin synthesis.[9-11] IUPAC name (2S)-2-(6-methoxynaphthalen-2-yl)propanoic acid. Molecular weight 230.25. Chemical formula$C_{14}H_{14}O_3$. t is an odorless, white to off-white crystalline substance. It is lipid-soluble, practically insoluble in water with a low pH (below pH 4), while freely soluble in water at 6 Ph. Liposomes are the most promising and broadly applicable of all the novel delivery systems. The poor stability associated with this system limits its long term storage. To overcome this issue Proliposomes are dry, free-flowing granular products composed of drug and phospholipid which, upon addition of water, disperse to form a multi-lamellar liposomal suspension. This paper reviews the method of preparation and evaluation of Proliposomes and highlights its potential to be exploited for different routes of administration.

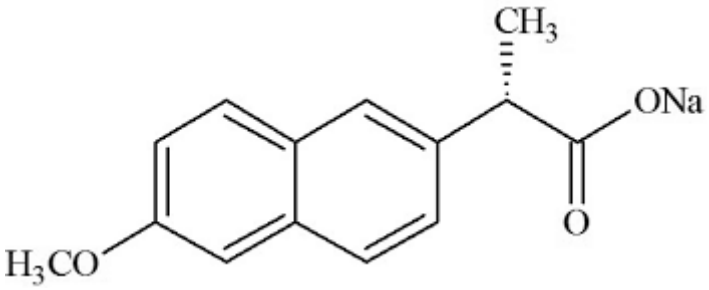

Figure 1: Chemical structure of Naproxen.

MATERIALS

Neproxen was purchased from Natco pharma pvt ltd Limited, Hyderabad, India, Hydroxypropyl methyl cellulose, Mannitol are from SD Fine-Chem. Pvt., Mumbai, India, Trimethanolamine from Dr. Reddy's laboratories, Hyderabad, Standard chemical reagents from SD fine chemical Ltd, Hyderabad. Methanol was of high performance liquid chromatography (HPLC) grade. All other reagents and solvents were of analytical reagent grade.

METHODOLOGY

Pre-Formulation Studies[12-25]

The overall objective of preformulation testing is to generate information useful for the formulation in developing stable and bioavailable dosage forms. The use of preformulation parameters maximizes the chances in formulating an acceptable, safe, efficacious and stable product.

Melting point determination

Melting point of Naproxen was set to determine by open cup capillary method.

ANALYTICAL METHOD DEVELOPMENT

Determination of absorption maxima

Absorption maxima is the wavelength at which maximum absorption takes place. For accurate analytical work, it is important to determine the absorption maxima of the substance under study.

Procedure

For the preparation of calibration curve stock solution was prepared by dissolving 100 mg of accurately weighed drug in 100ml of methanol (1mg/ml). Further 1ml of the stock solution was pipette out into a 100 ml volumetric flask and volume was made up with phosphate

buffer (7.4 PH). From this stock solution pipette out 1ml and dilute to 10 ml with phosphate buffer and subject for UV scanning in the range of 200-400 nm using double beam UV spectrophotometer. The absorption maxima was obtained at 270 nm with a characteristic peak.

Preparation of calibration curve

Using absorption maxima a standard curve was prepared in the concentration range of 0.5-2.5 µg/ml. from the second stock solution, pipette out 0.5, 1.0, 1.5, 2.0 and 2.5 ml into a series of 10ml volumetric flask and volume was made up to 10 ml with phosphate buffer PH 7.4 to get 0.5, 1.0, 1.5, 2.0 and 2.5 µg/ml of Naproxen respectively. The absorbance of resulting solutions were measured at 270nm and recorded. Concentration versus absorbance values were plotted.

Drug-excipients interaction studies by FTIR

The compatibility between pure drug and mannitol, phosphatidyl choline, cholesterol were detected by FTIR (Bruker Alpha- T) spectra. The potassium bromide pellets were prepared on KBR press (Horizon WC-56). To prepare the pellets the solid powder sample were ground together in a mortar with 100 times quantity of KBR. The spectra's were recorded over the wave number of 4000 to 600 cm^{-1}.

PREPARATION OF PROLIPOSOMAL GEL

Preparation of Naproxen loaded proliposomes

Proliposomes could be prepared by many methods including

a. Film deposition on carrier method
b. Crystal film method
c. Fluidized bed method
d. Powder bed grinding method
e. Freeze drying method
f. Spray drying method

Based on the laboratory conditions film-deposition on the carrier method was chosen to prepare Naproxen proliposomes.

Table No 1: Composition of proliposomal formulations (F1 to F9).

Excipients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Naproxen(mg)	200	200	200	200	200	200	200	200	200
HPMC K15M(mg)	100	100	150	150	50	150	100	50	50
PEG(mg)	150	100	50	100	100	150	50	50	150
Carbopol(mg)	25	50	75	100	125	25	50	75	100
Water(ml)	6	6	6	6	6	6	6	6	6
Methanol (ml)	4	4	4	4	4	4	4	4	4

The proliposomes containing Naproxen was prepared by film deposition on carrier method using vacuum rotary evaporator. There are various process variables which could affect the preparation and properties of the proliposomes. The optimization of Naproxen proliposomes was done by preparing the different formulations by varying the concentration of mannitol, phosphatidyl choline and cholesterol Mannitol (1 g, sieved with 100 mesh) was placed in 100ml round bottom flask which was held at 60-70^{0}C temperature and the flask rotated at 80-90 rpm for 30 min under vacuum. After complete drying the temperature of water bath was lowered to 20-30^{0}C.

Preparation of HPMC K15M gel base

1gm of HPMC K15M 934 was weighed and dispersed in distilled water. Then, propylene glycol was added and the mixture was neutralised by drop wise addition of 1% triethanolamine. Mixing was continued until the transparent gel was obtained and allowed to swell for 24 hours. Similarly 2% and 3% HPMC K15M gels were prepared.

Preparation of proliposomal gels

Proliposomes containing Naproxen (separated from the unentrapped drug) were mixed into the 1% HPMC K15M gel by using mortar and pestle, the concentration of proliposomes in the gel being 1%. All optimized formulations were incorporated into different HPMC K15M gels (1%,2% and 3%).

CHARACTERIZATION OF PROLIPOSOMES

Vesicle size and count

Average size and size distribution proliposomes were determined using optical microscope. A drop of distilled water was added to proliposome granules on a glass slide without a cover slip, and the process of liposome formulation was observed using optical microscope with 100X magnification.

Surface morphology

The surface morphology of proliposomes and plain mannitol particles were examined by scanning electron microscopy (SEM) after coating with gold. After gold coating of proliposome and plain mannitol particles, their surface morphology was viewed and photographed.

Drug content

Naproxen content in proliposomes was assayed by an UV-visible spectrophotometer. Proliposomes (100mg) were dissolved in 10ml methanol by shaking the mixture for 5 mins. One ml of the resultant solution was taken and diluted to 10ml with methanol. Then, aliquots were withdrawn and absorbance was recorded at 270nm using UV-visible spectrophotometer.

Entrapment efficiency

$$\text{Entrapment efficiency (\%)} = \frac{Ct - Cf}{Ct} \times 100$$

Ct – concentration of total drug

Cf – concentratin of free drug

Surface charge

The optimized proliposomal formulation was dissolved in phosphate buffer (pH 7.4) and made the serial dilutions until the clear solution was obtained. Then the sample was analyzed for surface charge using zeta sizer (Malvern).

Yield of proliposomes

After complete drying the proliposome powders were collected and weighed accurately. The yield of proliposomes was calculated using the formula

$$Percentage\ yield = \frac{Total\ weight\ of\ proliposomes}{\text{total weight of drug} + \text{weight of added materials}} \times 100$$

Physical appearance

All prepared gel formulations have been observed for their visual appearance, such as transparency, colour, texture, grittiness, greasiness, stickiness, smoothness, stiffness and tackiness.

P^H of formulation

pH measurement of the gel was carried out of the formulation was measured by using a digital pH meter (Lab India SAB 5000), dipping the glass electrode completely into the gel system. The observed pH values were recorded for all formulations (F1-F9) in triplicates.

Rheological properties

The rheological properties of prepared gels was estimated using a Brookfield viscometer pro D II apparatus, equipped with standard spindle LV1 with 61 marking. Sample holder of the Brookfield viscometer was filled with the gel sample, and then spindle was inserted into sample holder. The spindle was rotated at 100 rpm.

Homogeneity

The homogeneity of Naproxen proliposomal gels were checked by visual inspection. In this regard the gels were filled into narrow transparent glass tubes and were checked in light for the presence of any particulate or lump.

Drug Content

For determination of drug content, accurately weighed quantity (1 gm) of gel equivalent to 50 mg of Naproxen was dissolved in phosphate buffer (PH 7.4) and analyzed by UV-Vis Spectrophotometer (Lab India 3200) at 270 nm for drug content.

In vitro studies

Percent amount of drug release from semi permeable membrane

Franz diffusion cell was used for the in vitro drug release studies. Semi permeable membrane was placed between donar and receptor chamber of diffusin cell. Receptor chamber was filled with freshly prepared 30ml 7.4 PH phosphate buffer. proliposomal gel equivalent to 1gm was placed on semi permeable membrane. The franz diffusion cell was placed over magnetic stirrer (REMI 1ML) with 500rpm and temperature was maintained at 37 ± 1^0C. 5ml of samples were withdrawn periodically and replaced with fresh buffer. The withdrawn samples were periodically diluted and analysed for drug content using UV visible spectrophotometer (Lab India 3200) at 270nm.

IN-VITRO RELEASE KINETICS

There are several linear and non-linear kinetic models to describe release mechanisms and to compare test and Reference dissolution profiles are as follows:

- Zero order kinetics
- First order kinetics
- Korsmeyer-Peppas model
- Higuchi model

Korsmeyer-Peppas model

$$Mt / M\infty = Kt^n$$

Where, $Mt / M\infty$ is a fraction of drug released at time t,

k is the release rate constant and n is the release exponent.

Hixson-Crowell model

$$W_0^{1/3} - W_t^{1/3} = \kappa t$$

Where, W_0 is the initial amount of drug in the pharmaceutical dosage form

W_t is the remaining amount of drug in the pharmaceutical dosage form at time t

κ (kappa) is a constant incorporating the surface-volume relation.

Determination of similarity factor

The equation of similarity factor proposed by Moore and Flanner is represented in equation: $f2 = 50 \times \log \{[1 / (1 + (\Sigma (Rt - Tt) 2) / N)]1/2 \times 100\}$

Where, N = Number of experimental data.

RESULTS AND DISCUSSIONS

PRE-FORMULATION STUDIES

Melting point determination: Melting point of Naproxen was found to be in the range of 152-155^{0}C which compiles the standards thus indicating that purity of the drug sample.

Construction of calibration curve using phosphate buffer (P^H 7.4)

Table No 2: Standard calibration curve of drug in phosphateBuffer (PH 7.4).

S.No	Concentration	Absorbance
1	0.5	0.2251
2	1	0.4195
3	1.5	0.6407
4	2	0.8013

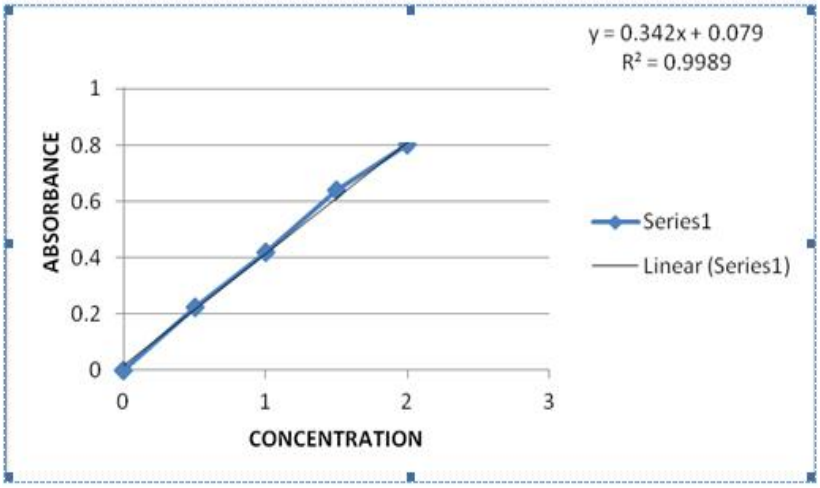

Figure 2: Standard calibration curve of drug in phosphate Buffer Characerization proliposomes and proliposomal gels.

Determination of vesicle size

Table No. 3: Particle size of proliposomal formulations.

S.No	Formulation	Average particle size (µm) for 100 particles
1	F1	5.34±0.023
2	F2	4.43±0.123
3	F3	2.65±0.076
4	F4	6.06±0.012
5	F5	4.34±0.231
6	F6	5.12±0.167
7	F7	3.21±0.221
8	F8	2.69±0.148
9	F9	2.34±0.321

Determination of entrapment efficiency

Table No. 4: Entrapment efficiency of proliposome formulations.

S.No	Formulation	Entrapment efficiency ± SD
1	F1	94.9±0.244
2	F2	85.12±1.48
3	F3	91.02±0.613
4	F4	96.5±0.205
5	F5	92.7±0.249
6	F6	94.1±0.509
7	F7	88.1±2.19
8	F8	89.2±0.817
9	F9	86.02±2.90

A positive correlation was observed for both variables phospholipid and cholesterol. Results show that with increase in the concentration of phospholipid and cholesterol entrapment

efficiency found to be increased. In the present study, the observed entrapment efficiency for all batches of Naproxen proliposome formulation in the range of 72 to 90%.

Among all Naproxen proliposomal formulations F1-F9 had maximum vesicle size and entrapment efficiency which were selected for the further study.

Drug content estimation

Table No. 5: Drug content of proliposomal formulations.

S.No	Formulation	%drug content ± SD
1	F1	95.03±0.543
2	F2	86.4±0.734
3	F3	93.7±0.664
4	F4	96.8±0.249
5	F5	94.7±0.984
6	F6	94.8±0.860
7	F7	92.4±1.70
8	F8	90.6±0.748
9	F9	87.5±0.953

The Naproxen content in the proliposomes were observed in the range of 86.4% to 96.8% at various drug to phospholipid ratios. From the above results it is concluded that F4, F1, F5 and F6 formulations showed maximum drug content when compare to other formulations.

Percentage yield of proliposomes

Percentage yield for F1 – F9 formulations was found to be with increase in the phospholipid concentration.

Table No 6: % Yield of proliposomes.

S.No	Formulation	Percentage yield
1	F1	93.4±0.324
2	F2	90.7±0.534
3	F3	89.5±0.654
4	F4	95.4±0.123
5	F5	94.3±0.221
6	F6	94.8±0.212
7	F7	88.7±0.321
8	F8	89.3±0.187
9	F9	86.5±0.265

In vitro studies

The cumulative amount of drug release of various proliposomal formulations and conventional (Conventional) Naproxen gel after 10hrs were shown in table 6.7.

Viscosity measurement

Rheological studies revealed that 1% carbopol gel showing better rheological properties when compared to 2% and 3% carbopol gels. So 1% carbopol was used for preparation of proliposomal gel. Viscosity of the gel was measured by Brookfield viscometer (LVDV II pro+). Viscosity of proliposomal gel showed 1156cps at 100rpm.

pH measurement

The pH of developed formulation was in accordance with human skin pH rendering them more acceptable. Therefore formulated proliposomal gel was suitable for topical application. The pH values of prepared proliposomal gels were within the limits of 5.5 to 5.8.

Stability studies

Stability of proliposomal formulation as well as gel formulation was carried out for 60 days at 8^0C, room temperature and 40^0C and analyzed for following parameters: visual appearance, drug content, entrapment efficiency of reconstituted liposomes(table 6.10). After 2 months of storage period the Naproxen proliposomes still appeared free flow and immediately form a liposomal dispersion on contact with water.

Table No 7: Stability study of the F4 proliposomal formulation.

Time (days)	Temperature(^{0}C)	Drug content	Entrapment efficiency
15	RT	95.4	94.0
15	8	95.2	93.5
15	40	94.5	92.6
30	RT	94.1	93.2
30	8	93.7	92.1
30	40	93.6	91.4
60	RT	93.5	92.5
60	8	92.3	91.5
60	40	91.2	90.7

The drug leakage of <1% of the initial load at refrigeration conditions is well within the limits and good for topical application.

Invitro Diffusion studies

Invitro diffusion studies were carried out by using 500ml of 7.4 pH phosphate buffer by using Franz diffusion apparatus. The diffusion studies were carried out for about 10hrs.

Table No 8: Invitro diffusion data.

Time(hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	20.22	14.26	15.02	17.21	14.66	16.22	21.10	19.50	23.54
2	32.65	20.65	22.55	29.53	28.44	25.45	32.86	31.88	38.33
3	49.68	39.29	40.89	40.12	43.85	36.89	43.24	49.28	44.25
4	55.24	59.55	48.22	56.84	65.88	42.83	66.49	61.11	51.01
5	60.35	77.58	54.48	66.85	79.25	58.46	74.85	78.96	66.86
6	74.24	80.65	59.77	76.63	96.12	62.70	84.25	82.58	78.98
7	88.28	98.63	66.62	85.12		75.23	95.57	94.66	84.26
8	97.55		74.35	97.70		84.26			96.99
9			88.99			97.99			
10			98.43						

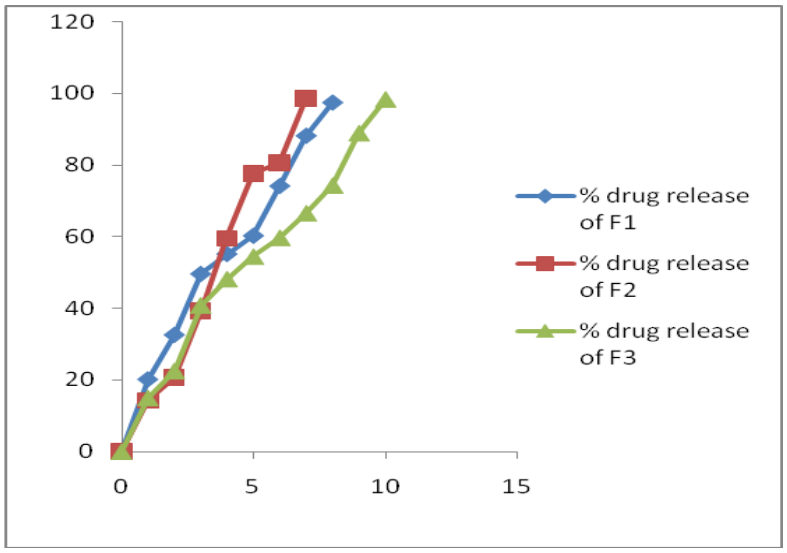

Figure 3: Cumulative drug release of formuations(F1-F3).

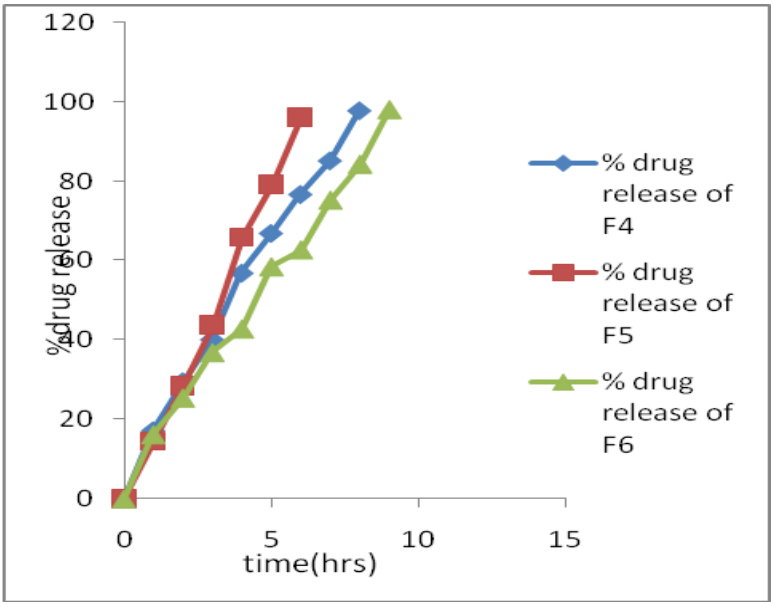

Figure 4: Cumulative drug release of formuations(F4-F6).

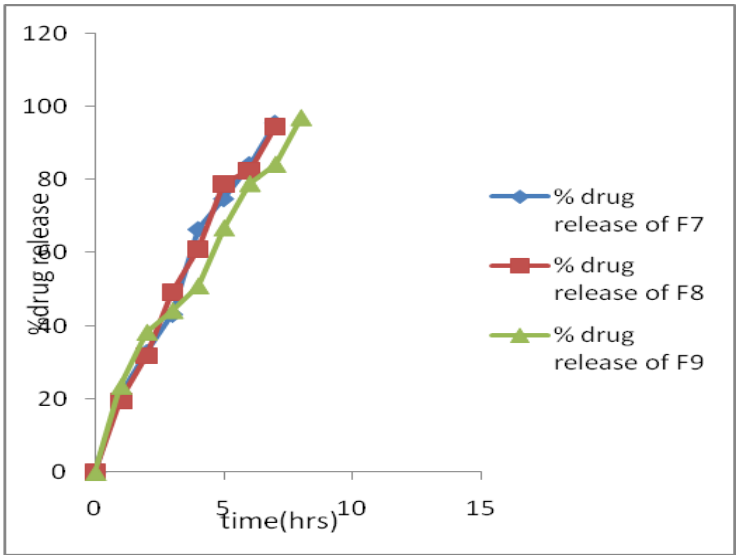

Figure 5: Cumulative drug release of formuations(F7-F9).

Release kinetics

Various mathematical models were selected to evaluate the kinetics and mechanism of drug release from proliposomal gel formulation. Best model was selected for release data which showed high correlation coefficient (r) value. In-vitro drug release over semi permeable membrane and skin was performed and release kinetics was calculated.

Table No. 9: Correlation coefficients (R^2) values of different kinetic models.

S.No	Formulation	Zero order R^2	First order R^2	Higuchi model R^2	Peppas model R^2	Release Exponent(n)
1	F1	0.9699	0.9889	0.9771	0.9802	0.257
2	F2	0.9972	0.9845	0.9583	0.9715	0.384
3	F3	0.9663	0.9899	0.9908	0.9916	0.418
4	F4	0.9905	0.9857	0.9889	0.9927	0.161
5	F5	0.9783	0.9949	0.9925	0.9959	0.325
6	F6	0.9875	0.994	0.989	0.9965	0.293
7	F7	0.9783	0.9819	0.9828	0.9859	0.502
8	F8	0.9926	0.9922	0.9812	0.9917	0.528
9	F9	0.982	0.9979	0.994	0.9948	0.405

The mechanism of release for the optimized proliposomal formulation based on regression coefficient (R^2) value. For most of the proliposomal formulation the R^2 value nearer to 1. Hence it can be concluded that the drug release follow peppas model.

The n value of peppas model of the proliposomal formulations are in the range of 0.1 to 0.5 which confirms that release of proliposomal formulation was fickian diffusion.

Release kinetic graphs of optimized formulation

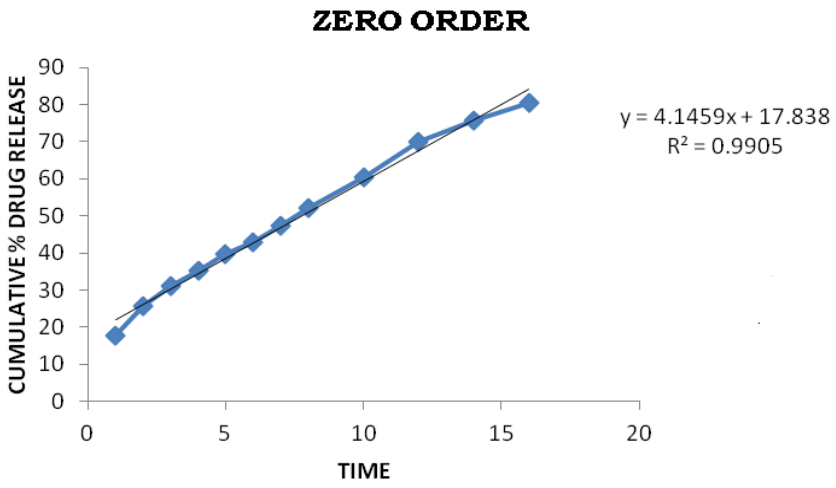

Figure 6: zero order plot of optimized proliposomal formulation (F3).

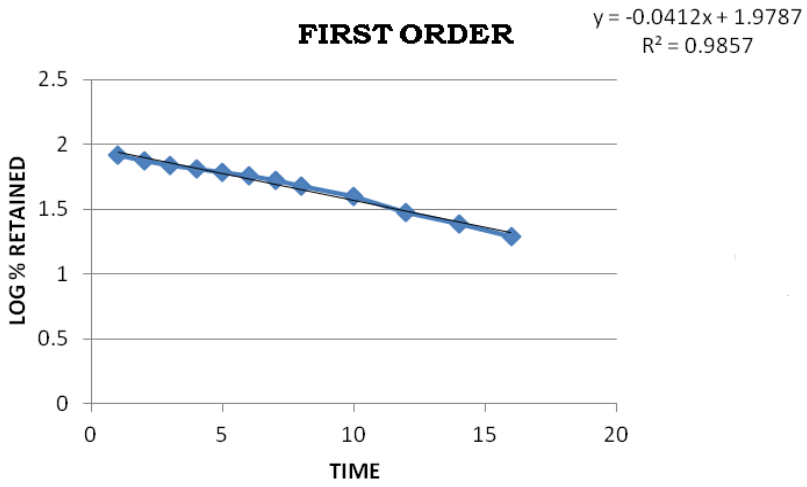

Figure 7: first order plot of optimized proliposomal formulation.

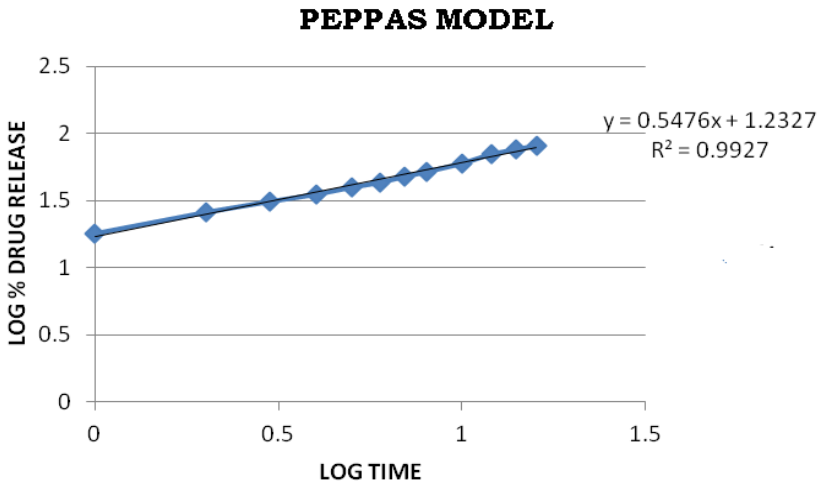

Figure 8: Peppas plot of optimized prolposomal formulation.

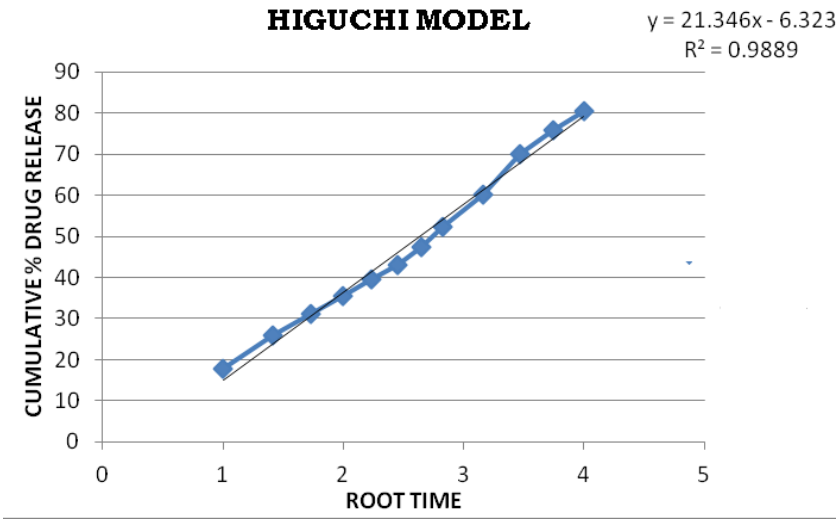

Figure 9: Higuchi plot of optimized proliposomal formulation.

The results clearly show that the proliposomal formulations were independent of the initial concentration following zero order kinetics which is ideal for topical drug delivery system. The release data showed regression value near to 1 and best fitted in pepps model indicating the drug delivery followed was fickian diffusion.

CONCLUSION

Preparation of gels using appropriate amounts of excipients to obtain stable gel formulation for anti-arthritic drugs. Variables such as amount of phospholipid, mannitol and cholesterol have a profound effect on vesicle size and entrapment efficiency. FTIR studies concluded that there is no interaction between drug and excipients. Rheological studies of all gels prepared with 1%, 2% and 3%w/w carbopol gave clear idea of concentration of carbopol (1%) is require for preparation of stable gel formulation. In-vitro studies of gels encapsulating anti-diabetic drugs were found to increase the skin permeation and deposition showing a sustain effect when compared to marketed gel (conventional gel). Stability studies performed for optimized gel formulation indicate that prepared gels have more stability at freezing temperature than that room temperature, suggesting the storage at low temperatures.

REFERENCES

1. Barry BW. Novel mechanism and devices to enable successful transdermal drug delivery. European J Pharm Sci., 2004; 14: 101-114.
2. Pavan Kumar. K; Radhika. P. R; Sivakumar.T. International Journal of Advances in Pharmaceutical sciences, 2010; 1,2.

3. Jain N, Talegonkar S, Jain NK. New ways to enter the blood stream: Emerging strategies in transdermal drug delivery. The Pharma Review, Sep–Oct, 2004; 41-60.
4. Drug delivery systems by Vinayak (Vasant) V. Ranade, 2002; 199-201, 205.
5. Modern Pharmaceutics, Fourth edition, Basic principles and systems by Gilbert S. Banker, Christopher T. Rhodes, 2002; 200.
6. Jain NK. Advances in controlled and novel drug delivery, New Delhi: CBS publication, 2001; 428-451.
7. Jain S, Bhandra D, Jain S and Jain NK. Transfersomes- A novel carrier for effective transdermal drug delivery. Controlled and novel drug delivery 1 st Edition. CBS publishers and distributors New Delhi, 1997; 426-451.
8. Vyas SP, Khar RK. Controlled drug delivery concepts and advances. Vallabh prakashan New Delhi. First Edition, 2002; 173-243.
9. Brutzkus JC, Varacallo M: Naproxen. [PubMed:30247840]
10. Wongrakpanich S, Wongrakpanich A, Melhado K, Rangaswami J: A Comprehensive Review of Non-Steroidal Anti-Inflammatory Drug Use in The Elderly. Aging Dis, Feb 1, 2018; 9(1): 143-150.
11. Todd PA, Clissold SP: Naproxen. A reappraisal of its pharmacology, and therapeutic use in rheumatic diseases and pain states. Drugs, Jul, 1990; 40(1): 91-137.
12. Vyas SP, Dixit DK. Targeted and Controlled Drug Delivery. CBS Publishers and distributors, 2002; 173-242.
13. Goel Anshu, Kumar Sandeep, Nanda Arun. “Membrane Engineered Long Circulating Liposomes in Cancer Therapy”. Eastern Pharmacist., 1996; 39(466): 39-42.
14. Jaiswal SB, Richasood KM. “Liposomal Drug Delivery System”. Eastern Pharmacist., 1987; 30(349): 53-59.
15. Chien YW. Novel drug drug delivery systems. Marcel Dekker Inc., 1992; 173-248.
16. Riaz M. “Liposomes Preparation Methods”. Pakistan Journal of Pharmaceutical Sciences., 1996; 19(1): 65-77.
17. Vladimir P. Torchilin. “Recent Advances with Liposomes as Pharmaceutical Carriers”. Drug Discovery., 2005; 4: 145-160.
18. Egbaria. K, Weiner. N. “Liposomes as Topical Drug Delivery Systems”. Advanced Drug Delivery Reviews., 1990; 5: 287-300.
19. Keon- hyoung song, Suk-jae chung, Chang- koo shim. “Preparation and Evaluation of Proliposomes Containing Salmon Calcitonin”. Journal Of Controlled Release, 2002; 84: 27-37.

20. Janga y. karthik et al., “Bioavailability Enhancement of Zaleplon Via Proliposomes: Role of Surface Charge”. European Journal Of Pharmaceutics And Biopharmaceutics, 2011.
21. Vandana gupta et al., “Formulation Development and In Vitro Characterization of Proliposomes for Topical Delivery 0f Aceclofenac”. Indian Journal Of Pharmaceutical Sciences., 2008; 70(6): 768-775.
22. Xiao yan-yu et al., “Preparation Of Silymarin Proliposome: A New Way to Increase Oral Bioavailability of Silymarin in Beagle Dogs”. International Journal Of Pharmaceutics., 2006; 319: 162-168.
23. Bo-young et al., “In Vitro Skin Permeation of Nicotine from Proliposomes”. Journal of Controlled Release, 1997; 49: 177-184.
24. Mallesh Kurakula et al., “Formulation and Evaluation of Prednisolone Proliposomal Gel for Effective Topical Pharmacotherapy”. International Journal of Pharmaceutical Sciences and Drug Research., 2012; 4(1): 35-43.
25. H. Xu et al., “Optimized preparation of vinpocetine proliposomes by a novel method and in vivo evaluation of its pharmacokinetics in New Zealand rabbits”. J. Control. Release, 2009; 140: 61-68.