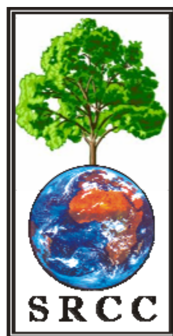

Scientific Reviews & Chemical Communications

Sci. Revs. Chem. Commun.: 1(1), 2011, 7-17

FORMULATION AND EVALUATION OF TOPICAL DRUG DELIVERY SYSTEM CONTAINING CLOBETASOL PROPIONATE NIOSOMES

M. ABRAHAM LINGAN, A. ABDUL HASAN SATHALI*, M. R. VIJAYA KUMAR and A. GOKILA

Dept. of Pharmaceutics, College of Pharmacy, Madurai Medical College, MADURAI – 625020 (T.N.) INDIA

(*Received : 04.10.2011, Accepted : 16.10.2011*)

ABSTRACT

The aim of the present study was to formulate topical gel containing clobetasol propionate niosomes to prolong the duration of action and prevent its side effects. The clobetasol propionate niosomes were prepared by altering the ratios between various non-ionic surfactants (Span 40, 60, 80) and cholesterol by three methods such as thin film hydration method, ether injection method and hand shaking method. The prepared niosomes were subjected to drug content analysis, entrapment efficiency, size analysis and *invitro* drug release studies. The higher entrapment efficiency (91.37%) was obtained with Span 60 niosomes (ratio of surfactant, cholesterol- 1 : 0.5) prepared by thin film hydration method was evaluated for its stability and formulated as gel formulation. The prepared niosomal gel (G2) and marketed gel (G3) were subjected to drug content analysis, *in vitro* drug release studies and *in vivo* pharmacodynamic studies. Our results suggested that the niosomal delivery of clobetasol propionate in carbopol gel base acts as a suitable topical drug delivery system to prolong the duration of action.

Key words: Clobetasol propionate, Niosomes, *Invitro*, Carbopol, *Invivo*, Pharmacodynamic.

INTRODUCTION

Controlled drug delivery systems are the dosage forms which are developed to achieve better patient compliance, modified drug release, delivery of drug at the site of action, more efficient administration of drugs by various routes and for better therapeutic effect[1].

Niosomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of non-ionic surfactants and cholesterol. Niosomes can be used to deliver both hydrophobic and hydrophilic drugs via transdermal route. Although niosomes were tried for various routes, it is used in the market for transdermal route (Novasome Products Such as 30% Petrolatum Novasomes and 10% salicyclic acid novasomes). Studies showed that an enhanced delivery of drugs was observed when niosomes was encapsulated. Niosomes increase skin penetration of drugs and it can act as local depot for sustained release of dermally active compounds.

Glucocorticosteroids are used topically for a large variety of dermatological conditions[2]. They benefit by virtue of their anti-inflammatory, immune suppressive, vasoconstrictor and antiproliferative

Available online at www.sadgurupublications.com

*Author for correspondence; E-mail: abdulhasan@hotmail.com; Mo.: 9443475400

actions. Even though glucocorticoids can be used to treat vast skin disorders it also have some side effects. Moreover the premature withdrawal of corticosteroids will result in relapse of the disease. Clobetasol propionate is a potent corticosteroid used as an anti-inflammatory; anti-pruritic and vasoconstrictor. Less frequent application of a superpotent corticosteroid in a suitable carrier may have prolonged action with less or minimal side effects. To achieve this goal, the drug encapsulating niosomes incorporated in a suitable gel base and evaluated for its controlled drug delivery

EXPERIMENTAL

Materials

Clobetasol propionate was obtained as gift sample from Apex Laboratories (P) Ltd., Chennai, India. Cholesterol, Sorbitan mono palmitate, Sorbitan mono stearate, Sorbitan mono oleate (S.D. Fine chemicals, Mumbai, India), Carbopol 934 (Dr.Milton laboratories, Chennai) were procured from commercial sources. All the other reagents and solvents used were of analytical grade.

Methods

Preparation of clobetasolpropionate niosomal formulations

Niosomes of clobetasol propionate was prepared by three methods.

(a) Thin film hydration method

(b) Ether injection method

(c) Hand shaking method

Different ratios of surfactant and cholesterol used in the preparation of niosomes were shown in Table 1.

(a) Thin film hydration method[3,4]

The surfactant, cholesterol and drug were weighed separately and dissolved in chloroform: methanol (2 : 1) mixture one by one until it gets completely dissolved. Then the above solution was taken in a rotary flash evaporator and the organic solvent mixture was completely evaporated at 60°C at 180 rpm to form a thin film on the wall of the flask. It was hydrated using distilled water for 1 hour with rotation. Then the niosomal dispersion was collected, cooled in an ice bath and sonicated using a probe type sonicator for three minutes at 150V.

(b) Ether injection method[5,14,15]

The surfactant, cholesterol and drug were weighed separately and dissolved in Diethyl ether: Methanol (1 : 1) mixture. Adequate amount of distilled water was taken in a beaker, placed over a magnetic stirrer and kept in a temperature between 55°C-65°C. The organic phase was injected into swirling aqueous phase at a rate of 0.25 mL/min through 14 gauge needle. The differences in temperature between phases cause rapid vaporization of ether and methanol resulting in spontaneous vesiculation leads to the formation of niosomes.

(c) Hand shaking method[6]

A thin film containing surfactant, cholesterol and drug was formed like thin film hydration method. It was hydrated with adequate amount of distilled water and shaken by hands in a water bath at 60°C until white niosomal dispersion was formed.

Determination of entrapment efficiency[7, 16]

Entrapment efficiency was determined by dialysis method. Cellophane membrane was soaked in Glycerol: water (1 : 3) mixture for 15 min and tied in an open ended tube. The niosomal dispersion was transferred into the tube and placed into a 250ml beaker containing 100 mL Distilled water and it was stirred by magnetic stirrer. The samples were taken every 15 min for 6 hours. The absorbance was measured at 242 nm by UV-spectrophotometer (Shimadzu UV-1700 Pharma spec, Japan) using distilled water as blank and the entrapment efficiency was calculated by the following formula.

Entrapment efficiency = % Drug content - % of maximum drug release of unentrapped drug.

Drug content of the niosomal preparations were determined by lysis method. Adequate amount of 50% n-propanol was added to the niosomal dispersion and shaken well until all the vesicles were completely lysed. It was diluted suitably with distilled water and the absorbance was measured at 242 nm by UV-spectrophotometer (ShimadzuUV-1700 Pharma spec).

Size analysis of niosomes[6]

The niosomes were analyzed by Scanning Electron Microscopy (SEM) for characterization size and shape of the vesicles. One drop of niosomalsuspension was mounted on a clear glass stub. It was air dried and gold coated using sodium aurothiomalate and visualized under scanning electron microscope.

Invitro release studies[8]

The pH of normal health human skin is between 4.5 and 6. However the pH value rises beyond 6, when a person actually suffers from a skin problem (or) skin disease. So the drug release studies of clobetasol propionate niosomes were done in distilled water (pH 7.4).

In vitro release studies were carried out by dialysis method. Cellophane membrane was soaked in Glycerin: water (1 : 3) mixture for 15 min and tied up in an open ended tube. The niosomal formulation was taken in the tube and placed into a receptor compartment containing distilled water, stirred by magnetic stirrer. The samples were taken periodically for 8 h and the absorbance was measured at 242 nm by UV-spectrophotometer (Shimadzu UV-1700 Pharma spec). The cumulative percentage drug released was plotted against time to find the drug release behavior of all niosomal formulations.

Stability studies[9]

The niosomal dispersion showing highest entrapment efficiency (F1) was stored in two different temperatures 4 ± 2^oC, 25 ± 2° C/60% RH ± 5% RH (ICH Guidelines) in an environmental chamber [Inlab equipments (Madras) Pvt. Ltd].The retention of drug in niosomal formulations was calculated immediately after the preparation and taken as 100% retention.The entrapment efficiency was estimated every week for 10 weeks.

Formulation of various gels[9]

From the in-vitro release studies, the best niosomal formulation was selected and incorporated into suitable gel base. The prepared gel containing plain drug (G1) and drug incorporated in niosomes (G2). The carbopolgel base (2.0%) was prepared by dispersing it inpurified water (freshly boiled and Cooled – 100 mL) by constant stirring. Triethanolamine (1.65 mL) was added and stirred until a viscous smooth gel was obtained.

Preparation of plain clobetasol propionate gel (G_1)[9]

The pure clobetasol propionate was incorporated in 2% carbopol gel base by trituration and stirred

by using a glass rod to get 0.05 % w/w of smooth homogenous Clobetasol propionate plain gel.

Preparation of Niosomal Clobetasol Propionate Gel (G_2)[9]

The best formulation (F1 - Span 60 : Cholesterol 1 : 0.5) was selected for the preparation of gel.Since the F1has more entrapment efficiency when compared to all the other formulations, it was selected to prepare niosomal gel. Niosomal dispersion was incorporated to the gel base and stirred using glass rod to get 0.05% w/w of smooth homogenous Clobetasol propionate niosomal gel.

Drug content studies of gels[3,7]

Clobetasol propionate plain and niosomal gel equivalent to 1mg of drug were weighed and diluted suitably using distilled water. The absorbance is measured at 242 nm by UV-spectrophotometer (Shimadzu UV-1700 Pharma spec.) to calculate the drug content. The niosomal gel was treated with 50% n-propanol before dilution to lyse the niosomal vesicles.

***Invitro* release studies of niosomal gels[8]**

In vitro release studies were carried out to compare the release behaviour of plain gel (G1), niosomal gel (G2) and marketed gel (G3) by dialysis method using cellophane membrane as a semi permeable membrane. The gel formulation wastaken inanopen ended tube tied with cellophane membrane and placed into a receptor compartment containing distilled water stirred by magnetic stirrer. The contents were uniformly rotated by a magnetic bead at 50rpm at $37^oC \pm 2^oC$. The samples were taken periodically and the absorbance was measured at 242 nm by UV-spectrophotometer (Shimadzu UV-1700 Pharma spec). The cumulative percentage drug release was plotted against time to compare the release pattern.

Pharmacodynamic studies of gel formulations[9-11]

The anti-inflammatoryactivity was carried out by carrageenan induced paw oedema method to compare the activity of the formulated niosomal geland marketed gel. After gets ethical clearance male albino rats of wister strain (150-200 g) were used for this study. The rats were fed with standard food and water. Food was withdrawn 12 hours before and during the experiment.

The animals were divided into three groups having four animals in each group. Group-I receiving normal saline without drug kept as control. Group II and group III receiving clobetasol propionate niosomal gel and plain gel (marketed) were applied in the right hind paw of the rats respectively. After half an hour of the gel application 0.1 mL of 1% carrageenan in normal saline was injected in sub plantar on the same right hind paw for all the animals. Then the paw oedema was measured by using plethysmometer at 0, 1, 2, 3, 4, 6 and 8 hrs respectively. Mean paw oedema was measured and the percentage inhibition of inflammation was calculated.

RESULTS AND DISCUSSION

Preparation of clobetasol propionate niosomal formulations

Eighteen formulations of clobetasol propionate niosomes were prepared by using non-ionic surfactants (Span 40, 60 and 80) along with cholesterol in different ratios (1 : 0.5 and 1 : 1) with the concentration of the drug being constant (5 mg) as shown in Table 1. The niosomal formulations were evaluated for entrapment efficiency and *in vitro* release studies.

Entrapment efficiency

The entrapment efficiency of the prepared niosomal formulations was measured by dialysis method. The entrapment efficiency was determined by subtracting the amount of drug dialysed from the total amount

of drug in the formulation. In all the formulations, the impact of cholesterol, surfactant and method of preparations on entrapment efficiency was significant. The results of entrapment efficiency were shown in Table 1 and Fig. 1.

Table 1: Composition and entrapment efficiency of various niosomal formulations

Formulation	Surfactant	Ratio of		Entrapment efficiency ± S.D (*n = 3)
		Surfactant	Cholesterol	
		Thin film hydration method		
F1	Span 60	1	0.5	91.37 ± 1.29 %
F2	Span 60	1	1	90.04 ± 2.06%
F3	Span 40	1	0.5	88.57 ± 1.39%
F4	Span 40	1	1	76.95 ± 1.99%
F5	Span 80	1	0.5	85.47 ± 2.42%
F6	Span 80	1	1	80.02 ± 2.37%
		Ether injection method		
F7	Span 60	1	0.5	58.44 ± 0.98%
F8	Span 60	1	1	56.85 ± 1.09%
F9	Span 40	1	0.5	42.52 ± 0.59%
F10	Span 40	1	1	38.12 ± 1.33%
F11	Span 80	1	0.5	39.19 ± 2.10%
F12	Span 80	1	1	32.84 ± 0.57%
		Hand shaking method		
F13	Span 60	1	0.5	83.68 ± 3.08%
F14	Span 60	1	1	79.79 ± 1.79%
F15	Span 40	1	0.5	77.55 ± 2.46%
F16	Span 40	1	1	72.70 ± 1.0%
F17	Span 80	1	0.5	70.61 ± 2.57%
F18	Span 80	1	1	65.97 ± 1.55%

Effect of cholesterol content[18]

Cholesterol is one of the common and essential additives in niosomal formulation. Incorporation of cholesterol was known to influence vesicle stability and permeability. The effect of cholesterol on clobetasol propionate entrapment was varied according to the nonionic surfactants used. Cholesterol was found to have little effect on drug entrapmentinto Span 40, Span 60 and Span 80 niosomes. The niosomes prepared using Span : Cholesterol 1 : 0.5 ratio showed higher entrapment efficiency in all the three methods shown in Table 1. Increasing cholesterol concentration, entrapment efficiency decreaseswhich may be due to intercalation of cholesterol in the bilayers[6,16].

This may be due to the following two conflicting factors,

1. With increasing cholesterol, the bilayer hydrophobicity and stability increased and permeability decreased which leads to efficiently trapping the hydrophobic drug into bilayers as vesicles formed.
2. In contrast, higher amounts of cholesterol may compete with the drug for packing space within the bilayer, hence excluding the drug as the amphiphiles assemble into vesicles.

Another study suggested that the decreasing the entrapment efficiency with increasing cholesterol ratio above a certain limit may be due to the fact that increasing cholesterol beyond a certain concentration can disrupt the regular linear structure vesicular membranes.

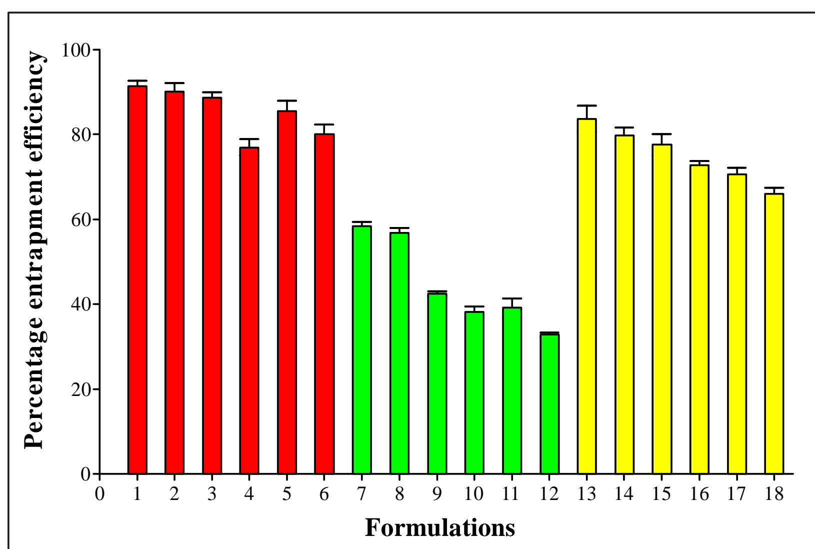

Fig. 1: Entrapment efficiency of various niosomal formulations

Effect of surfactants

Among all the formulations, F1 [span 60: cholesterol (1 : 0.5)] showed maximum entrapment efficiency compared with other formulations[13]. The entrapment efficiency increased in the order of Span 60 (C16) > Span 40 (C16) > Span 80 (C18), this could be due to the surfactant chemical structure. All span types have the same head group and different alkyl chain[18]. Increasing the alkyl chain length is leading to higher entrapment efficiency. Sp 60 and Sp 80 have the same head groups but Sp 80 has an unsaturated alkyl chain. In addition Sp 80 has the lowest transition temperature (Tc = -12ºC) amongst all tested spans (42ºC for Sp 40 and 53ºC for Sp 60). The span having the highest phase transition temperature provides the highest entrapment for the drug and vice versa.

The order of entrapment efficiency increased as the lipophilicity of the surfactant increased (HLB value decreased). The HLB value of the surfactant Span 60 was 4.7 whereas it was 6.7 and 4.3 for Span 40 and Span 80 respectively. Span 80 has the lowest HLB value but it has an unsaturated alkyl chain in its structure leads to lower entrapment efficiency[4,12,18,19]. It was concluded that by increasing surfactant concentration entrapment efficiency increases[16].

Effect of method

Among the three methods niosomes prepared by thin film hydration method showed maximum

entrapment efficiency than the other methodsdue to uniform and mechanical vortexing. The entrapment efficiency increases in order of

Thin film hydration method > Hand shaking method > Ether injection method

The entrapment efficiency of the formulation prepared by hand shaking method was more than ether injection method. This may be due to vortexing only carried out during hand shaking method, whereas in the case of ether injection process vortexing and injection takes place simultaneously. Moreover the vesicles obtained by hand shaking method were larger when compared to vesicles obtained by ether injection method. So due to this entrapment efficiency was more in hand shaking method when compared with ether injection method[20].

The entrapment efficiency of the formulation prepared by thin film hydration method was more, when compared with hand shaking method. This may be due to uniform and mechanical vortexing in thin film hydration method.

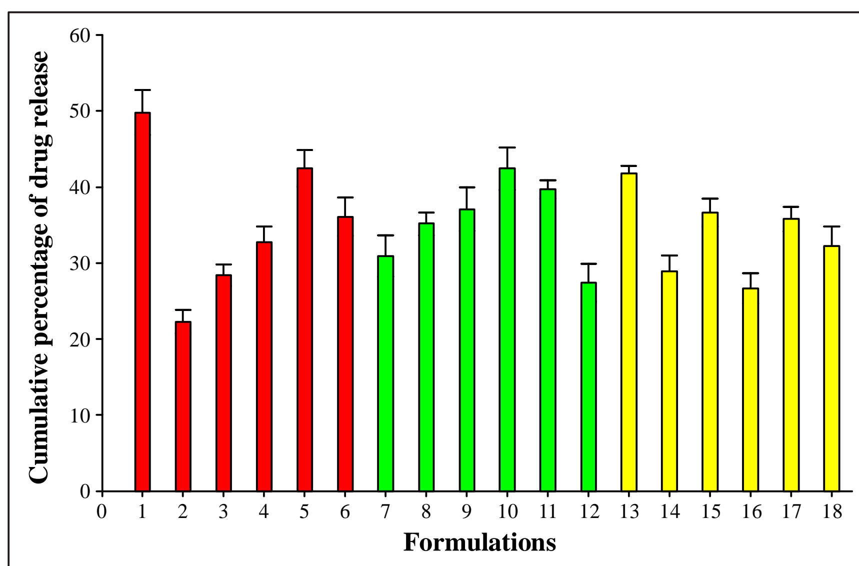

Fig. 2: Cumulative percentage of drug release from niosomal formulations after 12 hours

***Invitro* release studies[8]**

The cumulative percentage of drug release in 24 hrs from various niosomal formulations were shown in Fig. 2.

Among the six formulations from F1 to F6 prepared by thin film hydration method, F1 shows maximum drug release in 24 hrs. The order of decreasing percentage drug release in 24th hour were F1 > F5> F6 > F4 > F3 > F2.

Among the six formulations from F7 to F12 prepared by ether injection method, F10 shows maximum drug release in 24 hrs. The order of decreasing percentage of drug release in 24th hr were F10 > F11 > F9 > F8 > F7 > F12.

Among the six formulations from F13 to F18 prepared by hand shaking method, F13 shows maximum drug releasein 24 hours. The order of decreasing percentage drug release in 24th hr were F13 > F17 > F18 > F16 > F15 > F14.

In niosomal formulations, the experimental studies showed that the rate of drug release depends on the percentage of drug entrapment efficiency. This result was in conformity with the report of Samar Mansour[21]. From the non-ionic surfactants used (Span 40, 60 and 80) Span 60 containing formulation F1 showed higher drug release than other formulations. Hence it was chosen to formulate as niosomal gel (G2) to sustain the drug release rate.

Size analysis of niosomes[6]

The vesicle size of the prepared niosomes were observed and measured by Scanning Electron Microscopy. Most of the vesicles were found to be spherical in shape and the size ranged from 50 to 200 nm and shown in Fig. 3a, 3b and 3c. The size of niosomes in hand shaking method was larger when compared with the thin film hydration method and ether injection method.

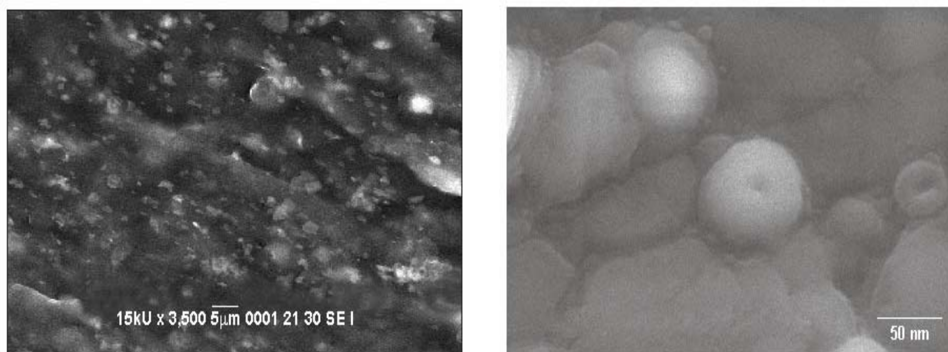

Fig. 3a: SEM photograph of F1

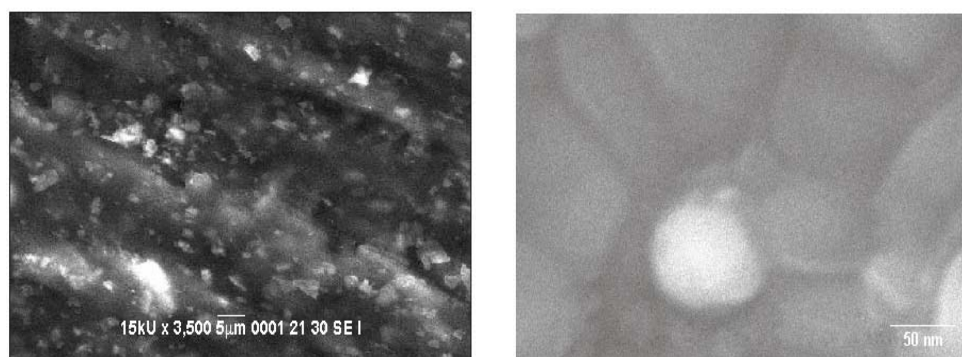

Fig. 3b: SEM photograph of F7

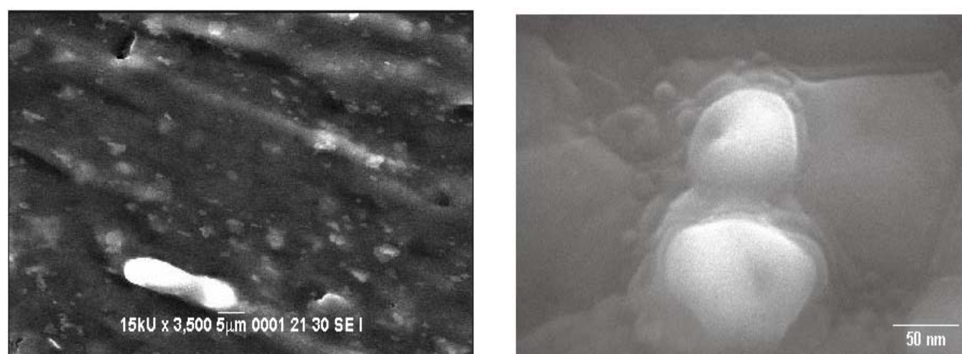

Fig. 3c: SEM photograph of F13

Stability studies[9]

Stability studies of the selected clobetasol propionate niosome formulation (F1) was performed by modified ICH guidelines (4 ± 2°C, 25 ± 2°C/60% RH ± 5% RH)[17]. The results were shown in Fig. 4. It showed that the drug retention capacity was decreased with increase in temperature and storage period. This may be attributed to phase transition of surfactant and lipid causing vesicles leakage at higher temperature during storage. Hence, it is concluded from the obtained data the optimum storage condition for niosomes was found to be 4°C.

Formulation of gels and drug content[3,7]

The prepared gel formulations were elegant in appearance and dispersed uniformly. The plain and niosomal gel showed the drug content of 94.57% and 96.21% respectively.

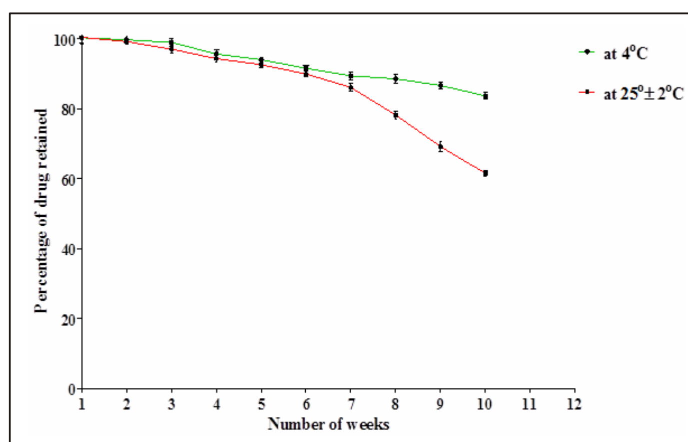

Fig.4: Precentage of drug retained in formulation

Invitro release studies of gels[8]

The release profile of the niosomal gels were shown in Fig. 5. The carbopolgel containing pure drug (G1) and the marketed gel (G3) showed the cumulative percentage of drug release 99.12% in 360 minutes and 98.43% in 300 minutes respectively. Thecarbopolgel containing niosomal formulation (G2) showed 51.58% of drug release in 24 hours. The cumulative percentage of drug release was decreased in the order of G3 > G1 > G2. The niosomal gel formulation showed controlled drug release due to the entrapment of drug in vesicles.

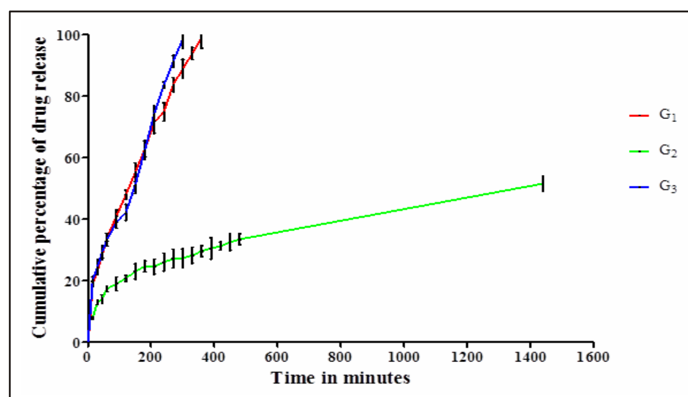

Fig. 5: Comparison of invitro release of various gel formulations G_1, G_2, G_3

Pharmacodynamic studies of gel formulations[10,11]

The percentage of reduction in paw oedema was gradually increased in the case of niosomal gel upto 8th hr, whereas in marketed gel gradually increasedupto 4th hr and later it declined on 6th and 8th hr shown in Fig. 6. The results revealed that the niosomal gel had a sustained as well as prolonged action than the marketed gel.

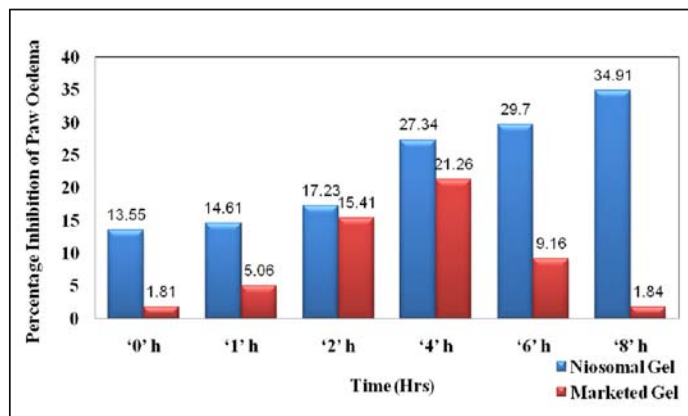

Fig. 6: Percentage inhibition of paw oedema

ACKNOWLEDGEMENT

The authors are thankful to Apex Laboratories (P) Ltd. for providing gift samples for this work. The authors are grateful to Anna University, Chennai, India for their technical assistance in carrying Scanning Electron Microscopy. Also, thankful to Dean, Madurai Medical College, Madurai and Department of Pharmaceutics for their kind support and encouragement to accomplish this work.

REFERENCES

1. J. S. Kulkarni, A. P. Pawar and V. P. Shedbalkar, Pharmaceutics and Pharmacokinetics, 1st Edition, CBS Publishers, New Delhi (2006) p. 40.
2. M. Fresta, G. Puglisi, J. Control. Release, **44**, 141 (1991).
3. Aliasgar Shahiwala, A. K. Misra, J. Pharm.Sci., **3**, 220 (2002).
4. A. R .Mullaicharam, R. S. R. Murthy, The Indian Pharmacist, **22**, 54 (2004).
5. J. N. Khandare, G. Madhavi and B. M. Tamhankar, The Eastern Pharmacist, **37**, 61 (1994).
6. Manjusha Malhotra, N. K. Jain, Indian Drugxs, **31**, 81 (1994).
7. Alok Namdeo, N. K. Jain, Ind. J. Pharm. Sci., **58,** 41 (1996).
8. J. N. Khandare, J. B. Hemant and U. R. Ramesh, Indian Drugs, **38**, 197 (2001).
9. Alok Namdeo, A. J. Mishra, P. R. Khopade and Nik Jain, Indian Drugs, **36**, 378 (1999).
10. J. N. Khandare, G. Madhavi, The Eastern Pharmacist, **38(452)**, 175 (1995).
11. R. A. RajaNaresh, U. V. Singh, N. Udupa and G. K. Pillai, Indian drugs, **30**, 275 (1993).
12. Toshimitsu Yoshioka, Brigitte Sternberg, Alexander, T. Florence, Int. J. Pharm., **105,** 1 (1993).
13. Shyamala Bhaskaran, L. Panigrahi, Ind. J. Pharm. Sci., **64**, 63 (2002).
14. Shyamala Bhaskaran, P. K. Lakshmi, Acta Pharma. Sciencia., **51**, 27 (2009).

15. Manivannan Rangasamy, J. Pharm. Res., **1**, 163 (2008).
16. A. Abdul Hasan Sathali, G. Raja lakshmi, Int. J. Pharmtech. Res., **2**, 2081 (2010).
17. S. Thamizharasi, A. Dubey, V. Rathi and J. C. Rathi, J. Young Pharmacist, **1**, 205 (2009).
18. Mahmoud Mokhtar, A. Omaima, Sammour, Int. J. Pharm., **361**, 104 (2008).
19. K. S. Chandra prakash, N. Udupa, Int. J. Pharm., **61**, R1 (1900).
20. V. Ravichandran, G. Velrajan, S. Raghuraman and D. Benito Jhonson, V. Sivan and V. Sankar, The Eastern Pharmacist, **518**, 113 (2001).
21. Samar Mansour, A. S. Guinedi and D. Nahed, Int. J. Pharm., **71**, 306 (2005).