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Lecithin Stabilized Organogel: Design and Development for Topical Application of Clobetasol Propionate

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ABSTRACT: PURPOSE- The purpose of this research was to design and study whether the topical application of Clobetasol Propionate would be effective by applying through novel delivery system like microemulsion based organogel.
METHODS- Novel formulation like lecithin-stabilized microemulsion based organogel was formulated using surfactant (soyabean lecithin), oil (isopropyl myristate), and mixture of distilled water and glycerol for topical delivery of Clobetasol Propionate. The phase diagram of microemulsion based organogel was constructed to determine the nature and extent of microemulsion region. Selection of oil was made on the basis of drug solubility.
RESULTS- The optimized formula of the organogel was 0.05% CP w/w, 32% lecithin w/w, 66% IPM v/w, 2% water and glycerol v/w (1:1). The effect of formulation variables on the release profile of the drug from the formulation was determined through excised rat skin. A significant decrease in drug release from formulations was observed with increase in concentration of lecithin from 32% to 62% w/w. The drug release increased remarkably as the loading was increased from 0.05% to 0.1% w/w. Increased oil concentration lead to increase in drug release from the formulation. Viscosity and transparency of formulations was observed to be directly effected by lecithin concentration. Skin retention of Clobetasol Propionate through comparative cutaneous deposition analysis was found to be 7.4% more for the developed organogel than marketed gel (Clobetasol$^{(R)}$). This system was capable of providing sustained drug release up to 10 hrs. Stability study revealed that the formulation was stable for 6 months. **CONCLUSIONS-** Based on the above results it could be concluded that Lecithin stabilized microemulsion-based organogel could be considered as efficient topical drug delivery system for Clobetasol Propionate.
KEYWORDS: Lecithin; Clobetasol Propionate (CP); lecithin-stabilized microemulsion-based gels (LMBG); Isopropyl myristate (IPM), Skin retention.

INTRODUCTION

Lecithin stabilized microemulsion-based organogels (LMBGs) are optically transparent, thermodynamically stable, viscoelastic and biocompatible jelly-like products, chiefly composed of hydrated phospholipids and appropriate organic liquid. Lecithin, a naturally occurring surfactant, is capable of forming reverse micelle-based microemulsions in an apolar environment[1-3]. It is believed that above a critical concentration of lecithin, the small reverse micelles tend to grow monodynamically into long flexible and cylindrical gaint micelles upon addition of a specific amount of water. These giant structures then build a continuous network with a high viscosity. LMBGs have attracted much attention as a biocompatible matrix for topical drug delivery because they are transparent, non-irritant, and stable and are capable of

solubilizing various guest molecules as well as release the drug molecule in sustained manner[4]. Clobetasol Propionate (CP), a superpotent (Class-1) corticosteroid is however associated with systemic adverse effects including erythema, skin atrophy, folliculitis and reversible HPA axis suppression[5]. Topical administration of CP offers the advantage of enhanced drug delivery to the affected sites with reduced incidence of systemic side effects. Marketed conventional dosage forms of CP for topical application (i.e. Creams, Gels, Ointments and Lotions etc) which are unable to cross the intact stratum corneum as skin is an exceptionally effective barrier to most of chemicals. Thus optimum concentration of drugs can't reach to the site of action. Therefore novel formulations like Lecithin stabilized microemulsion-based organogel was formulated for topical application of CP that makes the skin locally more permeable (due to the penetration enhancing capacity of IPM and lecithin). Thus it helps to obtain optimum drug concentration in the skin layer and avoids systemic side effects of CP.

In the present study, a novel formulation for topical application i.e. LMBG of CP was developed to facilitate the penetration of the drug through skin at sustained manner so as to obtain therapeutically effective concentration of the drug on the site of action.

MATERIAL AND METHODS

MATERIALS: Clobetasol propionate was provided by Cipla Pvt. Ltd as gift sample. Isopropyl myristate, Sodium Lauryl Sulphate (SLS), Disodium hydrogen phosphate, Potassium dihydrogen phosphate, Acetonitrile and Methanol (HPLC grade) and were purchased from Baroda Chemicals, Vadodara. Soyabean lecithin and glycerol was purchased from Himedia Pvt. Ltd, Mumbai. Double Distilled water. All other chemicals and reagents were of AR grade.

METHODS

Construction of calibration curves: 10 mg of Clobetasol Propionate was accurately weighed and dissolved in 100ml of Acetonitrile and methanol of HPLC grade (70:30 V/V) to make 100 ppm solution. Series of different concentrations 5 to 80 µg/ml CP in Acetonitrile and methanol of HPLC grade (70:30 V/V) were prepared to construct the calibration curves. Solutions were analyzed in HPLC at 241.5nm. Linear regression analysis of the corresponding plots showed a correlation coefficient of 0.9997 and regression equation as Y=0.034x+0.0198.

Preparation of drug loaded LMBGs: Drug containing LMBGs (10gms batch size) were prepared by first dissolving CP (5mg) in 6.6ml of oil (IPM). This solution was heated up to 40^0C and then the preweighed Lecithin (3.2gms) was slowly added to it with constant stirring on Magnetic Stirrer (Remi Instruments Pvt. Ltd, Mumbai) at 200rpm to obtain a homogeneous mixture of Lecithin in CP-IPM solution. To this mixture, 0.2ml of distilled water and glycerol mixture (1:1) was added through a micropipette and stirring was continued for 15 minutes. Thereafter the stirring was stopped and the samples were allowed to cool and set aside to obtain clear, homogenous organogels at room temperature[6].

Construct ternary phase diagram: To construct ternary phase diagram, different batches of LMBGs were prepared by the above method varying the concentration of Lecithin (32% to 72%) and IPM (66% to26%) with constant concentration of water and glycerol (2% in the ratio 1:1). The phase diagram of organogel was constructed using Chemix Software to determine the nature and extent of microemulsion region[7].

OPTIMIZATION OF LMBGS:

Formulation Optimization: Different LMBGs were formulated varying the Lecithin to IPM ratio and was optimized based on the parameters like transparency, physical stability[8]. Data of process optimization is given in Table-1.

Process Optimization: Different LMBGs were formulated varying the process parameters like temperature, rpm and was optimized based on the parameters like transparency, physical stability[8]. Table-2 provides the data of formulation optimization.

Stability Study: The optimized formulation was stored at three different temperature ranges for 6 months i.e., refrigerating condition (2^0C – 8^0C), room temperature and elevated temperature (50 ± 2^0C) and shelf life of the stored system was evaluated by visual inspection (phase separation and transparency), % Assay. In order to estimate the metastable systems, the optimized formulation was also centrifuged (Remi Laboratories, Mumbai, India) at different rpm like 5,000, 10,000 and 15,000 for 30 minutes at room temperature and observed for any change in its homogeneity[9]. The data are tabulated in Table-3.

Table 1: Results of process optimization:

Formulation Code No.	Formulation Composition				Transparency	Physical Stability
	L (%)	IPM (%)	W (%)	CP (%)		
F2	20	80	0.1	0.05	More	Not stable
F3	30	70	0.1	0.05	Moderate	Stable
F4	40	60	0.1	0.05	Less	Stable
F12	29	71	0.1	0.05	More	Stable
F13	31	69	0.1	0.05	More	More Stable
F14	32	68	0.1	0.05	Maximum	More Stable

Table 2: Results of formulation optimization:

Formulation Code No.	Formulation Composition		Transparency	Physical Stability
	Temp (^{0}C)	RPM		
F2	30	100	Less	Less
F3	40	100	Medium	Less
F3	40	150	Transparent	Medium
F3	40	200	Transparent but more bubbles	More
F4	50	200	Medium	Not Stable
F12	30	100	Medium	Medium
F13	40	100	Medium	Medium
F13	40	150	Transparent	More Stable
F13	40	200	Medium	Medium
F14	50	200	Medium	Not Stable

Table 3: Results of Stability:

Temperature (^{0}C)	Phase separation		Transparency		% of Assay	
	After 4 month	After 6 month	After 4 month	After 6 month	After 4 month	After 6 month
2^0C-8^0C	No	No	Yes	Yes	98.3±1.5	97.8±2.9
Room Temp	No	No	Yes	Yes	99.4±1.9	99.1±2.1
Elevated Temp (50 ± 2^0C)	No	No	Yes	Yes	99.2±0.9	98.6±1.7

Preparation of Excised Rat Skin: Albino rats of 8 weeks old were sacrificed by cervical dislocation technique and abdominal skin was removed. Samples i.e., full thickness hairless abdominal skin of the rat were prepared removing the hair, fat layer and then were cut into small circular pieces of 3.0cm diameter. All the pieces were carefully washed and transferred onto an aluminum foil, dried and stored at 4^0C until use.

Ex-Vivo Release Studies: Ex-Vivo Release studies of CP from the LMBGs were performed using Franz-diffusion cells (diameter = 3.0cm and volume = 23ml) through excised hairless abdominal rat skin respectively. The receptor compartment of the Franz-diffusion cells was filled with 23ml of Acetonitrile and methanol of HPLC grade (70:30 V/V) along with a magnetic bead. In Ex-Vivo Release study, the rat skin was carefully mounted between the donor and receptor compartment of the diffusion cell keeping the stratum corneum facing towards the donor side. The effective area of membrane available for diffusion was 7.06cm^2. Then 1.0gm (≅ 500µg of CP) of optimized LMBGs was uniformly spread on the membrane. The samples were withdrawn from the receptor compartment at predetermined intervals up to 10hrs and then replaced with same amount of Acetonitrile and methanol of HPLC grade (70:30 V/V). Drug concentration was

determined by HPLC at the wavelength 241.5nm. Cumulative rate of drug release was determined through rat skin[10]. The effect of CP concentration and lecithin concentration on diffusion rate through the excised rat skin from LMBGs was carried out.

Viscosity measurements: The viscosity of Lecithin/IPM/water-glycerol systems depends on the amount of Lecithin and water-glycerol incorporated into the organogel. Viscosity of each sample was measured using Brookfield LVDV & CP Viscometer (Brookefield, USA) using rheological software at a temperature of 25^0C. Viscosity of LMBGs with Lecithin to IPM ratio 30:70, 40:60, 50:50 and 60:40 were found to be 378.37, 789.66, 1351.07 and 1727.77 poise respectively.

Comparative Release Study: A comparative drug release study of conventional dosage form (Clobetasol$^{(R)}$), Optimized LMBG and plain drug solution was carried out through excised rat skin using Franz diffusion cell[11].

Estimation of the cutaneous deposition of Clobetasol Propionate: Hairless excised rat skin was used for the estimation of the cutaneous deposition of Clobetasol Propionate from optimized LMBG and Clobetasol$^{(R)}$ (Marketed Formulation). After being sacrificed, the abdominal skin of the rat was excised. The hair was removed carefully by using a new mechanical shaver. The excised hairless skin was washed with distilled water, collected and prepared into specific size 3.0cm in diameter and soaked in PB-5.8 for at least 10 minutes.

The acceptor compartment was filled with 23 ml of receptor medium with a magnetic bead. Prepared samples of skin were mounted on receptor chambers of two different Franz diffusion cells separately filled with diffusion medium along with magnetic bead keeping the stratum corneum facing towards the donor side. The temperature of diffusion cell was maintained at 37 ± 2^0C by circulating water through the diffusion cell. The conditions were stabilized on a magnetic stirrer for 30 minutes. Then a known weight of optimized LMBG and Clobetasol$^{(R)}$ (each $\cong$ 500 µg of drug) was applied on the skin separately and the donor part was mounted on it. The diffusion process was allowed to continue for 8 Hrs. After 8 Hrs the skin was carefully taken out from the Franz diffusion cells. The skin surface was carefully scrapped and then washed with known volume of SPB-5.8 to remove any residual gel remaining over the skin. The skin was then cut in to small pieces, homogenized and centrifuged at 10,000rpm for 10 minutes. The supernatant was withdrawn, diluted with SPB-5.8 suitably and then the drug content was determined by HPLC at 241.5nm[12].

RESULTS AND DISCUSSION:

Stability Study: The developed formulation was found to be stable for 6 months in room temperature and elevated temperature. But it was found to be unstable in cold temperature due to the freezing point (i.e.4^0C) of isopropyl myristate.

Viscosity measurements: From the results of rheological measurements, a significant increase in viscosity was observed with increase in Lecithin concentration due to the fact that long flexible and cylindrical gaint micelles were formed[13].

Effect of different formulation variables on diffusion of CP

(a) Effect of CP concentration on diffusion rate: The effect of CP concentration on diffusion rate through the excised rat skin from LMBGs was shown in Figure-1. Two LMBGs were formulated. F1 composed of 32% Lecithin, 66% IPM, 2% water and glycerol, and 0.05% w/w CP) and F2 composed of 32% Lecithin, 66% IPM, 2% water and glycerol (1:1), and 0.1% w/w CP). The effect of CP concentration on the diffusion rate from LMBGs was evaluated. A significant increase in the drug release was obtained in formulations containing 0.1% w/w of CP compared to those containing 0.05% w/w of the drug. The diffusion rate of formulation composed of 0.1% w/w CP was about 7.8 times (66.32µg/cm^2) higher than that obtained from the formulation with 0.05%w/w of the drug (8.5µg/cm^2). The data revealed that release rate of CP from LMBGs is directly proportional to drug concentration. Due to the concentration gradient of CP in the oil globule and the diffusion medium, CP released from the formulation and then diffused through the diffusion membrane.

Figure 1: Effect of CP concentration on diffusion rate from LMBGs across excised rat skin.

(b) Effect of Lecithin and Isopropyl myristate concentration on diffusion rate: The effect of lecithin concentration on diffusion rate through the excised rat skin from LMBGs was depicted in Figure 2. Effect of lecithin concentration (66% to 26%) on diffusion rate of CP from LMBGs composed of 2% water and glycerol, and 0.1% w/w CP was evaluated. A significant decrease in CP release was obtained as lecithin concentration was increased from 26% to 66% w/w in formulations.

The diffusion rate of CP were 65.14, 58.95, 50.43, 34.61, and 19.67 µg/cm^2 after 10hrs of diffusion from the formulations composed of 26% (and 74%), 36% (and 64%), 46% (and 54%), 56% (and 44%), and 66% (and 34%) of lecithin (and IPM) respectively. This effect may be due to the fact that at higher lecithin concentrations, there is more extensive entanglement of long cylindrical micelles with each other, forming a network-like structure with a very high viscosity. So after entrapment in the network, less amount of free drug is available which leads to decrease in the diffusion rate of CP across the rat skin.

Comparative Release Study: Optimized LMBG showed more drug release through excised rat skin than that of conventional dosage form (Clobetasol$^{(R)}$) and plain drug solution. This was due to the fact that isopropyl myristate and lecithin were acting as penetration enhancer. The release pattern was given in the figure-3.

Cutaneous deposition estimation of Clobetasol Propionate: Results showed that drug retained in the rat skin was 8.87% and 16.27% respectively for Clobetasol$^{(R)}$ and optimized LMBG 7.4% more drug deposition might be due to the fact that more amount of drug was penetrated through the intact stratum corneum.

CONCLUSION: From the above studies it was concluded that the formulation containing 32% w/w Lecithin and 66% IPM v/w, 2% v/w of water and glycerol mixture (1:1), and 0.05% w/w of CP was the optimized formulation and was stable for 6 months at room and elevated temperature. Due to the properties like biocompatibility, potential for skin penetration, sustained release, enhanced cutaneous drug deposition, lecithin stabilized microemulsion-based organogels may be considered to be promising vehicles for topical application of Clobetasol Propionate. However further studies in higher animals and human being need to be performed before this formulation can be commercially exploited.

Figure 2: Effect of Lecithin concentration on diffusion rate from LMBGs across excised skin

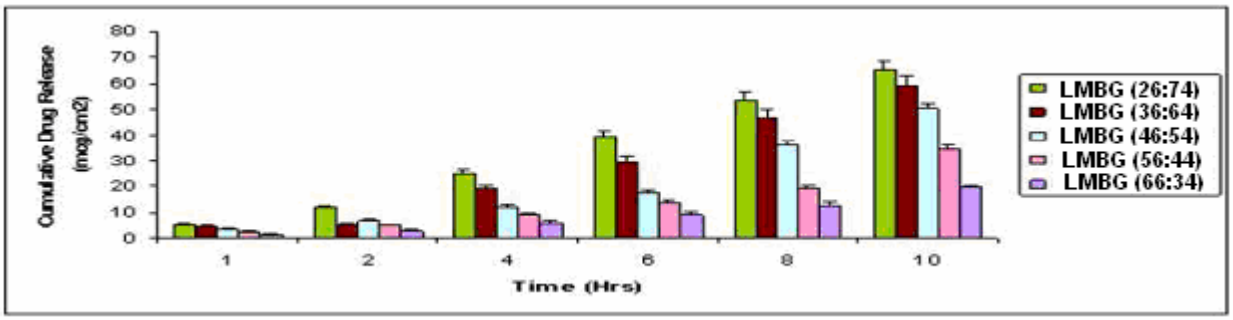
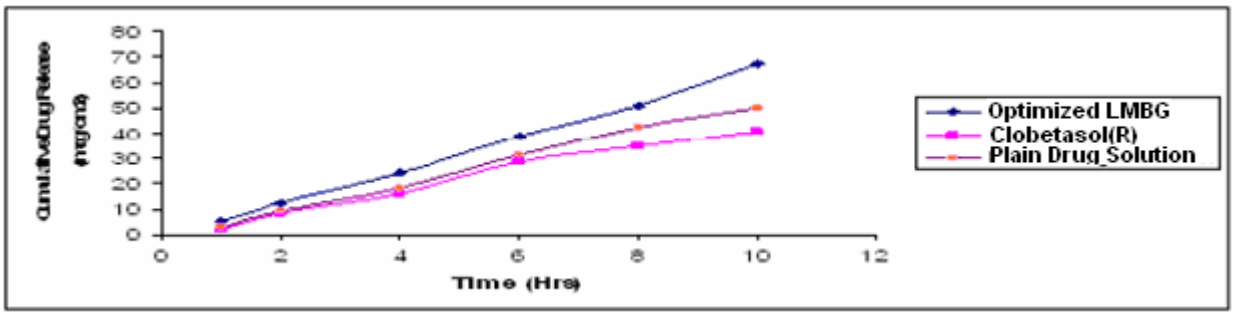


Figure 3: Data of comparative release study:



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