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Influence of Permeation enhancer on Ethosomes bearing Lamivudine for Transdermal Drug Delivery

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Abstract

The application of medicinal substances to the skin is a concept undoubtedly as old as humanity, the Papyrus records of ancient Egypt describes a variety of such medication for external use. Innovative techniques for drug delivery have been explored in human medicine in recent years. Transdermal drug delivery has gained much vital impact in dosage form; it has been impending as asubstitute to oral delivery. Several tactics for improving transdermal drug delivery were researched; Ethosome are ethanol derivatives of liposomes. Permeation enhancer has the ability to cause the perturbation of the skin and help in drug penetration. The ethanol effect of ethosome and permeation enhancer will synergistically enhance the drug penetration to deeper skin and systemic circulation. Lamivudineethosomal formulations were prepared and characterized for entrapment efficiency, fluorescence skin study and ex-vivo study. Propylene glycols (PG), oleic acid are penetration enhancer for lamivudine into skin. Ethosomes were prepared with propylene glycol (ETP) and linoleic acid (ETL) respectively. Ethosomes bearing propylene glycol and oleic acid has shown good flux values 1.5 fold and 2 fold than ethosomes (ET) respectively. The highest entrapment efficiency of ETP-2 is 64.6±1.5% and ETO-2 formulation is 68.2±4.1%. Increases in the concentration of both permeation enhancers decrease the entrapment efficiency. The fluorescence skin ex-vivo study of the ethosomes formulation reveals that oleic acid ethosomes has deeper penetration in the skin compared to ethosomes containing propylene glycol. The release of the ETP-2 and ETO-2 has both shown sustain release pattern for 12 hrs with reduced in lag time. Ethosomes containing oleic acid has better entrapment, enhancement of lamivudine penetration to deeper skin compares ethosomes containing propylene glycol.

Keywords: Ethosomes, lamivudine, oleic acid, propylene glycol, permeation enhancer.

Introduction

The solicitation of medicinal substances to the skin is a concept undoubtedly as old as humanity, the Papyrus records of ancient Egypt describes a variety of such medication for external use[1-3]. Innovative techniques for drug delivery have been explored in human medicine in recent years. Transdermal delivery is an important substitute dosage form to oral drug delivery system[4-9]. Stratum corneum (SC) of skin acts as barrier for transdermal route of drugs. Several tactics for improving transdermal drug delivery were researched. Polymers play important role in controlled release dosage formulation of transdermal delivery system[10]. Vesicular carriers are water-filled colloidal particles which are used to enhance the permeation of drugs[11-12]. Effective permeation enhancers are required to provide the drug molecule deeper into skin tissue for optimized therapeutic delivery of drug[3,4,7]. Ethosome is second generation of elastic liposome has ability to permeate drug into the skin. Ethanol intermingle with the lipid region of stratum corneum and escalates their fluidity and declining the density of the lipid multilayer by reducing the glass-transition temperature (T_g) of the lipids[2,13,14]. Drugs substances can be made permeable to stratum corneum by chemical penetration enhancers which has the ability to change the physicochemical properties of SC reversibly[15-16]. The increase in the drug solubility and improvement of its partition coefficient (skin/vehicle) is another mechanism explaining the action of penetration enhancers[17-18]. If the ethosomes were combined with permeation enhancer; it will boost the penetration of drug and to deeper skin tissue and show systemic action. Oleic acid is sorption enhancer which has the ability to enhances the permeation of drugs in skin by acting on the extracellular lipids of SC[18]. The lamellar sheet of SC contains ceramides as the major lipid[19]. Wartewig et al., investigated that oleic acid endorses phase separation by decreases T_m of ceramides[20-21]. Another permeation enhancer propylene glycol, well known for its multiple use in topical preparation, its mechanism of action for the penetration enhancing effect is postulated that the interaction of PEG and keratin in the SC which causes perturbation of the SC and enhances the penetration of drugs[22-24].

Lamivudine (3TC) is water soluble antiretroviral drug for treatment of HIV. It is an antiretroviral drug that decreases the amount of HIV in the body[25-26]. Lamivudine maintain viral suppression and decrease disease progression and help in build up the immune system[25,27]. Lamivudine has short biological half-life, soit requires frequent dosing as antiretroviral drugs are used lifelong in AIDS. Transdermal has numerous advantages

such less frequent dosing; maintain constant plasma concentration of drug in blood for longer time[25]. Ethosomes which are second generation of the vesicular carrier are used for transdermal delivery of lamivudine. Ethosomes with additional permeation enhancer boost the penetration of the lamivudine to deeper skin tissue and show systemic effect. The objective of the study was to estimate the effect of penetration enhancer (oleic acid and propylene glycol) in ethosomes containing lamivudine for transdermal delivery.

Material and Methods

Lamivudine was procured as a gift sample from Matrix (Mylan) Pharmaceutical Ltd Hyderabad; Phospholipid (soybean derived phosphatidylcholine) was obtained from Lipoid. Ethanol, propylene glycol, oleic acid, chloroform, isopropyl alcohol, cholesterol, polycarbonate membrane, dialysis membrane was procured from Himedia, Mumbai, India. Methanol and distilled water are of HPLC grade.

Characterization and Evaluation: Preparation of ethosomes formulation with permeation enhancer[28]: Ethosomes bearing lamivudine were prepared according to Touitou et al.,[28] with slight modification. The formulation system consists of phospholipids, ethanol, lamivudine/Rhodamine 123 and aqueous phase. In this method phospholipid (soybean derived phosphatidylcholine) and lamivudine/Rhodamine 123 is dissolved in ethanol with continue stirring in vessel maintained totemperature of 50°C. In separate vessel aqueous phase/ PBS 7.4 is heated to 50°C. The ethanolic mixture is added to the PBS mixture with continuous stirring at 800rpm for 30 minutes. The ethosomal preparation is passed through polycarbonate membrane for desired size of vesicles. Total volume of the preparation 25 ml and formula was given in table 1. In similar manner the ethosomal formulation containing propylene glycol and oleic acid respectively prepared[28].

Size and Zeta potential Analysis: Zeta potential and size of vesicles were analysed by dynamic light scattering method (DLS), (Malvern Zetamaster, UK)[25].

Drug Entrapment Efficacy: Ultracentrifugation method has been used to estimate the entrapment efficiency of drug in ethosome. 2ml of triton solution is added in 20ml ethosomal formulation and vortexed for 5 cycles for 15 minutes. In centrifuge tubes add the 2ml vortexed ethosomal formulation and using cooling centrifuge, it is centrifuged at 15,000 for 4 hours. After centrifuge, the clear liquid layer or supernatant liquid is decanted into volumetric flask and diluted with suitable solvent and the concentration of drug/lamivudine is estimated at 271nm in all samples. Similar procedure is followed for to all formulation containing bearing permeation enhancer[29]. The formula for the calculation of entrapment efficiency is.

Entrapment efficiency = {(Total concentration of drug -amount of drug in clear supernatant fluid)/Total concentration of drug} x100

Preparation of Dialysis membrane and human cadaver Skin: The healthy skin from the forearm region of a cadaver acquired from MY hospital, Indore, was incised, freed from fats and prepared for study. It was then cut into pieces of suitable size and treated with penetration enhancers such as Dimethyl sulfoxide (DMSO) and Dimethyl formamide (DMF).

Permeation studies of ethosomal formulation: All animal trials were performed according to the etiquettestandard by the Institutional Animal Ethics Committee (IAEC) of SCOPE, Indore. The *in-vitro* skin penetration of lamivudine from ethosome formulation containing with or without permeation enhancer was studied using diffusion cell. The temperatureand stirring was monitored and maintained in receptor cell at 37 ±0.5°C and 120 rpm respectively. Place prepared human cadaver skin between the donor and the receiver compartments. Apply the freshly prepared ethosome formulation on the upper surface of skin and the content of diffusion cell was kept under constant stirring. At regular interval withdraw 1ml of sample from diffusion cell and replace with fresh buffer PBS 7.4 to maintain skin condition. The withdrawn sample were analysed spectroscopic method at 271nm Triplicate experiments were conducted for skin permeation study. After the permeation study, human cadaver skin was analysed for accumulation of drug in the skin. The upper surface of skin was washed with PBS pH 7.4 and the whole skin is immersed in PBS. Analyse the both of solution and drug concentration was estimated by spectrophotometrically at 271 nm after suitable dilutions[30-31]. A cartesian plot was drawn between cumulative amount of drug versus time to determine the flux value of drug (Jss, $\mu g/cm^2/hr$). The slope of the linear portion of cartesian plot use to determined flux value[32].

HPLC Assay: The quantity of drug diffused in the receiver compartment during dialysis membrane and *ex-vivo* skin permeation experiments are analysed by RP-HPLC using methanol: water: acetonitrile (70:20:10 v/v) at flow rate of 1ml/min (Waters HPLC). The samples were analysed using PDA detector at 271nm.

Skin Fluorescence study: The skin permeation ability of ethosomal formulations (ET, ETP and ETO) were confirmed by fluorescence study. The fluorescent labelling will be carried out by preparing the optimized ethosomal formulations with fluorescence marker Rhodamine 123 indicator. Rhodamine 123 loaded formulations will be applied topically to rats. After post application of formulation, the rats will be forfeited and dorsal skin will be detached, cut into small pieces, fixed into fixative solution (3:1, absolute alcohol: chloroform) for 4 hours. The dorsal skinkept inethanol for 30 minutes and thereafter in mixture of ethanol and xylene for 60 minutes. The skin wax

blocks were prepared for microtomes section. The skin wax sections will beobserved under a fluorescence microscope[32-33].

Results and Discussion

Stratum corneum of skin acts as barrier for transdermal route of drugs. Phospholipids containing two sites of unsatuartion in fatty acid moiety are active while fully saturated fatty acids didn't exhibits any anti HIV activity[34]. Soybean derived phosphatidylcholine have two unsatuartion in fatty acid moiety and shows antiHIV activity[34-35]. Phosphatidylcholine self resembles into liposomes, a bilayer vesicles when it comes in contact with aqueous solvent. Effective permeation enhancers are required to provide the drug molecule deeper into skin tissue for optimized therapeutic delivery of drug. Ethosome is ethanolic liposome has ability to penetrate drug into the skin. If the ethosomes were combined with permeation enhancer, it will boost the penetration of drug into deeper skin tissue and show systemic action. Oleic acid act as permeation enchanter by lipid fluidization and lipid phase separation on skin.[33]It reduces the lag time of drug release from dosage form to skin by escalating the drug diffusivity in the SC to skin. Propylene glycol has ability to decrease the diffusional resistances of the skin barrier, possibly because drug kept in dissolved form in the glycol, diffuses through the skin[36-37]. Figure 1The size of the all batch ethosomes were analysed by zetasizer; it revealed that almost all ET, ETP_{1-3} and ETO_{1-3} are in nanometer size range from 356±12.5 nm to 434±24.8 nm. There is no significant change in size of vesicle with /without permeation enhancer. Zeta potential measurement study was observed to be −5.0 ± 0.1 to −8.0 ± 0.4 respectively, which revealed that according thumb rule; the vesicles are separated from each other and suspended in the formulation. Increase in propylene glycol show better zeta potential compared to increase in oleic acid concentration. The highest entrapment efficiency of ethosomal formulation is ET (63.4±2%), ETP-2(64.6±1.5%) and ETO-2 (68.2±4.1%). (table 2). Increases in the concentration of both permeation enhancers from 3 to 5% increase in entrapment due to itsupsurge in flexibility of membrane. Additional rise in concentration of penetration enhancer probably makes the vesicles more leaky in nature and decrease the entrapment efficiency. Oleic acid and propylene glycol both have shown effective permeation of lamivudine, oleic acid ethosomes shown faster permeation or reduction in lag time to propylene glycol ethosomes and ethosomes without permeation enhancer but the cumulative percentage releases of drug are similar (figure 2). Ethosomes bearing propylene glycol and oleic acid has shown good flux values 1.5 and 2.0 fold increase in flux rate than ethosome without permeation enhancer. The fluorescence photographs confirm that penetration enhancers augment the permeation of drugs and reduce lag time (figure 3).

Table-1
Composition of the ethosomes having different concentration of permeation enhancer

Formulation code	Egg derived phosphatidylcholine (%weight/weight)	Ethanol (ml)	Lamivudine (%weight/weight)	Permeation enhancer(%weight/weight)	
				Propylene glycol	Oleic acid
ET	2.0	30	0.4	-	-
ETP_1	2.0	30	0.4	3	-
ETO_1	2.0	30	0.4	-	3
ETP_2	2.0	30	0.4	5	-
ETO_2	2.0	30	0.4	-	5
ETP_3	2.0	30	0.4	7	-
ETO_3	2.0	30	0.4	-	7

Table-2
Characterization of the ethosomes for different formulation containing permeation enhancer

Formulation code	Entrapment Efficiency %	Vesicle Size nm	Zeta Potential (ζmv)
ET	63.4±2.3	356±12.5	–5.0 ± 0.1
ETP_1	63.1±1.5	412±14.9	–7.0 ± 0.5
ETO_1	64.3±1.9	412±21.4	–6.8 ± 0.3
ETP_2	64.6±1.5	389±11.7	–7.4 ± 0.4
ETO_2	68.2±4.1	402±20.1	–7.0 ± 0.2
ETP_3	59.3±2.4	434±12.9	–8.0 ± 0.4
ETO_3	61.7±1.9	403±10.4	–7.3 ± 0.6

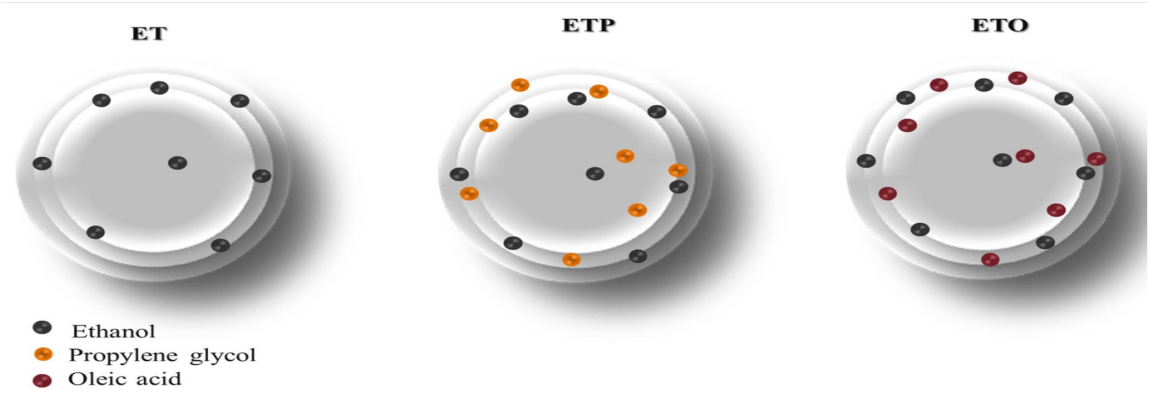

Figure-1
Schematic diagram representation of ethosomes bearing permeation enhancer oleic acid and propylene glycol

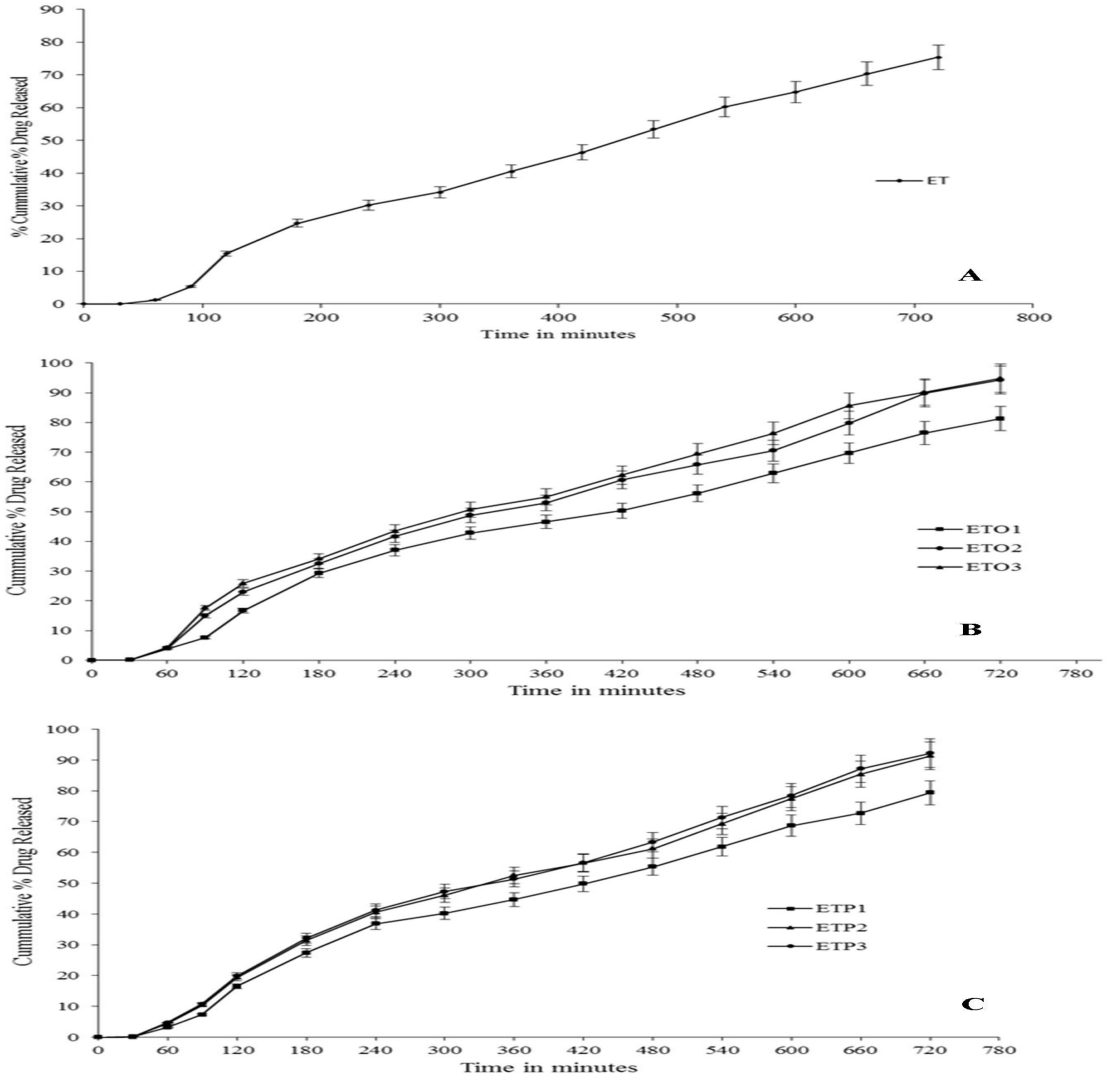

Figure-2
Invitro **release study of ethosomes bearing lamivudine A) Ethosomes B) Ethosomes with Oleic acid C) Ethosomes with Propylene glycol**

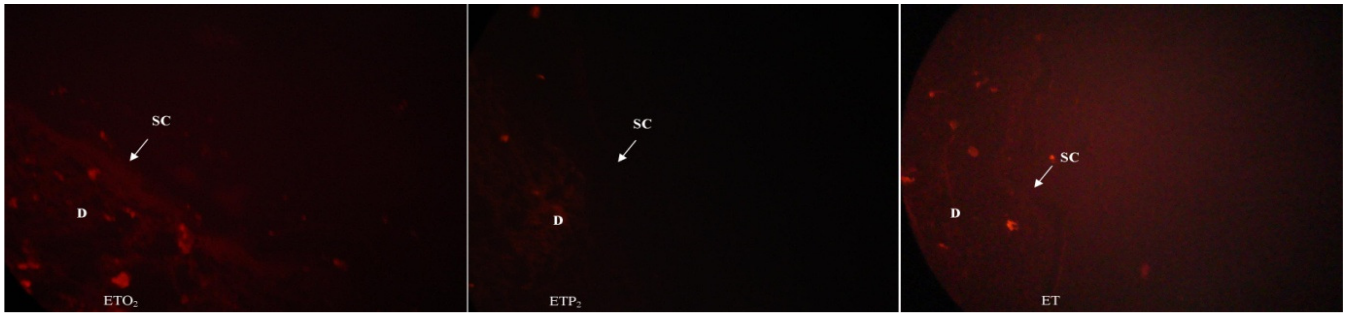

Figure-3
Florescence study of Ethosomes bearing Rhodamine ET, ETO_2, ETP_2 (SC: - Stratum corneum, D: - Dermis)

Conclusion

Ethosomes well known for its penetration of drugs into skin, utilizing the penetration enhancer it reduced the lag time of drugs penetrating into skin and enhances deeper penetration into skin. Ethosomes enhance permeation of drugs with its ethanol effect and show lipid perturbation. By adding penetration enhancer in ethosomes such as propylene glycol act by solvation of keratin within the stratum corneum and oleic acid endorses phase separation by declining melting temperature of ceramides; both have shown good result in term of penetration along with ethosomes formulation.

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