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Vijaya Rajendran
Assistant Professor, Department of Pharmaceutical Technology, Anna University, BIT Campus, Tiruchirappalli 620024, Tamilnadu, India

Correspondence:
Vijaya Rajendran
Assistant Professor, Department of Pharmaceutical Technology, Anna University, BIT Campus, Tiruchirappalli 620024, Tamilnadu, India

Effect of niosomes in the transdermal delivery of antidepressant sertraline hydrochloride

Vijaya Rajendran

Abstract

Objective: Sertraline hydrochloride is an antidepressant that undergoes hepatic first pass effect upon oral administration. Controlled delivery for prolonged period across skin is one of the solutions to improve its therapeutic efficacy. Here, niosomes has been used as a carrier for the transdermal transport of sertraline HCl. **Material and Method:** Sertraline HCl Niosomes were prepared by ether injection method using surfactants such as span 40(A), span 60(B) and span 80(C) along with cholesterol at a ratio of 1:1. Four different concentrations (200:200(Aa), 250:250(Ab), 300:300(Ac) & 350:350(Ad)) of surfactant and cholesterol were used for each surfactant. The niosomes were characterized in vitro for size and surface morphology by means of particle size analyzer and scanning electron microscope (SEM) respectively. Encapsulation efficiency was determined by high performance liquid chromatography. Sertraline HCl release and skin permeation studies were carried out using franz diffusion cell. At last, the optimized formulation was subjected to physical stability studies. **Results:** The SEM and size distribution analysis evidenced the formation of discrete, spherical niosomes. Higher encapsulation efficiency of 53.71%±3.2%, 51.18%±2.5% and 51.92%±2.7% were obtained for Ad, Bb and Bc respectively. A maximum sertraline HCl release of 70%±2.6% was obtained for Ad which showed a permeation of 2.71%±0.157% across mouse skin. A calculated 50.2%±0.9% of sertraline HCl was assumed to get accumulated in the skin layers and the niosomes were physically stable. **Conclusion:** Sertraline HCl niosome showed a slow and prolonged release of sertraline HCl through the mouse skin and thus holds promise for transdermal delivery.

Keywords: Sertraline hydrochloride, Niosomes, Span, Transdermal delivery, *In vitro* release and permeation.

INTRODUCTION

Sertraline HCl is a potent selective serotonin (5-hydroxy tryptamine, 5-HT) reuptake inhibitor (SSRI) which is administered orally in the treatment of major depression. Being a SSRI the most common adverse effects are gastrointestinal disturbances such as nausea, dry mouth, constipation, diarrhoea, decreased appetite, flatulence, vomiting, oropharynx disorder and dyspepsia. It undergoes extensive first pass metabolism with a daily dose of 25-100mg [1]. Sustained release formulations of antidepressants for oral [2] and transdermal route [3, 4] have been developed to improve the efficacy. Controlled release and enhanced bioavailability of sertraline HCl from eudragit based transdermal films has been reported earlier by the present author [5]. In the transdermal delivery, stratum corneum acts as a barrier for the transport of drugs across the skin [6] and thus various physical and chemical approaches are being tried to enhance the penetration of therapeutic molecules across the stratum corneum. Among the several enhancers, vesicular systems like niosomes and liposomes have been shown potential as penetration enhancer. These can act as a carrier for variety of drug molecules due to their structural properties. However, niosomes alleviate the disadvantages associated with liposomes such as chemical instability, variable purity of phospholipids and high cost [7].

Successful delivery of therapeutic agents across the skin using niosomes has been stated in the literature [8-10]. This vesicular carrier can also be served as solubilizing matrix, as local depot for sustained release or as rate limiting membrane for the modulation of systemic absorption of drugs via the skin [11]. Hence, the present investigation involved the development of sertraline hydrochloride niosomes for transdermal transport. The niosomes were subjected to size and surface analysis; *in vitro* drug release and permeation; stability studies. The role of surfactant type, its concentration in increasing the drug entrapment efficiency was also determined.

MATERIAL AND METHODS

Materials

Sertraline hydrochloride was obtained as a gift sample from Orchid Pharmaceuticals, India. Span 80, span 60 and span 40 were purchased from S.D. Fine Chemicals Ltd, India. Diethyl ether was obtained from Merck, India. Ethanol AR (99.9%) was obtained from Changshu Yangyuan Chemical Co. Ltd, China. Cholesterol was purchased from Qualigens Pvt. Ltd, India. Sodium acetate was obtained from Merck, Mumbai, India. Methanol (HPLC grade) was purchased from Rankem, India. Dialysis membrane was obtained from Himedia Laboratory Pvt. Ltd, India and Milli-Q water was used for all the experiments.

High Performance Liquid Chromatography (HPLC)

The HPLC (Shimadzu, Japan) analysis was carried out using the phenomenex C18 column (4.6x250mm, 5µm) as stationary phase. Acetate buffer: methanol (25:75) pH 4.1 was used as mobile phase. The flow rate was 1.0ml/min for 10min and the retention time was found to be 4.9min. The detection was made at 220nm using photo diode array detector. The regression coefficient value for the linearity plot was calculated in the concentration range of 5µg/ml to 25µg/ml.

Compatibility Study

The interaction between the drug and formulation excipients was analysed using Fourier transform infrared spectra (FTIR). The spectrum was recorded in NICOLET 6700 FTIR (USA) using potassium bromide pellets at a moderate scanning speed between $4000cm^{-1}$-$400cm^{-1}$.

Differential Scanning Calorimeter (DSC) thermogram of niosome formulation(Ad) was recorded (Universal V4-4A TA Instruments Q 200- V23, USA) at a scanning rate of 10°C/min over a temperature range of 0 to 250°C, under an inert nitrogen atmosphere at a flow rate of 20ml/min.

Preparation of Niosomes

The niosomes were prepared by slightly modifying the ether injection method [12]. Different micromolar concentrations (200:200, 250:250, 300:300 & 350:350) of span 40, span 60, span 80 and cholesterol were used at a ratio of 1:1 (Table 1). In the preparation of niosomes, a weighed quantity of sertraline HCl (10mg), non ionic surfactant and cholesterol were dissolved in sufficient quantity of diethyl ether. The solvent mixture was then injected into the aqueous phase (5ml) through a 22 gauge needle at a rate of approximately 0.25ml/min. The temperature of the aqueous phase was maintained at 60°C and the formulations were equilibrated at room temperature for 24h, stored in a refrigerator for further analysis.

Table 1: Formulation composition of niosomes

Formulation	Surfactant	Surfactant:Cholesterol (in mg)
Aa	Span 40	80.6:77.2
Ab		100.7:96.5
Ac		120.9:115.8
Ad		141:135.1
Ba	Span 60	86.2:77.2
Bb		107.7:96.5
Bc		129.3:115.8
Bd		150.8:135.1
Ca	Span 80	85.8:77.2
Cb		107.2:96.5
Cc		128.7:115.8
Cd		150.1:135.1

Size and Surface Morphology

Niosome suspension was placed and air dried on a cover slip and mounted using adhesive carbon tape. The vesicles were then splutter coated using gold in a vacuum evaporator. The size and shape of the vesicles were examined using SEM (TESCAN, Chezec republic) operated at 15kv accelerating voltage. The image was analysed by the software VEGA3**.** The size distribution was recorded using a particle sizing system (Santa Barbara, california, USA), sensor model LE 400-05 S. The elapsed time of data collection was 10s.

Encapsulation Efficiency of Niosomes

Before measuring the encapsulation efficiency, unencapsulated free drug was separated by filtering the niosomal dispersion through a 0.05µm filter (Millipore, USA) under vacuum [13]. The vesicles were washed with water to completely remove the free drug and then dispersed in aqueous solution. To one ml aliquot of the niosomal dispersion, equal volume of absolute ethanol was added to get a clear solution [14]. The sertraline HCl content in the solution was estimated by HPLC. The encapsulation efficiency was calculated from the ratio of the amount of drug entrapped to the total amount of drug added in the formulation.

In vitro Sertraline HCl Release [15]

The release of sertraline HCl from the niosome was examined under sink condition. A quantity of drug equivalent to 1mg present in the niosomal preparation was placed in a dialysis bag and was suspended in 50ml of phosphate buffer (pH 7.4). The temperature was maintained at 37°C±0.5°C under constant magnetic stirring (500rpm) (Remi, India). At predetermined time intervals, 1ml of sample was withdrawn from the beaker and the drug content was determined by HPLC.

In vitro Skin Permeation [16, 17]

Preparation of mouse skin

The full thickness abdomen skin capable of mounting between the two compartments of the diffusion cell was removed after shaving the hair from a female mouse aged 6 to 17weeks. The subcutaneous tissue was removed by surgical procedure using scalpel and scissor. The residual fat adhering with the dermis was wiped off using isopropyl alcohol. The thickness of the obtained whole skin was 0.81mm±0.04mm, as determined using eyepiece micrometer fitted on an optical microscope. Permission has been obtained from appropriate animal ethical committee for this study.

Permeation study

The skin was sandwiched between the receptor and donor compartment of a fabricated keshary chien type diffusion cell (Figure 1). The skin was fixed in the diffusion cell such that the dermal side was bathed with the fluid in the receptor compartment whereas the stratum corneum side was made to face upward the donor compartment. The receptor compartment contained 50ml of phosphate buffer (pH 7.4) and maintained at 37°C±0.5°C with stirring at 500rpm, while the donor compartment contained the niosomal formulation. 1ml sample was withdrawn from the receptor compartment at scheduled time intervals for 12h and replaced immediately with an equal volume of fresh phosphate buffer solution. The samples were assayed using HPLC. Finally, the amount of sertraline HCl accumulated in the skin was calculated by deducting the amount of drug in the receptor medium and in washing solvent (ethanol) from the initial drug content of the sample.

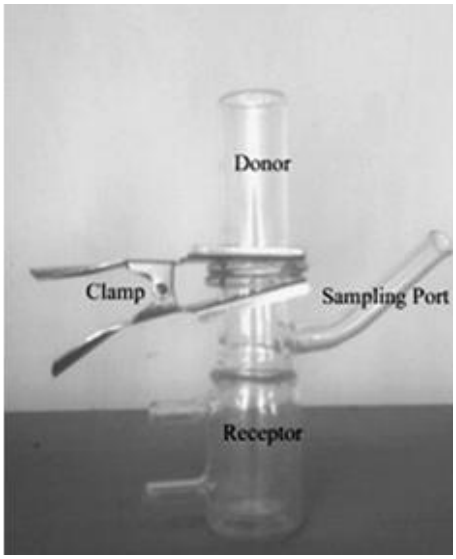

Figure 1: Keshary chien type diffusion cell

Physical Stability

Physical stability analysis was carried out for one week for the niosomal formulation Ad to assess any leaching of sertraline HCl from the niosome during storage at refrigerated temperature of 2 to 8°C. After storage, the vesicles were examined by HPLC for the encapsulation efficiency of sertraline HCl.

Statistical Analysis

Statistical analysis was carried out for all the experimental data using one way ANOVA and the level of significance was kept at $P < 0.05$.

RESULTS AND DISCUSSION

HPLC and Compatibility Study

For the HPLC analysis**,** the system and column were equilibrated with the mobile phase. Blank was injected followed by sample solution and the results were reported by area normalization method. The correlation coefficient value obtained was 0.9994.

FTIR spectral data were used to confirm the chemical stability of sertraline hydrochloride in niosome formulation. The FTIR spectra of pure sertraline hydrochloride, mixture of drug with each span and mixture of drug with cholesterol are shown in Figure 2. The spectra of pure sertraline hydrochloride showed peaks at 3479cm^{-1} (O-H stretching), 1577cm^{-1} (aromatic ring stretching) and at 1463cm^{-1} (CH_2 vibration). The bands at 1137cm^{-1} and 1020cm^{-1} were due to aromatic ring stretching. The peaks at 955cm^{-1} and 921^{-1} implies C-H twisting and NH_2 wagging.

The finger print characteristic vibration bands of drug appears in the FTIR spectra of drug and span mixture. However a slight shift in peaks for aromatic ring stretching was observed which might be due to association of aromatic head portion of span with the aromatic portion of the drug.

This aromatic ring association occurred at lower wave number in case of span 80. The band at 2999cm^{-1} corresponds to the C-H stretching of the methylgroup (CH_3) and at 1739cm^{-1} was due to C-H bending of ester carbonyl group present in span molecule.

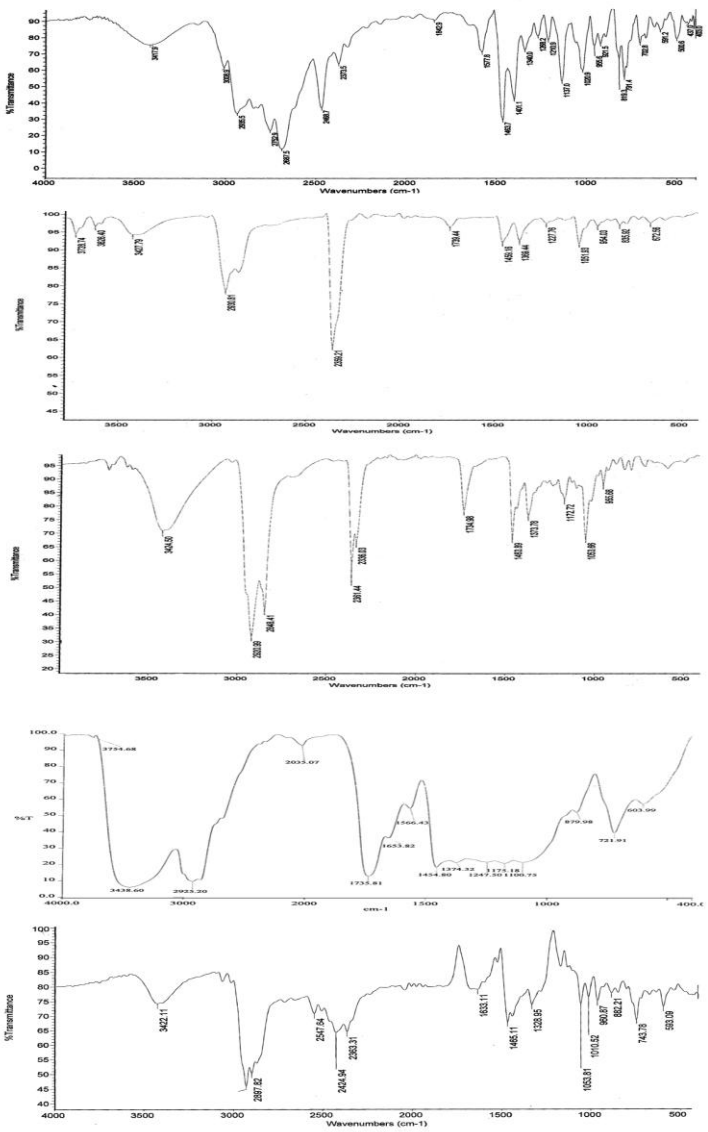

Figure 2: FTIR spectra of drug, drug + span 40, drug + span 60, drug + span 80 and drug + cholesterol

In case of cholesterol and drug mixture, the shifting of drug peak 2935cm^{-1} to lower wave number 2837cm^{-1} and shifting of 955cm^{-1} to higher wave number 960cm^{-1} had occurred. This might be due to the association of aromatic portions of drug and cholesterol and reduced affinity between polar portions of drug with entirely non polar cholesterol. The band at 882cm^{-1} was due to C-C-C stretching of cholesterol molecule. Hence, it has been confirmed that there is no significant chemical interaction had occurred between the drug and excipients except few association between them.

DSC thermogram of niosome formulation is shown in Figure 3. The thermogram of the drug showed a sharp melting peak at 235.95°C. The melting peak of cholesterol appeared at 133.23°C and melting peak of non ionic surfactant (span) was showed at 53.42°C. Physical parameter like melting point is an essential parameter to find out interaction between the excipient and active substance (drug). No additional melting point peak was observed in niosomal thermogram. The findings confirmed the formulation thermal stability and compatibility between drug and excipients since no modification with respect to melting point of drug, cholesterol and span 60 were observed.

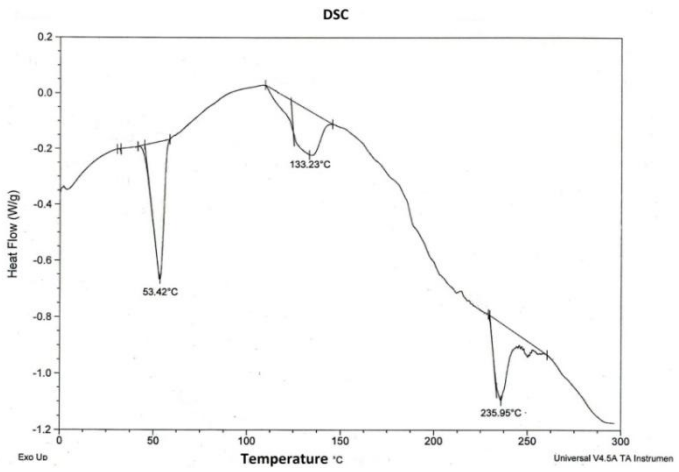

Figure 3: Differential Scanning Calorimetry Analysis of Niosome Ad

Size and Surface Analysis

The SEM and size distribution photographs of niosome Ad are shown in Figure 4 & 5 respectively. The microscopic analysis showed the formation of discrete, spherical vesicles having a smooth surface. The observed size of niosome ranged from 0.5 µm to 50µm with an average size of 3µm and the calculated polydispersity index (PDI) value was 0.04. The value of PDI <0.05 shows narrow size distribution. Further, the distribution graph demonstrates that, about 50% of the vesicle size was found to be less than 2µm. This showed that the method adopted might have resulted in the formation of large unilamellar niosomes.

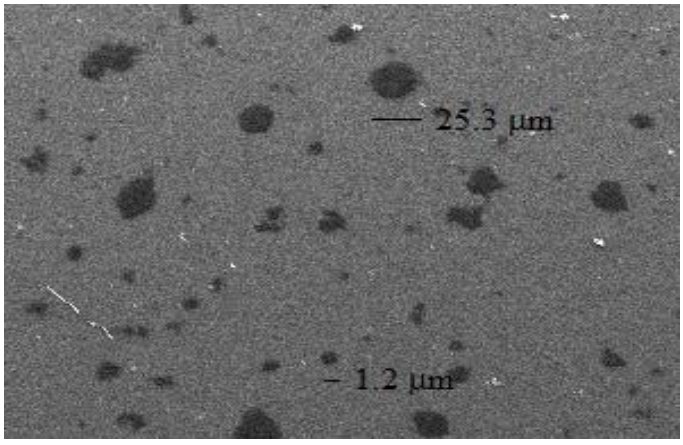

Figure 4: Scanning Electron Microphotograph of Niosome (Ad)

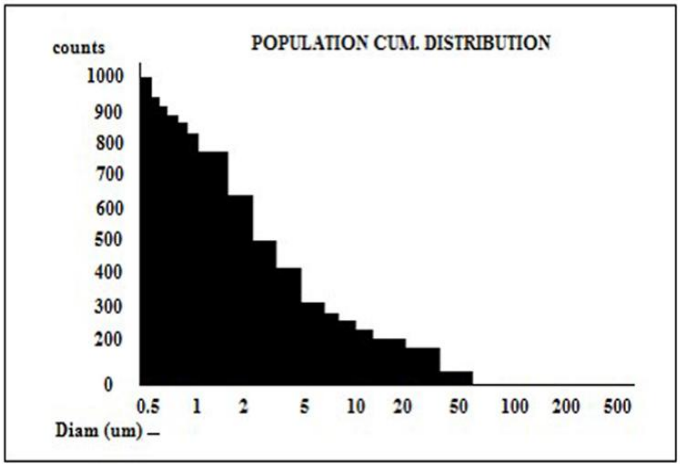

Figure 5: Size Distribution of Sertraline Hydrochloride Niosome Ad

Encapsulation Efficiency of Niosomes

The 1:1 ratio of surfactant and cholesterol was used in the niosome preparation, on the basis of the fact that this ratio is most beneficial for the efficient encapsulation of drugs [18]. The formulations Ad, Bb and Bc showed higher encapsulation efficiency of 53.71%±3.2%, 51.18%±2.5% and 51.92%±2.7% respectively. The lowest encapsulation efficiency of 9.35%± 1.2% has been observed for Ab. The results are shown in figure 6.

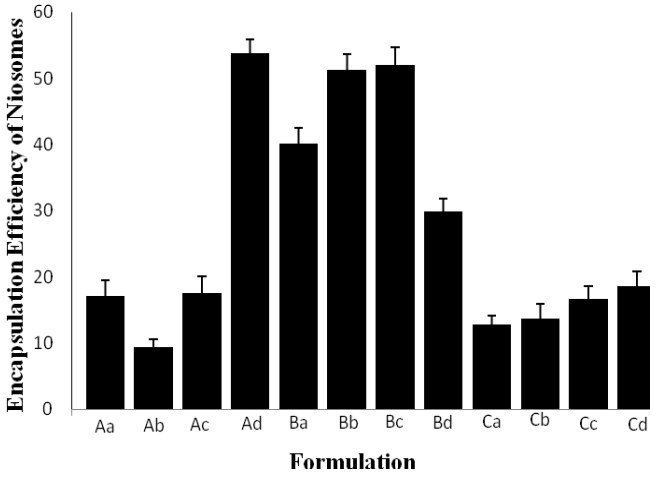

Figure 6: Percentage Encapsulation Efficiency of Niosomes

An increase in entrapment efficiency with increase in concentration of surfactant and cholesterol was observed which might be due to the increased liphophilic and hydrophilic ambience that could accommodate more amount of the drug [19]. However a decrease in entrapment efficiency beyond an optimal concentration of surfactant and cholesterol for the formulation B (span 60) may be explained as follows. With an increase in concentration of cholesterol, there is an increase in hydrophobicity as well as stability that prevents drug leakage. A further increase in cholesterol tends to deposit in between the bilayers and start excluding the drug from the niosomal bilayers as the amphipiles assembled into vesicles. Again the effect of cholesterol on entrapment efficiency depends on the phase transition temperature of surfactant. Below the phase transition temperature of surfactant, cholesterol tends to abolish the transition of gel to liquid state and make the membrane in less ordered condition which leads to the possibility of encapsulation of more amount of drug. Increasing cholesterol has been found to increase the membrane fluidity to the extent where the phase transition is abolished [20].

Comparatively smaller entrapment efficiency in case of span80 niosomes may be attributed to the presence of an unsaturated alkyl chain which introduces double bonds that cause a marked enhancement in their permeability which might lead to more leakage of drug through the bilayer [21]. Moreover, span 80 is liquid at room temperature and has the lowest phase transition temperature (T= -12ºC). Span 40 and span 60 are solid at room temperature and exhibit higher phase transition temperature (Tc) (42ºC for span 40, 53ºC for span 60) hence have higher encapsulation efficiency. The entrapment of the drug in span is directly proportional to the phase transition temperature and vice versa[18].

However an increase in entrapment efficiency of span 40 (Ad) niosomes might be due to their high HLB value of 6.7. The more hydrophilic nature of span 40 might have resulted in close association of head groups thereby preventing the leakage of encapsulated drug. This head

groups association of span 40 may also reject the incorporation of more cholesterol into the bilayers and thereby decreasing the rigidity of the membrane.

In vitro Sertraline HCl Release

Results of the *in vitro* release are shown in Figure 7. The study was performed for pure drug, formulations Ad, Bb & Bc on the basis of their higher encapsulation of sertraline HCl. The study revealed that the entire pure drug has got released in a time span of 3h. Formulations showed a controlled and continuous drug release with time and a maximum release of 70%±2.6% over a period of 12h was observed for Ad when compared with Bb and Bc, which have shown 49%±2.10% and 59.5%±2.5% of released drug respectively. The release of the formulations were significantly ($P<0.05$) different from each other. Further, the release profile was found to be apparently biphasic release process [22] i.e. an initial burst release for 2 to 3h followed by a slow release up to 12h. This is probably due to the ionization and release of the entrapped drug in the fatty acid chains of niosome upon dispersion in phosphate buffer till equilibrium is attained. Also, it has been reported that the highly ordered lipid particle cannot accommodate more amount of the drug and is the reason for drug expulsion [23]. A decrease in alkyl chain length of span 40 (Ad) that causes a marked enhancement in vesicle permeability might have resulted in increased drug release [14].

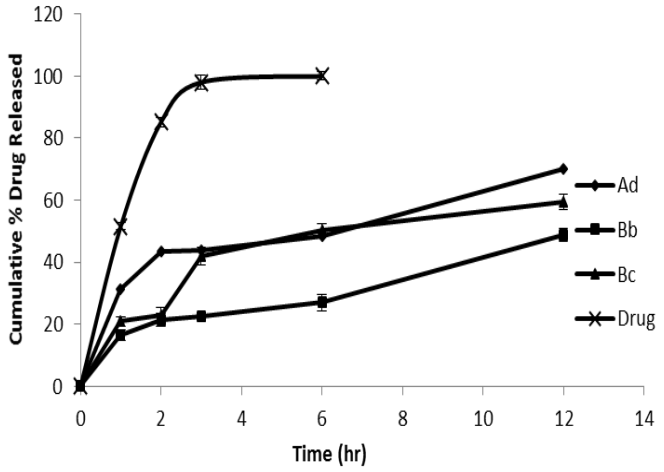

Figure 7: *In vitro* Release of Sertraline Hydrochloride Niosomes

In vitro Skin Permeation

The formulation Ad was subjected to permeation study on the basis of its highest drug release. The cumulative percentage drug permeated was 2.71%±0.52% through the animal skin. Figure 8 shows slow and prolonged permeation of the drug up to 12h. A lag time of 1.5h was exhibited by the formulation. The amount of drug accumulated in the skin was calculated as 0.502mg±0.009mg i.e. 50.2%±0.9% in 12h.This indicates that the sertraline HCl might get localized in the skin layers which could act as a depot providing slow release the drug for longer period. This would be useful in enhancing the bioavailability by maintaining the therapeutic plasma concentration for prolonged duration. The depot effect may due to the lipophillic surfactant and cholesterol components of niosome that increases the lipidity of its surroundings and the hydrophilic sertraline HCl diffuse slowly through this lipid rich surroundings.

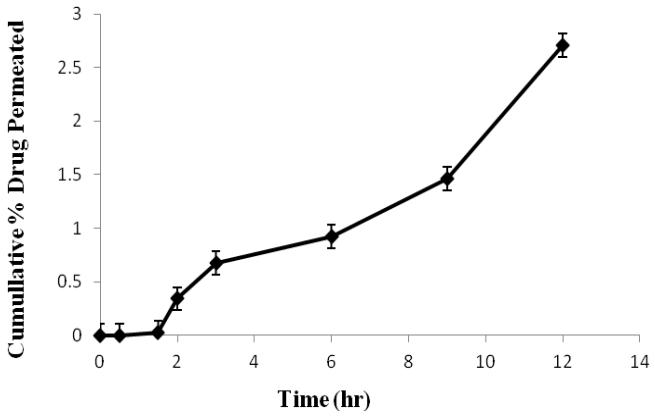

Figure 8: *In vitro* Skin Permeation of Niosomes Ad

Physical Stability

There was no much difference found with respect to encapsulation efficiency of niosomes after one week of storage at refrigerated condition. No fusion &/or aggregates were observed after this period. The results are tabulated in table 2. Here the physical stability study was conducted to identify any possible leakage of drug during storage. Any changes with respect to drug encapsulation has enormous influence over drug release and permeation properties of noisome. However the stability studies following ICH guidelines are to be conducted in future.

Table 2: Encapsulation efficiency of niosomes after storage at 2 to 8 °C

Formulation	Initial (%)	After one week (%)
Aa	17.18±2.3	17.09±2.02
Ab	9.35 ± 1.2	8.94±1.5
Ac	17.52±2.5	17.30±2.4
Ad	53.71±3.2	53.53±2.59
Ba	40.08±3.0	39.15±2.85
Bb	51.18±2.5	50.99±2.6
Bc	51.92±2.7	51.11±3.03
Bd	29.85±1.9	28.76±2.31
Ca	12.76±1.4	12.58±1.5
Cb	13.68±2.2	12.93±2.74
Cc	16.69±1.9	16.32±2.05
Cd	18.56±2.3	18.01±2.2

Values are mean ± SD, n=3

CONCLUSION

The sertraline hydrochloride niosomes showed a slow and prolonged release across the mouse skin and thus suitable for transdermal delivery.

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