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Research Article

PREPARATION AND CHARACTERIZATION OF IBUPROFEN LOADED TRANSFEROSOME AS A NOVEL CARRIER FOR TRANSDERMAL DRUG DELIVERY SYSTEM

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ABSTRACT

The goal of this study was to develop and evaluate the potential use of transfersome vesicles in the transdermal drug delivery of Ibuprofen. It was investigated by encapsulating the drug in various formulations of composed of various ratios of soya phosphatidylcholine ,span 80 and tween 80, prepared by lipid film hydration by rotatary evaporation method and evaluated for particle shape, size, zeta potential, entrapment efficiency (%EE),elasticity, stability, and in vitro skin permeation. The vesicles were spherical in structure as confirmed by Scanning Electron Microscopy and TEM, the vesicle size of best formulation for Span 80 and Tween 80 was 962 nm and 2250 nm respectively, and zeta potential (negatively charged) for Span 80 and Tween 80 was found to be -16.1 and -17.5 respectively. The %EE of ibuprofen in the vesicles was 47.8±2.2 and the elasticity of both increases with increase in surfactant conc. and were found to be 34.4±1.4 and 26.5±1.6. Stability studies for Transferosome were carried out for 5 weeks at 45^{0}C. In vitro skin permeation studies were carried by human cadaver skin using franz diffusion cell, and drug release after 24 hrs and flux was found 2.5824 and 1.9672 ug/cm^2/hr respectively. Fourier Transform Infrared Spectroscopy (FT-IR) and Differential Scanning Calorimetry (DSC) analysis indicated that the application of transfersomes significantly disrupted the stratum corneum lipid. It is evident from this study that transfersomes are a promising prolonged delivery system for Ibuprofen and have reasonably good stability characteristics. This research suggests that ibuprofen loaded transfersomes can be potentially used as a transdermal drug delivery system.

Keywords: Transferosome, Transdermal drug delivery, Analgesic and Antiinflamatory agent.

INTRODUCTION

Transdermal drug delivery systems (TDDs) offer a number of potential advantages over conventional methods such as injectable and oral delivery[1]. However, the major limitation of TDDs is the permeability of the skin; it is permeable to small molecules and lipophilic drugs and highly impermeable to macromolecules and hydrophilic drugs. The main barrier and rate-limiting step for diffusion of drugs across the skin is provided by the outermost layer of the skin, the stratum corneum (SC)[2]. Several strategies have been developed to overcome the skin's resistance, including the use of prodrugs, ion pairs, liposomes, microneedles, ultrasound, and iontophoresis [3-6].

Various types of liposomes (LPs) exist, such as traditional liposomes, niosomes, ethosomes, and transfersomes[3,7-12]. Various LPs have been extensively investigated for improving skin permeation enhancement. Liposomes are promising carriers for enhancing skin permeation because they have high membrane fluidity. Previous reports indicate that liposomes can deliver a large quantity of hydrophilic drugs (e.g., sodium fluorescein [12], carboxyfluorescein[13]), lipophilic drugs (e.g., retinoic acid[10] , tretinoin [11]), proteins, and macromolecules through the skin. Many factors influence the percutaneous penetration behavior of LPs, including particle size, surface charge, lipid composition, bilayer elasticity, lamellarity, and type of LPs[6,11]. Cevc's group introduced Transfersomes, which are the first generation of elastic vesicles. Transfersomes are prepared from phospholipids and edge activators. An edge activator is often a single-chain surfactant with a high radius of curvature that destabilizes the lipid bilayers of the vesicles and increases the deformability of the bilayers. Sodium cholate, sodium deoxycholate, Span 60, Span 65,Span 80, Tween 20, Tween 60, Tween 80, and dipotassium glycyrrhizinate were employed as edge activators. Compared with subcutaneous administration, transfersomes improved in vitro skin permeation of various drugs, penetrated intact skin in vivo, and efficiently transferred therapeutic amounts of drugs[8,13-15].

Ibuprofen (IBU) has low aqueous solubility, and it is a highly potent, nonsteroidal anti-inflammatory drug (NSAID) that is used for treatment of rheumatoid arthritis and osteoarthritis[16-18]. IBU shows similar efficacy for reducing pain and inflammatory symptoms, but it has lower toxicity than other NSAIDs. Although IBU is relatively potent and safe, its l imitations include low solubility , low incorporation in formulations, and low skin permeation[17].

In this study, vesicles were used as a novel IBU transdermal drug delivery system. The system was developed and evaluated for its physicochemical characteristics, such as particle size, surface charge, entrapment efficiency, loading efficiency, stability, and in vitro skin permeation. The type of vesicles (liposomes and transfersomes), the composition of lipid in the liposomes (cholesterol), and transfersomes(cholesterol and surfactants) were evaluated. Two surfactants were used for the preparation of transfersomes: Span80 and Tween 80.

MATERIALS AND METHODS

Materials

Ibuprofen was supplied as a gift sample by Micro Lab Baddi India. Span 80 and Tween 80 were purchased from SD fine Chemical, Mumbai, India and CDH laboratory pvt. Ltd. Mumbai, respectively. Soyaphosphatidylcholine was procured as a gift sample from HiMedia Laboratory pvt. Ltd. Mumbai. Other chemical and reagent were of analytical reagent grade.

Preparation of Ibupropen Loaded Transfersomes

Soya-phosphatidylcholine was taken in a round bottom flask. Span 80 or Tween 80 was put in the same round bottom flask. Ethanol was then added to the same flask. The drug was also loaded in the same RBM. These were then dissolved by shaking. Thin film was then formed by keeping it in the rotatory vaccum evaporator at 60C. This thin film was then hydrated by phosphate buffer saline to get the Transferosome[8]. Different variable parameters given in table 1.

Characterization Of Transferosomes

Morphology Imaging

Scanning electron microscope (SEM)

For SEM (JSM 6100,JOEL, Japan), one drop of Transfersomes were mounted on the stab covered with clean glass and coated with gold and were observed under the scanning electron microscope at an accelerating voltage of 20KV and photomicrographs of suitable magnification was obtained. The SEM of the formulation given in figure 3.

Table 1: Formulation code and variable used in preparation of transferosome.

S.No	Formulation code	PC:S	Drug(IBU)
1	IBU SP_1	95:5	100mg
2	IBU SP_2	90:10	100mg
3	IBU SP_3	85:15	100mg
4	IBU SP_4	80:20	100mg
5	IBU SP_5	75:25	100mg
6	IBU SP_6	70:30	100mg
7	IBU TW_1	95:5	100mg
8	IBU TW_2	90:10	100mg
9	IBU TW_3	85:15	100mg
10	IBU TW_4	80:20	100mg
11	IBU TW_5	75:25	100mg
12	IBU TW_6	70:30	100mg
13	PLAIN(IBUSP)	80:15	100mg

Where.IBU=Ibuprofen,SP=span,TW=tween80,PC=phosphatidylcholine,S=surfactant

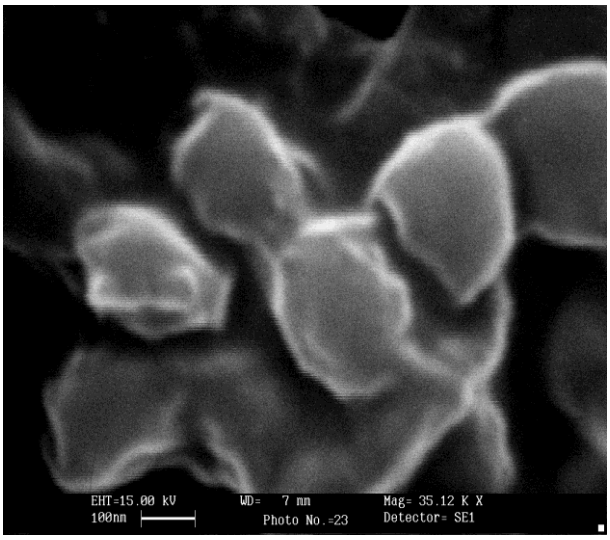

Figure 3:SEM Photograph of IBUSP3 Formulation

Transmission Electron Microscopy(TEM)

TEM (H-7500, Japan) was used to visualize the transfersomal vesicles. The sample were negatively stained magnification at an accelerating voltage of 100 kV with a 1% aqueous solution of phototungustic acid (PTA). After drying, the sample was viewed under the microscope at 10–100 k. The TEM of the formulation given in figure 2.

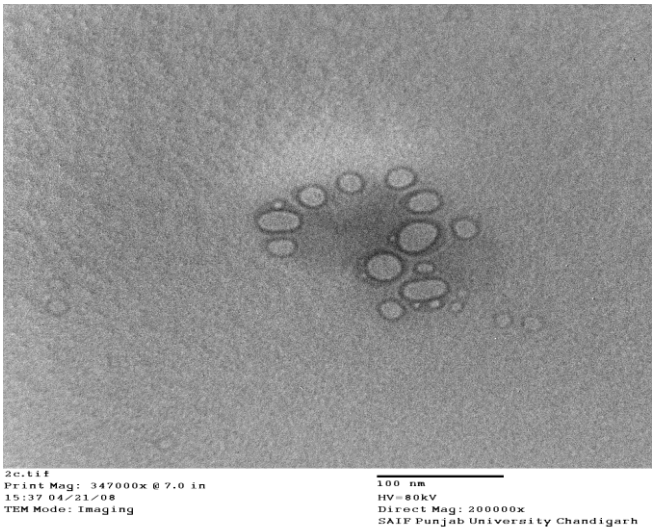

Figure 2: Tem Photograph Of Ibutw4 Formulation

Zeta Potential Measurement and Size distribution by dynamic light scattering (DLS)

The droplet size distribution and zeta potential of the transfersomes were determined by a DLS and computerized Malvern Zeta master inspection system (MAL, 500962, Malvern,UK)[25]. The data are shown in Table 2.

Turbidity measurement

Turbidity of the system was measured by Nephelometer[30]. The data are shown in Table 2.

Table 2: Characterization Of Transferosomal System

S.No	Formulation code	PC:S	Particle size	Zeta potential	Percentage entrapment	Elasticity
1.	IBU SP_1	95:5	1400		45.2±2.4	22.7±1.1
2.	IBU SP_2	90:10	1500		46.5±2.5	24.0±1.2
3.	IBU SP_3	85:15	962	-16.1	47.8±2.2	34.4±1.4
4.	IBU SP_4	80:20	1060	-16.0	42.2±1.9	17.1±1.6
5.	IBU SP_5	75:25	2960		40.5±2.5	19.6±1.4
6.	IBU SP_6	70:30	2250		42.2±1.9	22.2±1.3
7.	IBU TW_1	95:5	3640		43.4±2.5	17.1±1.4
8.	IBU TW_2	90:10	1890		43.8±1.9	22.7±1.1
9.	IBU TW_3	85:15	2520	-8.45	44.7±2.6	26.5±1.6
10.	IBU TW_4	80:20	2250	-17.5	44.7±2.6	26.5±1.6
11.	IBU TW_5	75:25	2510		40.5±2.3	22.2±1.3
12.	IBU TW_6	70:30	2510		40.5±2.3	19.6±1.4
13.	PLAIN(IBUSP)	80:15	6060	-4.96	40.5±2.2	15.1±1.4

Entrapment Efficiency (%EE) and Loading Efficiency

The concentration of Ibuprofen in the formulation was determined by UV analysis after disruption of the vesicles with Triton X-100 (0.5% w/w). The vesicle/Triton X-100 solution was centrifuged at 10,000 rpm at 4∘C for 10 min. The supernatant was filtered The entrapment efficiencies and the loading efficiencies of the Ibuprofen-loaded formulation were calculated by UV[28,30]. The data are shown in Table 2.

Elasticity measurements

The vesicle was extruded through the polycarbonate filter of 50nm pore diameter using a stainless steel pressure holder of 25mm diameter filter with 200ml capacity barrel at 2.5 bar pressure for 10min.vesicle size and volume of extruded suspension were compared before and after the extrusion. Elasticity was measured in term of deformability index which is proportional to J $(Rv/Rp)^2$, where J is wt. of the suspension ,which is extruded in 10min through a polycarbonate filter of pore size 50nm.Rv is size of the vesicle and Rp is the pore size of the membrane. The data are shown in Table 2.

In Vitro Skin Permeation Study

Human cadaver skin was used for the in vitro permeation experiment using locally fabricated Franz diffusion cell. The skin was clamped between the donar and the receptor chamber of diffusion cell with an effective diffusion area of 2.5 cm^2.The receptor chamber was filled with freshly prepared PBS (pH 7.4). The diffusion cell was

maintained at 32^{0}C and the solution of the receptor chamber was stirred continuously at 100rpm by using magnetic stirrer with hot plate (Remi equipment, Mumbai). The optimized formulation S3, T4 and liposome was gently placed in the donor chamber. At 0.5,1,1.5,2,3,4,5,6,7,8 and 24hr, 2.0ml of the solution in receptor compartment was removed and analyzed using UV spectrophotometer and replaced immediately with equal volume of fresh buffer[13,22]. The data are shown in Figure no.1.

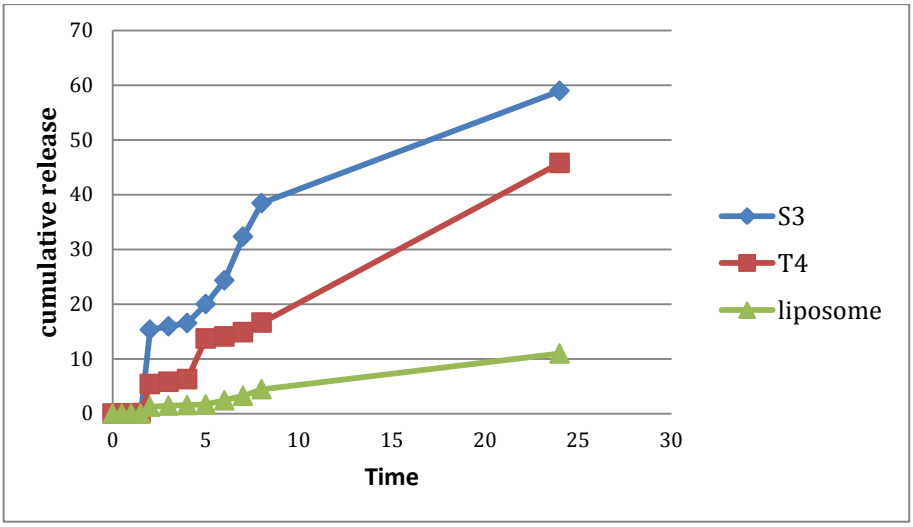

Figure 1: Drug Release Profile Of Different Formulation In Phosphate Buffer Saline Ph7.4 Across Human Cadaver Skin

Skin permeation and deposition studies

Dermatomed human cadaver skin from abdominal areas were obtained from CIMS, bilaspur and stored at -20C. By franz diffusion cell study was carried out. The receptor cell contain 10ml of PBS (pH7.4), which was constantly stirred for 24 hr at 37±1^{0}C using a magnetic stirrer at 100rpm. The donor compartment each containing 200ml of transferosomal formulation, (a)elastic liposomal formulation, (b)convectional liposomal formulation. Sample were withdrawn through sampling port at predetermined time interval over 24hr and analyzed for drug by UV[25-30]. The data are shown in Table 3.

Table 3: Permeation Parameters Of Ibu Across Human Cadaver Skin

S.No	FOR CODE	Jss (µg/ cm^{-2} / h)	P (cm/hr)× 10^{-4}	LT (hr)	D^d(Cm2/h) ×10^3	ER
1.	S3	2.5824	0.06	1.5	10	5.5
2.	T4	1.9672	0.036	1.8	8.3	4.2
3.	LIPOSOME	0.4722	0.0053	2.5	6	-

Where, Jss=transdermal flux, P=Permeability coefficient, LT=lag time, D^d=Diffusion coefficient, R=Correlation coefficient, ER=Enhancement Ratio(ratio of transdermal flux from transferosome formulation to liposome)

Storage-Physical Stability Evaluation of Transfersome

Transfersomes was evaluated for drug retentive potential at 3 different temperature condition i.e, 4-8^{0}C, 25±2^{0}C and 45±2^{0}C for a period of 5 weeks. The elastic liposome suspension were kept in sealed ampoules (10ml capacity). Sample were withdrawn periodically and analyzed for the drug content.

RESULTS AND DISCUSSION

It was found that the best formulation in term of this parameter was formulation no. 3 i.e SPC to S (85:15) for span 80 and SPC to S (80:20) for tween 80 the partical size of best formulation for Span 80 was 962nm, for tween80 this was 2250nm and for liposome this was 6060nm. We could see that the particle size decrease as the ration increased from 5 to 15% of span and than the particle size again increase from 20 to 30% ratio of S. This was same for tween 80 formulation.

A s Zeta potential is concerned, the least potential was found out to be -16.1, -17.5 and -4.96 for S3, T4 and liposomes.

The Turbidity increased till S3 and than started decreasing the same was true for formulation of tween80.

The percent entrapment was maximum for S3 (47.8±2.2) and minimum for S6 (42.9±2.6) and in case of tween80, maximum was found to be for T4 (44.7±2.6) and minimum for T6 (40.5±2.3) and for liposome it was (40.5±2.5). The data suggests that concentration with respect to the formulation represent the critical value upto, which the entrapment increased and beyond that it start decreasing.

The Elasticity was maximum for S3 (34.4±1.4) and minimum for S6 (22.2±1.3). In case of tween80, the maximum elasticity was for T4(26.5±1.6) and minimum for T1(17.1±1.4) and for liposome it was (15.1±1.4)

The result indicate that the elasticity of vesicle depend on both surfactant, concentration and type. With increase in surfactant concentration from 5 to 15%w/w, the elasticity vale increase and with further increase in surfactant concentration. Elasticity value of vesicle membrane decreases.

The shape and morphology of the Transferosome droplet was determined by TEM and SEM ,show the spherical shape and nano size range of vesicle. Demonstrating unilamillar structure under electron microscopic study confirming the vesicle characteristics.

In-vitro skin permeation for the determination of steady state transdermal flux (J),and permeation coefficient by franz diffusion cell (shown in graph). From above graph, it is concluded that S3 and T4 transferosomal formulation is a better vehicle for topical delivery of a drug than liposome formulation as the transdermal flux is 5-6 times more than the liposome and also having less lag time compared to liposome.

For storage stability characteristic, substaintial loss of drug at higher temperature was observe with more than 25% drug leakage

resultant after 5 week at 45^{0}C.however refrigerated conditions were found to be best, at all times the drug leakage was negligible.

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